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La Tapa
Maternidad Transgénica, 2007
Chino Benítez

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Tel. 5287-3827 Int. 73919 y 4523-6619
e-mail: revmedbuenosaires@gmail.com – http//: www.medicinabuenosaires.com

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Diagramación y Diseño: Andrés Esteban Zapata - aez.sgi@gmail.com

REUNIÓN CONJUNTA SAIC SAI&FAIC SAFIS 2022

LXVII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI) & 3ER CONGRESO FRANCO-ARGENTINO DE INMUNOLOGÍA (FAIC)

REUNIÓN ANUAL 2022 DE LA SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

16-19 de noviembre de 2022
Hotel 13 de Julio – Mar del Plata

EDITORES RESPONSABLES
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Dr. Emilio Malchiodi
Dr. Martín Vila Petroff
Dra. Caroline Lamb

JOINT MEETING SAIC SAI&FAIC SAFIS 2022

LXVII ANNUAL MEETING OF
SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXX ANNUAL MEETING OF
SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI) & 3RD
FRENCH-ARGENTINE IMMUNOLOGY CONGRESS (FAIC)

ANNUAL MEETING 2022 OF
SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

November 16-19, 2022
13 de Julio Hotel – Mar del Plata

RESPONSIBLE EDITORS
Dr. Daniel Alonso
Dr. Emilio Malchiodi
Dr. Martín Vila Petroff
Dr. Caroline Lamb

LA TAPA

Chino Benítez. Maternidad Transgénica, 2007

Técnica: Acrílico, *collage* sobre tela. Medidas 180 x 150 cm. Gentileza del autor

Artista plástico, arquitecto (FADU-UBA), diseñador. Realizó el *Workshop* “Crear Presencia” con la artista visual Lucia W. Meister en 2022 y Clínica de obra con E. Mac Entyre en el Centro Cultural Recoleta en 2008. Cursó seminarios de Posgrado en Artes Visuales con Juan Astica, J.C. Romero y Miguel A. Bengoechea en la Escuela Superior de Bellas Artes E. de la Cárcova-UNA entre 2004 y 2002. Su obra trata sobre temas como la naturaleza, la comunicación, el medio ambiente, los cuentos, la mitología, pero reformulándolos, al subvertir el rutinario orden de las cosas desde su imaginación razonada. Ha sido seleccionado en varios salones de artes visuales. En 2021 participó en la muestra “En nombre de la Libertad” en la Galería Adriana Budich; en 2018 participó en la Bienal de Area-Tec; en 2014 en el Salón de Pintura del Club Boca Jrs., exhibido en la Legislatura Porteña, donde recibió un premio *Mención*; en 2013 en el Salón de Artes Plásticas Manuel Belgrano en el Museo Sívori, así como también en el año 2007 en el mismo Salón, recibiendo un premio *Mención*; en el Salón Nacional de Artes Visuales, Palais de Glace, participó en 2006, 2007, 2008 y 2010; en el Salón Nacional B. Quinquela Martin, Museo Quinquela Martin en 2010 y 2008; en el Salón Nacional UADE en 2007; a través de la galería Sonoridad Amarilla participó en el proyecto “Biblioteka en Red” en arteBA 2004; y en el CCEBA en el mismo año y en 2003 fue elegido en el Salón de la Fundación Dante Alighieri, donde recibió un premio *Mención*; participó en la Bienal de Pintura AAGA en 2003 y 2009. En el exterior participó en la ART-FAIR de Atlanta, USA, en 2002; en la muestra colectiva de la McNeill Gallery en London, UK, en 2001; y en la muestra de artistas latinoamericanos en la Casa de Cultura Hispanoamericana en Milano, Italia, en 2000. Su obra fue exhibida individualmente en la Galería Adriana Budich en 2021; en la sala de arte del Art Hotel en Buenos Aires, en Clásica y Moderna; en el Espacio Giesso; y en la Sala Municipal de Arte E. Saracco en Neuquén.

chinobenitez.arq@gmail.com
Instagram: chino.b3nit3z
www.chinobenitez.blogspot.com

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Mensaje de Bienvenida de los Presidentes

Discurso del Dr. Daniel F. Alonso, Presidente SAIC.

Estimada comunidad científica del área biomédica,

Con mucha alegría les doy la bienvenida junto a los Dres. Emilio Malchiodi y Martín Vila Petroff a la LXVII Reunión de la Sociedad Argentina de Investigación Clínica (SAIC), que este año 2022 se realiza junto a la Sociedad Argentina de Inmunología (SAI) y la Sociedad Argentina de Fisiología (SAFIS), en una reunión conjunta que hemos denominado "del reencuentro" luego del aislamiento y las restricciones que impuso la pandemia COVID-19. Hemos arrimado a un total de 900 trabajos presentados, en una reunión conjunta de las más concurridas de los últimos años, en niveles incluso superiores a reuniones similares antes de la pandemia.

Mi reconocimiento en primer lugar para la Dra. Cristina Carillo y el Dr. Alejandro Curino, quienes me antecedieron en la presidencia de SAIC. Les tocó una situación sin precedentes y junto a sus respectivos equipos de trabajo supieron sostener las actividades de nuestra Sociedad y concretar con éxito a través de medios virtuales las reuniones de 2020 y 2021. Al respecto, Alejandro y Cristina coordinarán una mesa redonda donde podrán contarnos sobre los desafíos que vivieron y podremos debatir sobre lo que nos deja la pandemia en el ámbito científico.

Quiero destacar y agradecer la gran tarea del Consejo Directivo actual de SAIC en su conjunto, y en particular de los integrantes la Comisión Directiva, la Dra. Isabel Luthy, la Dra. Caroline Lamb, el Dr. Mariano Gabri y la Dra. Stella Ranuncolo, como también a la secretaria de nuestra Sociedad, Ivana Rossetto por todo el trabajo y el compromiso de siempre, y al contador Carlos Resnik por su acompañamiento profesional. Este año encaramos la adquisición de un inmueble como sede propia de SAIC en la ciudad de Buenos Aires, un anhelo postergado por mucho tiempo que finalmente hemos podido concretar. El logro no es sólo el mérito de esta gestión y del buen manejo de muchas gestiones previas, sino el resultado de una construcción colectiva en favor de un bien común, que ahora nos obliga a mirar nuevos horizontes y a encauzar un nuevo ciclo de crecimiento para SAIC.

En principio, la nueva sede debería constituir un espacio de encuentro presencial, pero que siempre soporte una buena conectividad a través de medios virtuales, asegurando a socias y socios de todo el país la posibilidad de participar en las distintas actividades a lo largo de todo el año. Pensamos en una sede moderna, preparada para la nueva normalidad híbrida del siglo XXI, que se erija en un eje para sostener el carácter federal de la gestión de SAIC y de todas sus actividades científicas biomédicas. En este sentido, y aunque pueda parecer contraintuitivo, muchos analistas empiezan advertir que el trabajo remoto, adecuadamente combinado a la actividad presencial en una sede cuando sea conveniente, está generando un mayor sentido de pertenencia y mayor compromiso con la cultura y los valores de una organización. De hecho, la comunidad científica siempre estuvo acostumbrada a trabajar en equipos distribuidos en distintos lugares, y aún en distintos países, mucho antes de la pandemia. Con lo cual no es difícil imaginar a la nueva sede de SAIC como una herramienta de encuentro para multiplicar ese trabajo colectivo.

A partir de los respectivos enfoques y los aportes de las tres Sociedades, para esta reunión 2022 buscamos brindar un abanico de temas científicos que cubran distintas áreas del conocimiento biomédico, a través de conferencias, simposios y mesas redondas. Además, el programa científico incluye las habituales sesiones de pósters donde la comunidad científica muestra por primera vez sus hallazgos recientes, una variada convocatoria a premios en distintas disciplinas y debates sobre asuntos científicos, educación en salud y cuestiones de género en el ámbito académico. Hemos puesto un interés especial en la medicina traslacional, entendida como un ámbito en el cual pueden confluir la investigación científica básica, la medicina asistencial e incluso las necesidades sanitarias. Tendremos varias actividades con esta tónica, incluyendo un Simposio que será coordinado por el Dr. Rodolfo Rey, quien es el Coordinador de la Red de Investigación Traslacional en Salud (RITS) del CONICET.

El impulso actual a la medicina traslacional debería ser capitalizado como una gran oportunidad para que SAIC vuelva a conectar con sus orígenes, acercándose más a la actividad médico-hospitalaria. En su etapa fundacional en la década de 1960, SAIC pretendió contribuir al desarrollo de la investigación básica y aplicada en el ámbito de la medicina, con un fuerte contenido fisiológico y clínico. El auge de los conocimientos en biología molecular y celular, sumado a los avances en biotecnología a partir de la década de 1990, promovieron una expansión sorprendente hacia estas disciplinas, con un paulatino predominio de las investigaciones del ámbito biomédico básico. Sin descuidar esta actual fortaleza de la comunidad científica que se reúne hoy en SAIC, tal vez sea el momento oportuno para mirar nuestras líneas de trabajo y nuestra experiencia bajo la perspectiva de las necesidades médicas insatisfechas y valorar si es factible volcar nuestras capacidades a la aplicación clínica. Tengo el convencimiento de que existen las condiciones para que esto ocurra, pero en ocasiones las capacidades se encuentran en "islas" de conocimiento y en esfuerzos algo aislados. Sólo faltaría establecer los puentes para avanzar hacia abordajes multidisciplinarios y transdisciplinarios en problemas concretos, tomando como referencia algunos modelos exitosos. De alguna manera, la pandemia ha visibilizado la potencial capacidad del sistema científico y tecnológico para responder de esta manera.

Desde las últimas gestiones, hemos encarado iniciativas en favor de esta mirada, como fue la incorporación de SAIC al Foro de Sociedades Científicas, Organizaciones de la Sociedad Civil y Universidades, y el establecimiento de convenios de colaboración académica, científica y tecnológica con grupos y sociedades médicas. En 2021, se firmó un primer convenio con la Sociedad Argentina de Pediatría (SAP) y este año con la Sociedad Argentina de Hematología (SAH), la Asociación Argentina de Salud Pública (AASAP) y el Grupo Argentino de Investigación Clínica en Oncología (GAICO). En todos los casos, ya se han iniciado reuniones y actividades conjuntas, y la perspectiva es que estos puentes con el campo médico y sanitario sigan creciendo en las próximas gestiones. En particular, se encuentra avanzada la posibilidad de que SAIC se posicione para acreditar Residencias Hospitalarias, contribuyendo a la formación de residentes y dando soporte a una capacitación sólida en investigación, con el dictado de cursos y la oferta de pasantías en laboratorios.

Luego de dos años nos volvemos a encontrar en Mar del Plata, la misma ciudad que nos recibió tantas veces. Deseamos que este reencuentro tan esperado nos lleve a una discusión enriquecedora de los resultados de los trabajos de investigación y que nuevamente resulte en un motor generador de futuras colaboraciones. Muy en especial pretendemos que la reunión vuelva a representar un ámbito adecuado para el intercambio productivo entre becarios, investigadores jóvenes e investigadores de mayor experiencia. Más allá de la utilidad que hemos aprendido a valorar de las herramientas virtuales, sin dudas estas reuniones presenciales son irremplazables.

Dejo formalmente inaugurada la Reunión Anual Conjunta 2022.

Discurso del Dr. Martin Vila Petroff, Presidente SAFIS.

Estimados colegas y amigos,

Es un verdadero placer inaugurar y darles la bienvenida junto a los Dres. Daniel Alonso, Presidente de SAIC, y Emilio Malchiodi, Presidente de SAI, a la Reunión Anual de Sociedades de Biociencias 2022 o como preferimos llamarla nosotros: "La Reunión del Reencuentro". Y realmente lo sentimos así dado que será la primera reunión presencial de nuestras sociedades desde la realizada en 2019. Los años vividos en pandemia nos movilizaron de diversas maneras. Sufrimos, nos adaptamos, nos reinventamos y aprendimos nuevas formas de hacer ciencia y de comunicarnos. Tal es así que la reunión anual de SAFIS 2021, que fue 100% virtual, tuvo un record de inscriptos, de resúmenes recibidos y de simposios y conferencias con una participación internacional sin precedentes. Eso nos generó una enorme satisfacción, y nos dio la ventaja de tener en un congreso local asistentes internacionales. Sin embargo, soy un firme defensor de las reuniones presenciales porque creo que permiten una interacción entre los asistentes al congreso que es difícil de lograr en las plataformas virtuales. Teniendo esto en cuenta, y aprovechando lo aprendido y adquirido en la pandemia, este año tenemos un programa científico sumamente estimulante y provocativo que incluye prestigiosos invitados nacionales y la participación de renombrados científicos extranjeros que por primera vez participaran en los simposios de manera virtual.

Fiel a la costumbre de SAFIS esta reunión propicia la investigación interdisciplinaria, la discusión crítica de las investigaciones y la incorporación de nuevas tecnologías en el área de estudio de la fisiología humana. La asociación virtuosa con SAIC y SAI multiplica el abordaje multidisciplinario y genera un terreno fértil que seguramente redundará en el avance de la ciencia nacional. Para garantizar el cumplimiento de este objetivo hemos organizado un programa científico con foco en los más recientes avances de la ciencia fisiológica, destacando nuevos principios y mecanismos que promuevan o rompan el ciclo salud-estrés-enfermedad. El programa incluye 2 conferencias, la primera a cargo del Dr. Shey-Shing Sheu del Centro de Medicina Traslacional de la Facultad de Medicina de la Thomas Jefferson University, Philadelphia, Estados Unidos y la segunda dictada por la Dra. Ana Maria Genaro del Instituto de Investigaciones Biomédicas, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina, 4 simposios temáticos y una mesa redonda de debate científico en la que discutiremos si la modernidad liquida de Bauman afecta la forma en la que hacemos ciencia. Además, la Comisión de Educación de SAFIS ha organizado un interesante taller participativo titulado "Entre la fisiología que investigamos, la que enseñamos y la que la sociedad necesita", y poniendo especial énfasis en la participación de becarios e investigadores jóvenes, se incluye un simposio organizado por la Comisión de Investigadores Jóvenes, que abordará la temática del uso de transferencia génica como estrategia terapéutica. Finalmente, y como sello de las actividades de nuestra sociedad, se realizarán las clásicas secciones de posters (en este caso mezcladas con posters de las 3 sociedades) y los premios SAFIS y María Cristina Camilión de Hurtado, destinado a los trabajos del área cardiovascular.

Es importante destacar que este año, SAFIS ha logrado un importante convenio con la revista Frontiers Physiology que brinda la posibilidad a los socios de SAFIS y a los participantes del congreso a que envíen un trabajo completo para su publicación a un volumen especial (Topic Issue) basado en el congreso y titulado: Health-Stress-Disease Triangle. Pathophysiological Focus and Perspectives. Para más información dirigirse a: https://www.frontiersin.org/research-topics/43533/health-stress-disease-triangle-pathophysiological-focus-and-perspectives.

Finalmente quiero expresar un especial agradecimiento a los integrantes del Consejo Directivo de SAFIS por el enorme trabajo realizado durante estos 2 años de gestión y a Valeria Cassaza, Secretaria de SAFIS, por su apoyo permanente. Asimismo, mi agradecimiento al CONICET y al FONCYT que nos han brindado apoyo económico y a la Firma Microlat y la Familia Camilion de Hurtado que realizaron el aporte económico para los premios SAFIS y Camilion de Hurtado, respectivamente. Hago también extensivo mi agradecimiento a todos los invitados nacionales e internacionales, coordinadores, jurados y asistentes que desinteresadamente contribuyen a la excelencia de nuestra reunión.

En nombre de la Consejo Directivo, de la Comisión de Docencia y de Jóvenes Investigadores de SAFIS les doy la bienvenida a la "Reunión del Reencuentro" a la espera de que compartamos un congreso verdaderamente memorable.

Discurso del Dr. Emilio Malchiodi, Presidente SAI.

Estimadas/os colegas,

Con enorme placer damos por inaugurada la Reunión Anual de Sociedades de Biociencias 2022, con las sociedades hermanas SAIC y SAFIS, y que este año cuenta, además, con la realización del 3er Congreso Franco Argentino de Inmunología, organizado por SAI y los queridos colegas franceses.

Afortunadamente, gracias a la vacunación y los cuidados implementados, luego de 2 años de impedimentos por la pandemia, en esta ocasión podemos reunirnos de forma presencial, lo que nos hace realmente muy felices.

Visto el programa científico y la enorme convocatoria, creemos que este año tendremos un excelente Congreso, que para SAI-FAIC incluye más de 300 inscriptos, más de 230 resúmenes aceptados, 6 postulaciones al premio Leonardo Satz, y 4 postulantes al novedoso premio de Inmunología Clínica, que hemos instituido para este año. Además del honor de un premio otorgado por SAI-FAIC, estos premios contarán con un monto de $120.000, gracias al generoso aporte de las empresas Hemoderivados de Córdoba (Leonardo Satz) y EGLE-Tx de Francia (Inmunología Clínica).

Son numerosas las empresas que han contribuido financieramente a la realización de este evento, para las cuales es importante que los inscriptos visiten los stands de la muestra comercial. Agradecemos también, muy especialmente, al CONICET y MinCyT por los aportes en subsidios para la realización de Reuniones Científicas. En ocasión de este 3er Congreso Franco Argentino de Inmunología, muy particularmente debemos agradecer el apoyo financiero que han realizado desde Francia, la Université Paris-Est Créteil Val de Marne, el Instituto Curie y la empresa EGLE-Tx.

El programa científico incluye, lo que para nosotros constituye la parte más importante de todas, que es la exposición y defensa de todos los resúmenes aceptados, para los que designamos a científicos destacados a los que les agradecemos enormemente su trabajo ya que es la parte más fructífera y participativa del Congreso. Esto sin menoscabar a los destacados simposios proyectados, 3 de ellos organizados en forma conjunta con SAIC, que contarán con la presencia de excelentes expositores de Francia, Argentina y otras regiones, a los que les agradecemos fervientemente su presencia y compromiso para engalanar el Congreso. Como broches destacados, hemos organizado 3 conferencia plenarias con temas de enorme actualidad y con expositores de altísimo nivel científico, que esperemos disfruten.

Como todos los años, los evaluadores han seleccionado 4 de los trabajos presentados a Premios para su defensa pública el sábado. Vista la calidad de los candidatos, los evaluadores han tenido y tendrán un enorme trabajo para definir los ganadores, por lo que también agradecemos su presencia y predisposición.

Quiero también destacar y agradecer el compromiso y dedicación de los miembros de CD de SAI y muy especialmente a la Secretaria y la Tesorera, sobre las que ha descansado la mayor parte del trabajo.

Sin más, los invitamos a compartir un cóctel de recepción, ¡pero sobre todo a reencontrarse y departir con tantos colegas a los cuales hace tanto que no vemos!

SAIC I LECTURE *Wednesday, November 16, 14-15 hr*
Chairs: Ana Maria Buzaleh - Laura Sabina Varela.

ALTERED EPIGENETIC MECHANISMS AND BEHAVIORAL DEFICITS DERIVED FROM PERINATAL MALNUTRITION: LESSONS FROM A MOUSE MODEL.

Mariela Chertoff[1,2], Carolina D Alberca[1,2], Estefania Fesser[1], Bruno Berardino[1] and Eduardo T Cánepa[1,2]
[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Neuroepigenética y Adversidades Tempranas, Buenos Aires, Argentina. [2]CONICET-Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales – CONICET (IQUIBICEN), Buenos Aires, Argentina.

Perinatal period is a critical stage for individual development. The quality of the embryonic environment and postnatal experiences have a great influence on the emotional and cognitive development of the infant and the adolescent. Early life adversities can modulate the expression of molecules involved in neuronal plasticity contributing to alter developing neuronal circuits trajectories. We study the impact of perinatal protein malnutrition, using a both sexes mice born from dams fed with normal protein diet (NP-20% of protein) or low protein diet (LP-8% of protein) during pregnancy and lactation. Interestingly, malnourished dams provide a lesser and more fragmented maternal care than their normally fed counterparts. This altered behavior was associated with an increased anxiety-like behavior and a reduction on glucocorticoid receptor in the hippocampus. In the pups, through a preclinical PET analysis, we found evidence of changes in glucose metabolism in the hippocampus and prefrontal cortex of LP mice, suggesting a potential alteration in the function of these areas. Therefore, we performed in F1 mice different behavioral tests in order to evaluate emotional status, social cognition and memory. We analyzed the performance of NP and LP animals in learning and memory tests that aim to evaluate the social cognition. As a result, we found that contextual recognition memory, social interaction and social memory were impaired in LP mice. In addition, anxiety-like behavior was increased in LP mice. In order to understand the molecular mechanisms behind this, we focus on DNA methylation and stress pathways, finding an increased expression of DNMT3b and Gadd45b in P21 malnourished females but not in males. We also observed a deregulation of the expression in genes linked to the glutamatergic and gabaergic pathways in the upstream maintenance of the E / I balance in adults LP mice. We observed that environmental enrichment (EE) partially reverts the emotional and cognitive deficits. The 5hmC seems to be an important epigenetic mechanisms of brain adaptation, so, we examine the distribution of 5hmC on ventral hippocampus and we found several anxiety-related genes differentially 5hmethylated on malnourished mice, which are reverted after growing on EE. Together, these findings represent a critical step toward understanding the molecular effects of the environment on the mechanisms that underlie behavioral disorders caused by perinatal protein malnutrition.

SAIC II LECTURE *Wednesday, November 16, 17-18 hr*
Chairs: Mariano Gabri - Analía Reinés

EARLY-LIFE PATHOGENS SELECT FOR A HUMAN-SPECIFIC CD33 ALLELE THAT PROTECTS FUNCTION OF GRANDMOTHERS

Dr. Ajit Varki
University of California, San Diego, USA

The myelomonocytic receptor CD33 (Siglec-3) inhibits innate immune reactivity by extracellular V-set domain recognition of sialic acid-containing “self-associated molecular patterns”. Existing knowledge about early-life pathogens and human CD33-evolution suggests that grandmothering emerged in humans.

SAI I EMBO LECTURE *Wednesday, November 16, 17-18 hr*
Chairs: Eloisa Arana

INFORMING VACCINE DESIGN BY DEFINING THE RULES OF ANTIBODY RESPONSES

Facundo Batista
Ragon Institute of MGH, MIT and Harvard, Cambridge MA, USA; Department of Immunology, Harvard Medical School, USA; Department of Microbiology, Harvard Medical School, USA.

The antibodies generated by B cells underly the efficacy of almost every licensed human vaccine. Understanding the process by which B cells respond to antigens is, therefore, key to rationalizing the design of vaccines for known and emerging pathogens. On activation, B cells produce two waves of antibodies. The second, high-affinity wave originates with B cells which have undergone rounds of proliferation, somatic hypermutation, and selection within the germinal centers. Much of my career has been dedicated to the molecular mechanisms of B cell activation: the site and structure of the encounter between antigen and B cell receptor (BCR) and the subsequent signaling process. In recent years, however, we have expanded our work into vaccinology more directly, using CRISPR/Cas9 to enormously expedite the production of humanized mice for vaccine research. With this mouse platform, and the tools we developed to analyze basic B cell responses, we have identified antibody feedback processes with serious implications for the design and provision of booster shots, including for SARS-CoV-2, developed a new approach to the improvement of prophylactic monoclonal antibodies for malaria, and provided preclinical validation for novel immunogens for HIV and other infectious diseases. A deeper understanding of the fundamentals of B cell responses and advanced model systems will both be necessary to keep vaccinology moving forward.

SAFIS I LECTURE *Wednesday, November 16, 19-20 hr*
Chair: Julieta Palomeque

CALCIUM, ATP, AND ROS: A MITOCHONDRIAL LOVE-HATE TRIANGLE

Shey-Shing Sheu
Center for translational medicine, department of medicine, Thomas Jefferson University, Philadelphia, USA

In cardiac muscle cells, the majority of ATP is generated by mitochondria via oxidative phosphorylation. During the process of electron transport chain activity, a small number of electrons slip into oxygen resulting in the subsequent production of reactive oxygen species (ROS). An optimal level of ROS serves as key signaling molecules to regulate cellular activities and functions. However, excessive amounts of ROS cause oxidative stresses and lead to cell injury and death. Ca2+ is a key regulator of mitochondrial function and acts at several levels within the organelle to stimulate ATP synthesis and modify ROS generation. However, the dysregulation of mitochondrial Ca2+ homeostasis is now recognized to play a key role in several pathologies. For example, mitochondrial matrix Ca2+ overload can lead to enhanced generation of ROS, triggering of the permeability transition pore, and cytochrome c release, leading to apoptosis and necrosis. My talk will highlight the delicate balance among Ca2+, ATP, and ROS homeostasis for ensuring a healthy cardiac performance. Moreover, I will discuss why the perturbation of this balance can lead to cardiac arrhythmias and heart failure.

SAIC III LECTURE *Thursday, November 17, 9-10 hr*
Chair: Stella Maris Ranuncolo - Anahí Vijnovich

THE DIAGNOSIS OF LYMPHOMA IN THE ERA OF PERSONALIZED MEDICINE.

Elaine S Jaffe
Hematopathology Section, Laboratory of Pathology, Center for Cancer Research.

The definitions of follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are evolving in the era of personalized medicine. Early stages of the evolution of FL have been recognized. Two histological manifestations of early lesions are in situ follicular neoplasia and duodenal type FL. Additionally, FL frequently undergoes histological transformation, the most common form being DLBCL. High-grade B-cell lymphoma with double hit, with translocations involving BCL2 and MYC are important clinically. Rarer forms of transformation include classic Hodgkin lymphoma (CHL) and histiocytic sarcoma. In addition to conventional FL associated with the BCL2 translocation, alternative forms of BCL2-negative FL have been observed. These are heterogenous clinically

and genetically, with a major subtype characterized by CD23 expression, and cooperating mutations in STAT6 and CREBBP. Other entities of follicle cell derivation arise in young patients and include pediatric type FL, testicular FL and a large B-cell lymphoma with IRF4 rearrangement. Historically DLBCL was separated into only two histological variants, centroblastic and immunoblastic. Today we recognize greater clinical and molecular diversity. The WHO classification of 2017 recommended (1) the segregation of activated B cell and germinal center B cell derived DLBCL, (2) the identification of high-grade B-cell lymphoma with double hit, and (3) the recognition of an aggressive lymphoma that may resemble Burkitt lymphoma, currently designated in the International Consensus Classification as Large B-cell lymphoma with 11q aberration. Recent studies have highlighted the genomic complexity among aggressive B-cell lymphomas. NGS and mutational profiling have identified clinically significant genetic subgroups. It is hoped that these data ultimately will lead to targeted therapy based on the genetic profile.

SAIC IV LECTURE *Thursday, November 17, 12-13 hr*
Chairs: Dra. Mariana Malvicini - Dra. Karina Gomez

SYSTEMS BIOLGY APPROACHES TO UNDERSTAND IMMUNITY TO MALARIA

Diana S. Hansen
The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Unlike many viral and bacterial infections, which can induce life-long protection after a single exposure, the acquisition of immunity to Plasmodium parasites is strikingly less efficient. Individuals in malaria endemic areas develop clinical immunity only after many years of exposure to the parasite. Clinical immunity is not sterilising but prevents symptomatic episodes by substantially reducing parasite burden, with adults often experiencing asymptomatic malaria. Historically, asymptomatic malaria has been viewed as beneficial and required to help maintain clinical immunity, thereby reducing the risk of symptomatic infections. However, emerging views suggest that these infections are detrimental and constitute a parasite reservoir that perpetuates transmission. To define the impact of asymptomatic malaria, we pursued a systems approach integrating antibody responses, high-dimensional mass cytometry, and transcriptional profiling of individuals experiencing symptomatic and asymptomatic Plasmodium vivax and Plasmodium falciparum infection in a malaria endemic area of Indonesia. Antibody responses to specific parasite invasion ligands, defined populations of classical and atypical memory B cells and a TH2 cell bias were associated with reduced risk of symptomatic infection. Despite these protective responses, asymptomatic malaria featured an immunosuppressive transcriptional signature with upregulation of several pathways involved in the inhibition of T cell function, and CTLA-4 as a predicted regulator in these processes. As proof of concept, we demonstrated a role for CTLA-4 in the development of asymptomatic recrudescent parasitemia in infection models. The results suggest that asymptomatic malaria is not innocuous and might not support the induction of immune processes to fully control parasitemia or efficiently respond to malaria vaccines. Our research provides a framework to consider screening and treatment these chronic infections to further elimination programs and to improve malaria vaccine efficacy in pre-elimination settings.

SAIC V *ALFREDO LANARI* **LECTURE** *Thursday, November 17, 14-15 hr*
Chair:Marianela Candolfi

FROM THE BENCH TO THE BATTLEFIELD: LETS TALK ABOUT THE PANDEMIC, SCIENCE AND GENDER

Andrea Gamarnik
Fundación Instituto Leloir-IIBBA CONICET, Buenos Aires, Argentina

In this talk, I will give an overview of my curiosity for science and discuss gender issues that I have faced throughout my career. Also, I will share the main ideas that were developed in the molecular virology laboratory that I have directed since 2001 at the Institute Leloir, Buenos Aires, Argentina. Over the past 20 years, we uncovered molecular mechanisms of dengue virus replication, provided new paradigms for viral adaptation to mosquito and humans, dissected novel functions of viral enzymes, and provided information for designing viral attenuation for vaccine development. I am convinced that rigorous basic science is necessary for innovative solution to our problems. The pandemic forced us to create new ways of working in science and to communicate among different disciplines. In March 2020, we created the COVIDAR group for helping to cope with the emergency. In this context, we developed a serologic test to evaluate the immune response against SARS-CoV-2. The test was approved and produced for detecting IgG and IgM antibodies against the spike protein. About 1.5 million tests were freely distributed to public and private health institutions for evaluating immune responses in hospitalized

patients and seroprevalence studies in nursing homes and neighborhoods. The COVIDAR group also cooperated with the Ministry of Health for evaluation of immune responses elicited by the vaccines applied in Argentina. In this process, we learned that improving communication, implementing collaborative approaches and assembling transdisciplinary teams are essential for tackling any complex scientific problem, including a pandemic.

SAIC VI -SAI II LECTURE *Thursday, November 17, 15-16 hr*
Chairs: Dr. Matías Ostrowski - Dra. Daniela Papademetrio

EFFECTS OF TUMOR-DERIVED EXTRACELLULAR VESICLES AND THEIR SUBTYPES ON MYELOID IMMUNE CELLS

Clotilde THERY
INSERM U932, Institut Curie, PSL research university, Paris, France*

All cells, including tumors, release diverse types of extracellular vesicles (EVs), which can transfer complex signals to surrounding cells. Tumor-derived EVs have been often described as promoting tumor growth and/or metastasis, in particular by manipulating the anti-tumor immune responses. However, different types of EVs, originating from different subcellular compartments (e.g. endosome-derived exosomes, plasma membrane-derived ectosomes or microvesicles), are likely to bear different functions. Specific markers to distinguish these different classes of EVs are still difficult to define. Furthermore, in many EV isolation methods, EV preparations also contain co-isolated components, that can contribute to the EV functions studied. Consequently, the specific composition and functions of subtypes of tumor-derived EVs and the other extracellular nanoparticles still need to be defined. We will present our recently developed approaches to identify putative markers of subtypes of EVs among heterogeneous EV populations released by tumor cells, ways to distinguish functions of EVs and co-isolated non-EV components within EV preparations, and our recent observations that, in triple-negative breast cancer, tumor-derived EVs may carry anti-tumoral, and thus beneficial functions for the patient.

SAI III *LEONARDO SATZ* **LECTURE** *Thursday, November 17, 18-19 hr*
Chair: Mariana Maccioni

REGULATORY T CELLS AND CANCER: A TRANSLATIONAL APPROACH

Eliane Piaggio
Translational Immunotherapy team, INSERM U932, Institut Curie, Paris, France.
Egle-Therapeutics (https://egle-tx.com), Paris, France.

Regulatory T lymphocytes (Tregs) play an important role in the homeostasis of the immune system, preventing the development of autoimmune diseases. However, Tregs also inhibit the immune response against cancer, having a determining impact on the survival of cancer patients and their response to treatment. We have studied immune cells from the blood, tumor-draining lymph nodes (LNs), and primary breast and lung tumor tissue. We have shown that tumor invasion in LNs is associated with an accumulation of both CD8+ T effector cells, and of suppressive Tregs and myeloid cells. While CD8+ and CD4+ effector T cells from the tumor fail to proliferate and produce effector cytokines, CD8+ and CD4+ T cells from invaded LNs and non-invaded LNs are highly functional. At odds, Tregs are equally suppressive in LNs and tumors, as they can better adapt to the harsh conditions of nutrient deprivation, and hypoxia imparted by the tumor. Thus, removal of LNs in cancer would encompass the elimination of functional anti-tumor T cells which could likely be more appropriate targets of immunotherapies than the exhausted T present in the tumor. Consequently, in certain cases, – as in clinically LN negative patients, or in particular neo-adjuvant settings before surgical treatment – conserving the LNs but eliminating the suppressive Tregs could represent a valuable therapeutic strategy. Based on our recent results using single-cell multiomics technologies and showing that Tregs adapt to the tumor microenvironment acquiring unique molecular traits, we hypothesized that identification of the unique characteristics of tumor-Tregs should allow their selective targeting and allow the generation of strong anti-tumor immune responses, while sparing all-body Tregs and avoiding generalized immunosuppression. I will develop in this talk the current strategy that we are developing in the team and at Egle-Tx, a spin-off of Institut Curie to eliminate tumor-associated Tregs in patients.

SAIC VII LECTURE *Friday, November 18, 9-10 hr*
Chair: Dr. Hernán Farina

DISSEMINATED CANCER CELL DORMANCY: A HOMEOSTATIC SEED AND SOIL PARTNERSHIP

Julio A. Aguirre-Ghiso
Department of Cell Biology, Department of Medicine, Cancer Dormancy and Tumor Microenvironment Institute, Albert Einstein Cancer Center, Gruss-Lipper Biophotonics Center, Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine, Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Increasing evidence shows that cancer cells can disseminate from early-evolved primary lesions much sooner than the classical metastasis models predicted. Here we reveal at a single-cell resolution that mesenchymal- and pluripotency-like programs coordinate dissemination and a long-lived dormancy program of early disseminated cancer cells (early DCCs). Using various in vitro and in vivo genetically engineered mouse models of metastasis, single-cell RNA sequencing, and human sample analysis, we reveal how in early breast cancer lesions and early DCCs the transcription factor ZFP281 induces a permissive state for heterogeneous mesenchymal-like (M-like) transcriptional programs. This program is further sustained by signals derived from tissue resident macrophages in the lung. These programs also carry a dormancy signature and are absent in proliferative primary tumors and metastasis. Importantly, the absence of the M-like signatures in human breast tumors correlates with high-risk recurrence. FGF2 and TWIST1 induce ZFP281 expression, and the latter transcription factors cooperate to induce the M-like state. ZFP281 not only controls the early spread of cancer cells but also locks early DCCs in a dormant state by preventing the acquisition of an epithelial-like proliferative program and consequent metastasis outgrowth, which is associated with the downregulation of CDH11 and upregulation of CDH1. We identify ZFP281 and the M-like dormancy program as drivers of early dissemination and barriers that early DCCs must overcome to initiate metastatic outgrowth. This dormancy program is further reinforced by homeostatic tissue resident macrophages that suppress metastatic reactivation.

SAI IV LECTURE *Friday, November 18, 19 hr*
Chair: Dr. Emilio Malchiodi

HISTORICAL REVIEW IN THE CELEBRATION OF 50 YEARS OF ARGENTINIAN SOCIETY OF IMMUNOLOGY (SAI) 1972-2022

Gabriel A. Rabinovich
Laboratorio de Glicomedicina. Instituto de Biología y Medicina Experimental (IByME). Buenos Aires. Argentina.

The Argentine Society of Immunology (SAI) was created in 1972 and its first president was Dr Alois E. Bachmann, a physician and Professor of Microbiology and Bacteriology at the Faculty of Medical Sciences of the University of Buenos Aires, and its vice-president was Dr Christiane Dosne-Pasqualini a Doctor in Experimental Medicine graduated from McGill University in Montreal, Canada who was a CONICET researcher at that time. The subsequent year, Dr Pasqualini was appointed president of the Argentine Society of Immunology. At the moment of its foundation, our society had only 71 members. Today the Argentinean Society of Immmunology gathers more than 500 members from 65 research institutes and 16 hospitals throughout the country, having close ties with different public universities in our country and abroad. Perhaps anticipating this growth, Dr Bachmann called this foundational milestone an “immunological hatching”. At that time, the meetings of immunologists were held in the context of the Immunology Club. Among those present were the Argentinian Clinical Research Society members such as Dr Pasqualini, Dr María Marta Elizalde, Dr Marta Braun, Dr Clelia Riera, Dr Elsa Vottero de Cima, Dr Alicia Mazzoli, and Dr Bachman, Dr Londner, Dr Morini, Dr Manni, and Dr Ricardo Margni, along with youngest researchers such as Alberto Fossati, Silvia Hajos, Martín Isturiz, and Leonardo Fainboim.

Thus, in 1972, the First Argentinian Congress of Immunology was held, and the Latin American Association of Immunology (ALAI) was created. In the 1980s, the SAI members were internationally recognized as part of international societies such as the Latin American Immunology Association (ALAI, currently ALACI due to the incorporation of Caribbean Immunology Societies) and IUIS (International Union of Immunological Societies).

The words of one of the pioneers Dr Alberto Fossati summarize the role of the SAI in our country: *“The scientific activity carried out by the SAI throughout this half-century of life has certainly been intense. It always required a great effort from its leaders and partners who had to face critical situations -especially in the economic or political sphere- that were solved while always protecting our society. This commitment and devotion allowed for the continued growth and quality of Immunology in science and technology in our country. Over the years, our*

society has become a forum for exchanging knowledge and far-reaching collaborative work that fills us with satisfaction".

Currently, the SAI has an outstanding responsibility in the dissemination and debate of scientific knowledge through numerous courses, sponsorships, seminars, webinars, and conferences, as well as participating in the spread of articles in different media. The broad representation of women among its members and on its board of directors is also noteworthy. SAI's contribution to the society is unquestionable and aims to democratize knowledge, creating bridges between researchers and health care professionals by answering the questions raised concerning clinical cases involving immunology. This 50-year tour of the Argentine Society of Immunology prepares us to face new challenges that involve other scenarios in which the immune system will be a key player.

The commemoration lectureship of the 50th anniversary of the Argentinian Society of Immunology will be presented by Dr Gabriel A. Rabinovich. Gabriel is an active, outstanding, and generous member of our society with a solid commitment to scientific progress that widely embrace SAI's spirit. His distinguished career has been devoted to immunology and glycobiology, contributing enormously to our society in several aspects. During his career, Gabriel has been recognized worldwide as a pioneer in glycoimmunology, making relevant discoveries on galectin-glycan interactions and their impact in the regulation of immune responses. His findings, reflected in more than 300 publications in leading journals, provide novel mechanistic insights and therapeutic targets in a broad range of pathophysiologic settings including cancer, autoimmune inflammation, and infection.

Text by: Laura Cervi, Griselda Moreno, and Mariana Salatino

A SWEET ADVENTURE FROM AN UNEXPECTED DISCOVERY TOWERS THE DESIGN OF NOVEL THERAPEUTIC AGENTS IN AUTOIMMUNITY, INFECTION AND CANCER

Gabriel A. Rabinovich

Laboratorio de Glicomedicina. Instituto de Biología y Medicina Experimental (IByME). Buenos Aires. Argentina.

SAFIS II LECTURE *Friday, November 18, 18-19 hr*
Chair: Graciela Cremaschi.

OBESITY-STRESS INTERRELATIONSHIP. ASSOCIATED COGNITIVE AND METABOLIC IMMUNE DISORDERS

Ana Maria Genaro

Institute of Biomedical Research (CONICET-UCA), Buenos Aires, Argentina.

Chronic exposure to stressful situations and consumption of hypercaloric diets are common conditions in modern society and have been identified as predisposing factors for different disorders. Moreover, it has been suggested that an adverse intrauterine environment may result in deleterious effects on the offspring "per se" or after exposure to a challenge later in life. In this context, we study whether exposure to prenatal stress (PS) or adult stress could lead to the development of obesity and associated metabolic, behavioral, and immune disorders. Also, we search for peripheral markers as predictors of these disorders. Taking into account the relevance of the inclusion of sex as a biological variable in research, our investigations were carried out in both males and females. We studied the effect of chronic stress (CS) in the adulthood in C57Bl/6J mice that were feed with control or high fat diet (HFD) after weaning. Results indicate that males are more susceptible than the females in modulating metabolic and cognitive functions under HFD and CS. In both sexes HFD induced weight gain, fat accumulation, insulin resistance, high cholesterol; but only males exposed to CS showed: i) impaired glucose tolerance with higher glucose levels, ii) increased IFNgamma mRNA expression in hippocampus, suggesting a greater neuroinflammatory response, iii) poorer cognitive performance related to a decrease in hippocampal and spleen BDNF mRNA expression. In addition, we analyzed the impact of PS exposure in cognitive performance and the development of obesity and metabolic alterations. In BALB/c mice, we found that PS females but not males exhibited an impairment in spatial memory in parallel with a decrease in BDNF, an increase in glucocorticoid receptors and an alteration of Th1/Th2 in the hippocampus and peripheral lymph nodes as well. Moreover, PS females were more resilient to PS metabolic consequences; but under a HFD, an increase in glucose and insulin levels and higher visceral adipose tissue mRNA expression of leptin, resistin and IL-1beta was found, suggesting a pro-inflammatory profile. In addition, they also presented an increase in body weight and adiposity and a rise in cholesterol levels. The emerging results of this research could promote the identification of new therapeutic interventions to prevent the progression and/or heritability of these disorders.

SAIC I SYMPOSIUM *Wednesday, November 16, 11-13 hr*
PRE-CLINICAL MODELS UTILITY
Chairs: Martina Crispo

PRECLINICAL MODELS IN THE LABORATORY ANIMAL BIOTECHNOLOGY UNIT (UBAL)

Martina Crispo
Unidad de Biotecnología en Animales de Laboratorio, Institut Pasteur de Montevideo, Uruguay.

Research with preclinical murine models is of great relevance for the continuous advancement of biomedicine. In Uruguay there is a great development of this type of models that provide knowledge of Vanguard. Our Technological Unit carries out its own research, as well as provides regional support from high level in the field of the generation of genetically modified murine models using the techniques pronuclear microinjection, homologous recombination in embryonic stem cells, lentiviral injection, transposons and the revolutionary CRISPR/Cas system. In addition to these techniques, we offer cryopreservation, in vitro fertilization and embryonic rederivation for the maintenance of the generated mouse lines. Since 2007 we work with national and international biotechnological companies carrying out biological in vivo tests in mice, under GLP standards. This presentation will show the main preclinical models of the UBAL for your information.

FROM THE BENCH TO (HOPEFULLY) THE BEDSIDE: PRECLINICAL DEVELOPMENT OF A NOVEL DRUG FOR THE PREVENTION AND TREATMENT OF OBESITY AND ITS COMORBIDITIES

Carlos Escande, PhD
Laboratory of Metabolic Diseases & Aging
Institut Pasteur Montevideo, Uruguay

Obesity-related type II diabetes (diabesity) has increased global morbidity and mortality dramatically. Previously, the ancient drug salicylate demonstrated promise for the treatment of type II diabetes, but its clinical use was precluded due to high dose requirements and concomitant side effects. Recently, we showed that the nitroalkene group of unsaturated nitro-fatty acids can be attached to different molecular scaffolds to confer beneficial drug actions. In this study, we designed a nitroalkene derivative of salicylate, 5-(2-nitroethenyl)salicylic acid (SANA), and assessed its effects in murine diet-induced obesity (DIO). The data show that SANA reduces DIO, liver steatosis and insulin resistance at doses up to 40 times lower than salicylate. Furthermore, SANA showed: a) improved metabolic beneficiary effects when compared to Metformin, and b) weight-loss effects comparable to Liraglutide. Analyses of adipose tissue revealed SANA-mediated stimulation of mitochondrial respiration, via a creatine-dependent heat production pathway. Indeed, depletion of creatine resulted in the loss of SANA action. Together, these data demonstrate SANA as a candidate for the treatment of obesity and type II diabetes. Based on that, we aimed to follow the pre-clinical development necessary to perform a Phase IB clinical trial in human subjects. SANA complied with all the required safety requirements from regulatory agencies, strongly suggesting that it may be suitable for further clinical development.

BIOIMAGES APPLIED TO COMPLEX PRECLINICAL TRIALS

Hugo H Ortega
Centro de Medicina Comparada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina. Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

The discovery of a new active pharmaceutical ingredient includes the study of the synthesis processes, isolation from a natural source or biotechnological production, the characterization of its activity and the complete preclinical phase, including the toxicological and biodistribution evaluation. Efforts to improve the efficiency of this process are being permanently implemented in translational research. In this sense, the need for tools that accelerates the evaluation process of new therapies, taking advantage of technological evolution in the sciences, has

been highlighting. In vivo imaging systems (bioluminescence, fluorescence, ultrasound, etc.) are cutting-edge technologies due to their advantages of high sensitivity, non-invasiveness, non-radioactivity and low operative cost. In vivo bioluminescence and fluorescence systems have the ability to visualize cellular processes and biological interactions, without the need to sacrifice the animals, makes it possible to obtain serial images. These images provide anatomical and functional data that can be obtained quickly, without interfering with the other variables under study. These tools are extremely useful in in vivo pharmacokinetic and pharmacodynamic studies, to understand different aspects of the absorption, distribution, metabolism, and excretion of new drugs, having important advantages over traditional methods since they do not require the development of complex complementary analytical methods. The intensity and location of the bioluminescent signal can facilitate the adequate identification of end-points and targets organs. In addition, in efficacy and proof of concept studies, in vivo bioluminescence is very useful to visualize cancer cell tumor progression, monitor stem cell behavior and immune cell response, and follow implants rejection in transplanted tissues. For example, the ability to detect micro-metastases and their localization, or the study by doppler ultrasound imaging of the vascularization of tumors, have direct translational relevance. We can conclude that in vivo imaging techniques are extremely useful for regulatory preclinical studies as well as in concept tests to accompany the findings obtained by other methods.

PRECLINICAL MODELS OF FIBROTIC DISEASES

ANA ROMO[1,2,3]

[1] RADBIO S.A.S. Sunchales, Santa Fe, Argentina. ana.romo@radbiopharm.com. [2] Instituto Tecnológico de Chascomús (CONICET-UNSAM). Chascomus, Buenos Aires, Argentina. [3] Escuela de Bio y Nanotecnologías (UNSAM). San Martín, Buenos Aires, Argentina

Fibrosis is a pathological wound healing process in which connective tissue replaces normal parenchymal tissue. Defined by the uncontrolled accumulation of extracelular matrix (ECM) proteins, fibrosis affects almost all adult tissues causing scarring and thickening, and leading to organ dysfunction and failure. It is estimated that fibrosis causes around 45% of all deaths in the industrialized world, however, to date, there are no effective therapeutics to treat or prevent fibrosis. Extended efforts are being focused on the discovery and development of new drugs intended to treat different types of fibrotic diseases. Fibrosis research is heavily reliant on animal models, but variations in the molecular basis of the fibrosis establishment, and differences between animal and human fibrosis imply a careful selection of the models and experimental designs. Here we will discuss a diversity of animal models of fibrotic diseases, highlighting their advantages and limitations from a translational perspective.

SAIC II SYMPOSIUM *Wednesday, November 16, 15-17 hr*
TRANSLATIONAL RESEARCH IN GENOMICS.
Chairs: Cecilia Fernandez - Carlos David Bruque

GENETIC ANALYSIS OF PATIENTS WITH MULTIPLE CONGENITAL ANOMALIES AND ISOLATED CONGENITAL HEART DISEASE

LILIANA DAIN 1, 2

1 Centro Nacional de Genética Médica-ANLIS; 2 Facultad de Ciencias Exactas y Naturales UBA, Buenos Aires

Congenital anomalies (CA) are prenatal clinically significant birth defects resulting from morphological disturbances in the process of human development affecting infant morbidity and mortality, regardless of its pathogenesis, etiology, and time of diagnosis. The etiology of these defects is widely recognized as heterogeneous, with the contribution of genetic and environmental factors. Around 5% to 10% of CA are due to environmental and maternal causes, whereas around 40% are known to have a direct genetic cause, either chromosomal, polygenic, or single-gene defects. Although largely studied in several populations, the genetic contribution to CA in Latin America is less documented. CA affect 3-5 % of newborns, representing the second leading cause of infant mortality in Argentina. Among CA, patients presenting multiple congenital anomalies (MCA) have a prevalence at birth of 2.26 per 1000 births whereas congenital heart defects (CHD) are the most frequent CA, with prevalence at birth of 4.06 per 1000 births. Considering their severity and birth prevalence we aim at analyzing the genetic causes in Argentinian patients affected with MCA and isolated CHD. We will present the results obtained after applying a sequential algorithm to identify different genetic causes in 366 recruited patients (172 with MCA and 194 with isolated CHD) born between June 2015 and August 2019 at public hospitals. Different techniques were applied, ranging from cytogenetic studies. MLPA, array-CGH and next generation sequencing (NGS). Altogether, we were able to determine the genetic contribution in 27.5% of the analyzed patients. Moreover, following NGS analysis, 5 patients presented novel pathogenic or likely pathogenic genetic variants in different genes described for the first

time worldwide.

COMPREHENSIVE TRANSCRIPTOMIC ANALYSIS FOR THE IDENTIFICATION OF LONG NON-CODING RNAs (LncRNAs) WITH CLINICAL IMPLICATIONS IN THE MOLECULAR SUBTYPES OF COLORECTAL CANCER.

Ezequiel Lacunza

CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina.
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Colorectal cancer (CRC) is a frequently lethal disease with heterogeneous prognosis and response to treatment. Most CRC progress from normal epithelium, through a benign precursor adenoma, through the accumulation of genetic alterations in oncogenes and tumor suppressor genes, and additional epigenetic aberrations implicated in disease initiation and progression. Multi-omics studies have revealed the transcriptional landscape of CRC, allowing patients to be classified according to 4 consensus molecular subtypes (CMS1-4). CMS1-immune comprises the majority of tumors with microsatellite instability (MSI) and is characterized by the infiltration of activated immune cells. CMS2-canonical and CMS3-metabolic show epithelial features, with high WNT and MYC signaling predominantly in CMS2 and metabolic reprogramming in CMS3. CMS4 comprises the most similar cancers to mesenchymal ones, with high stromal infiltration and poor patient prognosis. The clinical utility of the CMS classification resides in the possibility to estimate survival (prognostic value) and select patients for both chemotherapy and currently used targeted agents (predictive value). Long non coding RNAs (LncRNAs) are defined as RNAs that do not code for proteins and exceed 200 nucleotides in length. LncRNAs play key roles in the regulation of chromatin dynamics, gene expression, epigenetic regulation, growth, and differentiation. An increasing number of studies have demonstrated the aberrant expression of LncRNAs in human cancers, assigning them a promising role as diagnostic and prognostic biomarkers. The deregulation of LncRNAs has been shown to be closely related to the development and progression of CRC. Currently, there are no LncRNA signatures that are distinctive of precancerous CRC lesions or of the molecular subtypes that define tumors. The lecture will go over these topics, followed by a comprehensive transcriptomic bioinformatics analysis for the identification of clinically relevant LncRNA signatures in the context of the CMS classification of CRC in premalignant lesions and tumors. These repertoires of LncRNAs could be evaluated in screening colonoscopy biopsies and plasma samples from patients in order to consider them as specific molecular tools that may have diagnostic, prognostic and/or predictive value.

ADULT NEURODEGENERATIVE DISEASES: THE MOLECULAR BIOLOGY LABORATORY IN A CLINICAL CONTEXT

Ezequiel I. Surace

Laboratorio de Enfermedades Neurodegenerativas (LEN), Instituto de Neurociencias Fleni (INEU-CONICET).

Adult-onset neurodegenerative diseases (ENA) represent a heterogeneous group of disorders characterized by the pathological accumulation of misfolded proteins in various locations of the nervous system. ENAs may have a familial presentation (with typical Mendelian modes of inheritance) or an apparently sporadic presentation. The study of the genomic variants involved in these pathologies involves family segregation analysis (genetic evidence) and the functional evaluation of the variants on the physiological function of the encoded protein in relevant in vivo and in vitro models. Likewise, the development of specific biomarkers in different biofluids has represented a paradigm shift in the diagnosis and prognosis of these diseases, as well as a fundamental contribution in the evaluation of the efficacy of possible treatments in the clinical research phase. This dissertation will deal with aspects of translational research work in cohorts of patients from Argentina with different ENAs. In this framework, cases related to Alzheimer's disease (AD) with autosomal dominant inheritance and people with trisomy of chromosome 21, who have an increased risk of presenting early-onset AD, will be discussed. Likewise, the contributions of molecular genetics in cases of frontotemporal dementia and amyotrophic lateral sclerosis, conditions that belong to the same clinical-pathological continuum, will be discussed.

SAI I SYMPOSIUM *Wednesday, November 16, 15-17 hr*
UNRAVELING IMMUNE ENVIRONMENT AT MUCOSAL SURFACES
Chairs: Virginia Pasquineli - María Virginia Gentilin

UNRAVELING INTESTINAL IMMUNITY: FOCUS ON RHYTHMS, MACROPHAGES AND LITERATURE

Silvia Correa
Departamento de Bioquímica Clínica. CIBICI-CONICET, Facultad de Ciencias Químicas- Universidad Nacional de Córdoba, Argentina

A key aspect of intestinal immunity is to understand the predominantly tolerogenic nature of this mucosa and to characterize its alteration in intestinal diseases. To provide new evidence in this field, we have been working on three topics: (1) to determine whether critical functions of gut immunity are synchronized with some biological rhythm, we studied relevant parameters at different times of the day in C57BL/6 mice. By using multivariate statistical tools, we found oscillations that represent at least three possible scenarios with different conditions for tolerance induction. At the end of the dark phase when feeding occurs, the entry of luminal antigens, the release of soluble factors, and the presence of certain subsets of leukocytes converge at the mesenteric lymph nodes (MLN). In this window, the tolerogenic potential seems stronger, favoring the induction of specific regulatory populations that express homing receptors to recirculate to the lamina propria. (2) to demonstrate whether the increased IgG content observed in the intestinal lumen during inflammation can trigger signaling through the IgG-CD64 axis, we studied the frequency of IgG+IgA+-coated fecal bacteria in the DSS-induced colitis model. In these mice, the percentage of IgA+IgG+ bacteria increase rapidly, the luminal content shows a superior ability to coat fresh bacteria, and these double-coated bacteria are a potent stimulus for phagocytic activity of lamina propria macrophages involving CD64 receptors and Dectin-1. (3) to study the representativeness of the components involved in the interactome of human Inflammatory Bowel Diseases (IBD) (cells, molecules, genes, and biological processes), we analyzed their relationships in the last three decades. Over time there was an increase in the components added to the IBD network and greater connectivity within and between functional levels. However, some over-representation was observed and 82% of the components appearing in the literature are absent or show low frequency, suggesting that many aspects of the proposed IBD interactome still have weak experimental support in humans.

FOOD ALLERGY: LESSONS FROM JUVENILE POLYPS

Cecilia Muglia
IIFP-Instituto de Estudios Inmunológicos y Fisiopatológicos - CCT La Plata, Centro Científico Tecnológico CONICET, Argentina

Food allergies, including cow´s milk protein allergy (CMA), are adverse immune response to food proteins, which are growing worldwide, affecting 5-10% of children and 1-3% of adults. These disorders can be classified as IgE-, non-IgE- and mixed allergic reactions. IgE mediated allergies are of concern because of the risk of severe reactions such as anaphylaxis. Evidence regarding the mechanisms of IgE production in the human gut are scarce, and little is known about IgE memory cells. We have been working for several years on colorectal juvenile polyps (JP) from children sensitized to cow´s milk proteins (CMP). These polyps, which promote rectal bleeding, are routinely removed by colonoscopy, and constitute an excellent human tissue to study the mucosal underlying mechanisms that provoke food allergies. We reported a prominent cellular infiltrate in JP rich in mononuclear cells, eosinophils, and mast cells. We demonstrated the presence of active germinal centers, with a high frequency of CD20+ cells (B cells), ki67+ cells (proliferating cells) and AID+ cells (cells undergoing class switch recombination and hypermutation). The stroma also showed to contain IgE+ plasma cells (IgE+CD138+ cells). All these features refer as active and Ig-producing germinal centers in the stroma of polyps, and we demonstrated for the first time that the human colon is a mucosal site for production of IgE. Furthermore, we described in the polyp stroma a Th2 inflammatory environment, with high levels of IL-4 and a high IL-13/IFN-γ ratio compared to the surrounding tissue, and the presence of milk-specific T cells expressing gut homing integrins. Finally, JP are rich in pro-inflammatory and type-2 chemokines secreted by epithelial cells, which may play a central role in the promotion of the local allergic response and development of polyps. In conclusion, our findings reveal the role of epithelial cells in the inflammatory environment and IgE synthesis found in JP and indicate a potential link between JP and food allergy.

BRUCELLA ABORTUS AND THE RESPIRATORY MUCOSA: INVASION MECHANISMS AND INNATE IMMUNE RESPONSE

Pablo Baldi
Cátedra de Inmunología Investigador Principal – IDEHU. Facultad de Farmacia y Bioquímica – UBA, Argentina

Brucella spp. frequently infects humans and animals by the respiratory route but rapidly disseminates to other organs. We found that *B. abortus* (and other smooth *Brucella* species) can infect and replicate in human and murine lung epithelial cells (alveolar and bronchial), lung fibroblasts, and alveolar macrophages (AM). The pathogen can also infect and translocate a model of bronchial epithelial barrier (epithelial cells grown over a collagen matrix embedded with lung fibroblasts). All these cell types produce varying levels of proinflammatory cytokines and/or chemokines in response to the infection. Several innate immunity pathways and receptors are involved in these responses and in the control of the respiratory *Brucella* infection. TNF-α and CXCL1 (KC) responses in AM are mediated by TLR2 recognition, and CFU of *B. abortus* after intratracheal infection are higher in AM from TLR2 KO mice than in wild type controls. Inflammasomes mediate the secretion of IL-1β and CXCL1 in the lungs in response to *Brucella*, and mice lacking inflammasome components have an impaired control of the infection. STING mediates the production of proinflammatory cytokines and IFN-β by *Brucella*-infected lung cells, and STING KO mice have increased bacterial counts in lungs and peripheral organs after intratracheal infection. Despite these pulmonary immune responses to *Brucella*, the pathogen can disseminate rapidly to other organs after airborne infection and can also persist in the lungs for long periods. Murine lungs exhibit a mild inflammatory response during the first week of infection, in part due to bacterial Btp proteins that interfere with TLR signaling. The ability of the pathogen to survive, replicate and egress from the bronchial model for several days, and its capacity to downmodulate MHC-II expression in infected AM may contribute to its long pulmonary persistence and systemic dissemination.

MODULATION OF INTESTINAL INFLAMMATORY RESPONSE BY PROBIOTIC YEAST AND FERMENTATION METABOLITES

Martin Rumbo
IIFP-Instituto de Estudios Inmunológicos y Fisiopatológicos - CCT La Plata, Centro Científico Tecnológico CONICET, Argentina

Intestinal mucosa is exposed to a complex mixture of microbiota and food-derived molecules. Epithelial cells as well as immune cells located in the epithelial compartment and *lamina propria* are able to sense and react to a great variety of these compounds, triggering homeostatic or inflammatory circuits depending on the integration of signals received. In this complex scenario, dysregulation of modulatory circuits contributes to different types of pathologies, often with local and systemic impact. The identification of dietary interventions that may shape modulatory circuits is of great interest. In our group we have characterized the capacity of different yeast strains isolated from fermented dairy products such as kefir, to modulate innate immune response in different scenarios. Probiotic yeasts (of *Saccharomyces* and *Kluyveromyces* genus) have the capacity to modulate TLR signaling in epithelial cells by inhibition of NFkB pathway activation and enhance epithelial cell survival under oxidative stress. In the talk we will discuss the effects of yeast administration in different *in vivo* scenarios, including small intestinal inflammation models, such as irinotecan-induced mucositis and large intestine inflammatory models such as acute colitis and colon cancer induced by mutagen administration/DSS-induced chronic colitis model. Furthermore, due to their origin, kefir-isolated yeasts from *Kluyveromyces* genus have the capacity to ferment lactose, being able to use cheese whey or cheese whey permeate as substrate, offering an attractive technological alternative to increase value of these problematic subproducts. Conservation of probiotic traits when yeasts are grown on these industrial substrates will be discussed.

SAFIS I SYMPOSIUM *Wednesday, November 16, 17-19 hr*
THE INFLAMMATORY PROCESS IN THE GENESIS OF MULTIPLE PATHOPHYSIOLOGICAL MECHANISMS
Chairs: Martin Rumbo - Carolina Caniffi

ANTI-INFLAMMATORY RESPONSE OF THE ANGIOTENSIN-(1-7) MAS RECEPTOR: DIALOGUE WITH OTHER RECEPTORS

Mariela M. Gironacci
Dpto. Qca. Biológica, IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad Nacional de Buenos Aires

Inflammation has been shown to play an important role in the mechanisms involved in the pathogenesis of hypertension. Different subpopulations of cells involved in innate and adaptive immune responses, such as mono-

cyte/macrophages and dendritic cells on one hand and B and T lymphocytes on the other hand, play roles leading to vascular injury in hypertension. The components of the renin-angiotensin system play an important role in inflammation. While it is well established that angiotensin (Ang) II elicits proinflammatory properties in a range of target organs, Ang-(1-7), a component of this system synthetized mainly by angiotensin-converting enzyme 2 from Ang II, counter-regulates Ang II effects. Ang-(1-7) exerts its actions through Mas receptor (MasR). Activation of ACE2/Ang-(1–7)/MasR axis has been described to display anti-inflammatory actions in several experimental models, including ischemic stroke, atherosclerosis, pulmonary fibrosis, acute lung injury and arthritis. MasR belongs to the G protein-coupled receptors (GPCRs) family. Although GPCRs are able to operate as monomers, there is increasing evidence about the ability of GPCRs to form and function as heterodimers/heteromers that exhibit distinct pharmacological, trafficking and functional properties as compared to their parent monomeric or homodimeric/homomeric GPCRs. Efforts have focused over the past two decades on the identification of GPCR complexes as well as on their signaling properties. In our lab we have been investigating how the anti-inflammatory properties of MasR are influenced by interaction with others GPCRs. GPCRs heteromerization not only brings forth a plethora of drug target combinations, but also gives an opportunity to carefully tweak the structure and function of one or more GPCRs involved in the complex, with the final goal of improving therapeutic strategies.

THE SYNTHESIS OF INTERLEUKIN-1 BETA BY RENAL TUBULAR CELLS: AN UNEXPECTED ORIGIN OF INFLAMMATION IN DIABETIC KIDNEY DISEASE

Jorge F. Giani[1,2], Luciana C. Veiras1, Ellen A. Bernstein[1], Zakir Khan[1,2], Derick Okwan[3], DuoYao Cao[1], Faizan Ahmed1, Ryan M. Williams[4], David Gibb[2], Kenneth Bernstein[1,2]

[1]Departments of Biomedical Sciences and [2]Pathology, Cedars-Sinai Medical Center, Los Angeles, CA. [3]Department of Pathology, Stanford University, Palo Alto, CA. [4]Department of Biomedical Engineering, The City College of New York, New York, NY.

Hypertension is twice as prevalent in diabetic compared to non-diabetic populations and is a major risk factor for the development of end-stage renal disease. We observed that after 4 weeks of high salt diet, male db/db mice, a model of diabetes and obesity, displayed salt-sensitive hypertension, accompanied by the accumulation of proinflammatory (CD80+) macrophages and higher levels of interleukin-1β (IL-1β) in the kidney compared to non-diabetic db/+ controls. Our data show that tubular epithelial cells initiate renal inflammation by releasing IL-1β that further activates renal macrophages to propagate the inflammatory response and impair kidney function. Immunofluorescence analysis showed that the higher IL-1β expression of db/db mice is primarily localized in the renal tubules. In addition, flow cytometry analysis of renal cells showed that db/db mice have higher IL-1β expression in tubular (E-cadherin+) cells (21 ± 2 vs. 8 ± 3 % of E-cadherin+ cells, P<0.001) compared to db/+ controls. To evaluate the role of tubular IL-1β in salt sensitivity, 7-mo-old db/db (n=6) mice were injected with a siRNA against IL-1β encapsulated in PEG nanoparticles that specifically accumulate in the renal tubular epithelium resulting in a site-specific suppression of IL-1β. Notably, db/db mice treated with IL-1β-siRNA displayed reduced levels of tubular IL-1β (by immunofluorescence), low CD80+ macrophages (20 ± 14 vs. 50 ± 12 CD80+ macrophages, P<0.001), and no salt sensitivity (BP was 109 ± 5 vs. 123 ± 8 mmHg, P<0.05) compared to db/db treated with a non-targeting sequence. To evaluate whether IL-1β mediates macrophage polarization towards a pro-inflammatory phenotype in diabetes, another cohort of 2-mo-old diabetic db/db mice (n=6) were transplanted with a bone marrow of IL1RKO mice. In these mice, named db/db-IL1RKO, immune cells cannot respond to IL-1β. db/db receiving wild-type (WT) bone marrow (db/db-WT) were used as control. Mice were studied at 7-mo-old. Compared to db/db-WT, db/db-IL1RKO did not develop salt-sensitivity (blood pressure was 110 ± 7 vs. 122 ± 9 mmHg, P<0.05), and had less CD80+ macrophages, (21 ± 4 vs. 54 ± 5 CD80+ macrophages, P<0.01), despite an accumulation of tubular IL-1β. In conclusion, using two different experimental models, our data show that tubular epithelial cells are a major source of IL-1β in diabetes. This cytokine promotes a pro-inflammatory phenotype of macrophages that impairs kidney function leading to salt sensitivity.

MICROGLIA AT THE CROSSROADS OF THE NEUROVASCULAR DAMAGE INDUCED BY INTRACELLULAR PATHOGENS

Guillermo Hernán Giambartolomei

Instituto de Inmunología, Genética y Metabolismo (INIGEM). CONICET-Universidad de Buenos Aires

For a long time, the central nervous system (CNS) has been considered an immuneprivileged organ. Recent advances in this field clearly demonstrate that the CNS is a highly immunologically active organ, with complex immune responses mostly based on innate immune processes. Invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. Many of the associated neurocognitive and neurovascular symptoms of neurobrucellosis might be the result of the inflammatory response induced by *Brucella*

activation of innate immune responses in the CNS. We have described different mechanisms whereby microglia activated by *Brucella* elicit an inflammatory deleterious response that affects the integrity of the neurovascular unit, damaging glial cells, neurons, and the integrity of the blood brain barrier. Culture supernatants (CS) from *B. abortus*-infected microglia induced activation of brain microvascular endothelial cells (HBMEC). Activation of HBMEC was dependent on IL-1β because CS from *B. abortus*–infected microglia deficient in caspase-1 and apoptosis-associated speck-like protein containing a CARD (ASC) failed to induce HBMEC activation. Both absent in melanoma 2 (AIM 2) and Nod-like receptor containing a pyrin domain 3 (NLRP3) are involved in IL-1β secretion. Neutrophil and monocyte migration across HBMEC monolayers was increased by CS from *Brucella*-infected microglia in an IL-1β –dependent fashion, and the infiltration of neutrophils into the brain parenchyma upon intracranial injection of *B. abortus* was diminished in the absence of AIM 2 and NLRP3. Additionally, we have demonstrated that, due to *B. abortus* infection, microglial primary phagocytosis (phagoptosis) actively induces neuronal death, without inducing neuronal apoptosis. This phenomenon was due to microglia-TLR2 activation. *B. abortus*-activated microglia secrete nitric oxide (NO) and increase their phagocytic ability. NO induced the exposure of eat-me signal on neurons (phosphatidylserine, PS). Blocking PS-binding protein milk fat globule epidermal growth factor-8 (MFG-E8) interaction, or microglial vitronectin receptor-MFG-E8 interaction was sufficient to prevent neuronal loss without inhibiting microglia activation. Our results indicate that innate immunity of the CNS set in motion by *B. abortus* contributes to the activation of the blood–brain barrier and neuronal demise observed during neurobrucellosis.

CANONICAL AND NON-CANONICAL PATHWAYS OF INFLAMMASOME ACTIVATION

Martin Rumbo
Instituto de Estudios Inmunológicos y Fisiopatológicos IIFP-CONICET-UNLP – Centro Asociado CICPBA

Canonic inflammasomes are proteolytic complexes assembled by homotypic interaction between multidomain cytosolic proteins –so called inflammasomes- that among its domains contain a sensor domain that controls oligomerization. There are several canonic inflammasomes, that differ in their multidomain organization. These differences result in different sensing capacity, being some inflammasomes such as AIM2 able to be activated by interaction with intracytosolic DNA, whereas other are activated by bacterial derived molecules such as flagellin in the case NLRC4 inflammasome. NLRP3 inflammasome, one of the most studied complexes, is activated through different signals such as K+ ionic flux, ROS or mitochondrial DNA released in the cytosol. Once assembled, these complexes are able to recruit Caspase 1 in most of the cases mediated by adaptor protein called PYD-linked apoptosis-associated speck-like protein (ASC). Recruitment of caspase 1 to the assembled inflammasome activates its proteolytic activity, being able to degrade and activate pro-gasdermin D, pro-interleukin 1 (pro IL-1) and pro-interleukin 18 (pro IL-18). Once cleaved, gasdermin D oligomerizes forming pore-like structures in the plasma membrane, allowing the secretion of IL-1 and IL-18 and disrupting cell homeostasis. Non-canonic inflammasomes are complexes formed by different caspases (typically caspase 11 in mouse and caspases 4 and 5 in humans) that are able to sense and be activated by the presence of intracytosolic bacterial LPS. Once activated, these caspase complexes are able to cleave progasdermin D, triggering an oligomerization process that results in the formation of a gasdermin D membrane pore. Usually, ionic fluxes generated by this process activate NLRP3 canonic inflammasome, reinforcing the inflammatory response. Due to their expression in several cell types and their capacity to sense multiple signals that represent loss of homeostasis, inflammasomes are central to a variety of physiopathologycal processes. During the talk, central aspects of canonic and non-canonic inflammasome biology will be discussed, specially related to recognition of bacterial-derived structures such as outer membrane vesicles.

SAIC III-SAI II SYMPOSIUM *Wednesday, November 16, 18-20 hr*
IMMUNOMETABOLISM
Chairs: Daiana Vota - Pilar Aoki

TARGETING IMMUNOMETABOLISM IN HOST DEFENSE AGAINST MYCOBACTERIUM TUBERCULOSIS

Luciana Balboa
Instituto de Medicina Experimental (IMEX), CONICET-Academia Nacional de Medicina, CABA, Argentina

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a major global health problem accounting for millions of deaths annually. Although our immune system is endowed with immune cells specialized in controlling bacterial growth (e.g., M1 macrophages), Mtb has developed different strategies to impair the functionality of these cells. Moreover, Mtb can also disrupt the adaptive immune response by impairing the activation of

naïve T cells induced by dendritic cells (DCs). Several studies have recently revealed that the cellular metabolism may determine the effector functions of immune cells. For instance, upon bacterial infection, M1 macrophages switch their metabolism toward aerobic glycolysis, which is coordinated by the Hypoxia-Inducible Factor 1-alpha (HIF-1α) and leads to faster production of ATP as well as metabolic intermediates that support the production of proinflammatory cytokines and antimicrobial peptides. On the contrary, how the metabolism influences human dendritic cell biology is much less explored. Our team is focused on unraveling the immunometabolic pathways that regulates leucocytes' functions and their impact on the control of TB. To study the impact of TB-associated microenvironment on the functionality of M1 macrophages, we used the acellular fraction of pleural effusions from TB patients (TB-PE). Interestingly, we found that M1 macrophages exposed to TB-PE displayed a lower expression of HIF-1α and a reduced glycolytic activity, impairing the control of the bacterial growth. We have also shown that the pharmacological stabilization of HIF-1α expression restores glycolysis and pro-inflammatory properties resulting in a better control of the Mtb burden. Importantly, the downmodulation of HIF-1α was driven by omega-3-derived lipids mediators. Regarding to the metabolic regulation of DCs function, we showed that Mtb triggers glycolysis in DCs which promotes their migration into lymph nodes in a HIF-1α-dependent manner. Interestingly, DCs from TB patients displayed a reduced glycolytic capacity and are unable to migrate upon Mtb stimulation, while HIF-1α stabilization restores these properties. These findings may explain the delayed accumulation of activated CD4+ T cells in the lung reported in TB. In summary, a further understanding of the metabolism of immune cells and their specific functions during TB pathogenesis can lead to the development of immunotherapies capable of promoting Mtb clearance.

INFLAMMATION, METABOLISM AND GLIAL CELL ACTIVITY IN NEURODEGENERATION.

Juan Beauquis, Ángeles Vinuesa, Amal Gregosa, Melisa Bentivegna, Carlos Pomilio, Melina Bellotto, Nicolás González Pérez, Jessica Presa and Flavia Saravia.

Laboratorio de Neurobiología del Envejecimiento, IBYME-CONICET and Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

The neurodegenerative process involves a series of cellular and molecular changes that eventually lead to neuronal dysfunction and death. It occurs in the course of neurological diseases like Alzheimer's, metabolic diseases as Type 2 diabetes and obesity, and during the brain ageing process. Chronic inflammation, brain metabolic deficits and aberrant protein accumulation are common features in these scenarios. Glial cells could play a central role in the installment or the resolution of theses brain changes. In our laboratory we are interested in understanding how astrocytes and microglia respond to pathological stimuli to detect therapeutic opportunities. Glial cells could exacerbate or amplify the damage through the adoption of pro-inflammatory phenotypes and, also, through the loss of homeostatic and metabolic functions. Using models of insulin resistance and lipotoxicity, we detected brain and glial changes that are typically found in the neurodegenerative process. Fatty acid-induced inflammation leaded to microglial activation with NFkB nuclear translocation, increased IL1b expression, decreased IL4 expression, loss of phagocytic capacity and mitochondrial defects. Also, microglia amplified this response activating astrocytes through soluble factors and extracellular vesicles. This interaction was prevented with the pharmacological inhibition of ceramide synthesis, suggesting a role for this metabolic pathway. In parallel, using experimental models of Alzheimer's disease, we have detected glial reactivity, impaired hippocampal insulin signaling and autophagic defects that could promote neurodegeneration. In vitro, the adoption of pro-inflammatory glial phenotypes was accompanied by autophagy deficiency, detected through the accumulation of p62+ and LC3+ intracellular vesicles. Also, astroglia displayed decreased Akt phosphorylation and lower levels of insulin receptors than control cells, suggesting impaired insulin signaling. Concomitant glial mitochondrial defects indicate a cellular metabolic deficiency. Finally, we found that dietary and pharmacological interventions could modulate some of these pathological changes. Altogether, our results suggest that glial reactivity and cellular metabolic changes are concurrent events in experimental models of neurodegeneration. We believe that these results would contribute to a better understanding of the pathophysiology of the neurodegenerative process and, ultimately, to design therapeutic approaches.

METABOLIC TRAINED IMMUNITY POTENTIATES SARS-COV-2-INDUCED INFLAMMATION

Pedro M Moraes-Vieira

Department of Genetics, Microbiology and Immunology, Institute of Biology, University of Campinas

Covid-19 is a systemic disease caused by the severe acute respiratory syndrome coronavirus 2 (Sars-Cov-2). A dysregulated immune response, with an exaggerated production of inflammatory cytokines, and an enrichment of myeloid cells in the lungs are linked to severe symptoms and higher risk of death. Recently, we described

that diabetic people has a higher risk of severe COVID-19 because Cov-2 depends on glucose to replicate and enhance the inflammatory profile of CoV-2-infected monocytes in a HIF-1a/glycolysis-dependent manner. Also, we observed that the mechanism responsible for increased COVID-19 disease in obese people is due to a type of trained immunity, which we named metabolic trained immunity.

METABOLISM IN THE SINGLE CELL ERA

Rafael Argüello
Aix Marseille University, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, France.

Metabolic reprogramming is a hallmark of cancer and immune responses. Different immune cells can adopt pro or anti-tumoral functions that are correlated with the dependence of different energetic metabolism pathways. Metabolic reprogramming involves dramatic changes in cellular levels of NAD+, Lactate, Acetyl-CoA, SAM and other metabolites that are substrates of the enzymes that mediate epigenetic modifications. These links between metabolic changes in cancer and immune cells, their impact on epigenetics, gene expression and cell function are poorly understood. Especially due to the lack of adapted technologies that allow to study metabolism and epigenetics with single cell resolution in physiologically relevant contexts. I will present our recent development and applications of a method to monitor functional metabolism with single cell resolution, and our first results using this technology, called SCENITH in combination with measurements of epigenetic marks of the chromatin (Epic-SCENITH). Altogether, SCENITH enables to characterize the metabolic landscape of cancer and immune cells in patient samples. We envision to use SCENITH as a personalized medicine approach to predict the efficacy of therapies against cancer and other diseases.

SAIC IV-SAI III SYMPOSIUM *Thursday, November 17, 10-12 hr*
B CELLS IN HEALTH AND DISEASE
Chairs: Marta Zerga - Adriana Gruppi

BIOLOGY OF HODGKIN LYMPHOMA

Ralf Küppers
Institute of Cell Biology (Cancer Research), University of Duisburg-Essen, Essen, Germany

Various key aspects of the pathogenesis of Hodgkin lymphoma (HL) are currently under intensive investigation. This presentation aims to highlight several recent developments in the field. Our understanding of the mutational landscape of Hodgkin and Reed/Sternberg (HRS) cells is evolving, based on exome and whole genome sequencing studies of isolated HRS cells. For IgD-expressing cases of lymphocyte predominant HL, a recent study provided compelling evidence for a role of combined antigenic and superantigenic triggering of the lymphoma cells and/or their precursors by Moraxella catarrhalis derived antigens in lymphoma pathogenesis. Gene expression studies of HRS cells point to a close relationship of these cells to the rare normal CD30+ B cells. HRS cells indeed highly and consistently express CD30 on their cell surface, which is used for its diagnosis and also for targeted therapy with drug-conjugated CD30-specific antibodies. However, the role of CD30 in the pathogenesis of cHL is not well understood and controversially discussed. We established a CRISPR/Cas9 system in CD30-positive lymphoma cell lines for efficient knockout of CD30. Characterization of CD30-depleted cHL cell lines identified a growth disadvantage under competitive growth conditions and CD30-knockout cells showed increased cell death. Furthermore, contribution of CD30 signaling to the high MYC activation signature of cHL cell lines was identified. These results point to an important role of CD30 expression by HRS cells for the pathobiology of cHL.

DYSREGULATION OF SUPER-ENHANCER NETWORKS IN B CELL LYMPHOMA

Laura Pasqualucci
Institute for Cancer Genetics. Department of Pathology and Cell Biology. Columbia University. Nueva York, Estados Unidos.

TARGETING B CELLS GENETICALLY

Klaus Rajewsky
Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlín, Alemania.

MECHANISMS OF GERMINAL CENTER B CELL CONFINEMENT AND DISRUPTION IN LYMPHOMAGENESIS

Jason G Cyster, Antonia E Gallman, Erick Lu, Jagan R Muppidi, Finn D Wolfreys
Howard Hughes Medical Institute and Department of Microbiology & Immunology, University of California, San Francisco, USA

The G13-protein coupled receptors S1PR2 and P2RY8 promote the confinement and growth regulation of germinal center (GC) B cells, and loss of either receptor is associated with B cell lymphomagenesis in humans. We identified the novel metabolite S-geranylgeranyl-l-glutathione (GGG) as a P2RY8 ligand. The mechanisms controlling GGG distribution in lymphoid tissues had not been defined. We found that gamma-glutamyltransferase-5 (Ggt5) expression in follicular dendritic cells (FDCs) is required for GGG catabolism and confinement of P2RY8-expressing cells to GCs. We identified the ATP-binding cassette subfamily C member 1 (Abcc1) as a GGG transporter and showed that Abcc1 expression by hematopoietic cells was necessary for P2RY8-mediated GC confinement. Furthermore, we found that P2RY8 and GGG negatively regulate trafficking of B and T cells to the bone marrow (BM). CRISPR Cas9 mediated deletion of P2RY8 from human T cells increased their BM homing. Our accumulated knowledge of the factors determining sphingosine-1-phosphate (S1P) and GGG distribution helps advance understanding of how disruptions in S1PR2 and P2RY8 function contribute to B cell lymphomagenesis.

SAFIS II SYMPOSIUM *Thursday, November 17, 11-13 hr*
"AQUAPORINS" 30 YEARS AFTER THE DISCOVERY OF AQP1: NEW ROLES, REGULATIONS AND CLINICAL IMPLICATIONS
Chairs: Claudia Capurro - Gisela Di Giusto

AQUAPORINS (AQP) IN THE BRAIN, THEIR CONTRIBUTION TO CEREBROSPINAL FLUID HOMEOSTASIS, AND THEIR ROLE IN HYDROCEPHALUS

Miriam Echevarría[1,2], Francisco Mayo León[1,2], Lourdes González Vinceiro[1,2], Laura Hiraldo González[1,2], José Luis Trillo-Contreras[1], Claudia Calle Castillejo[1], Pablo García-Miranda[1], Javier Villadiego[1,2] and Reposo Ramírez Lorca[1,2]
[1]Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital. (HUVR)/Spanish National Research Council (CSIC)/University of Seville, Seville 41013, Spain. [2]Department of Physiology and Biophysics, University of Seville, Seville 41009, Spain

AQP1 and AQP4 have been proposed to play a significant role in cerebrospinal fluid (CSF) production and its homeostasis. The first, expressed mainly in epithelial cells of the choroid plexus, and the other, highly abundant in ependymal cells, glia limitans, and perivascular astrocyte foot processes. Recently, our work has demonstrated that both proteins participate in different aspects of CSF homeostasis such as ventricle load and drainage, contribute to the build-up of intraventricular pressure and play a role in distensibility capacity of the ventricular system. By magnetic resonance imaging in AQP1-/-, AQP4-/-, double AQP1-/--AQP4-/- knockout mice and wild-type (Wt) mice controls, we evaluated ventricular volume and confirmed that both AQPs have a significant participation in CSF production. Our data are consistent with significant extrachoroidal CSF formation mediated by AQP4 and support the idea of an important CSF production/absorption process, sustained by efflux/influx of water between the brain capillaries and interstitial fluid. In aged animals, exposure to hypoxia produces ventriculomegaly and cognitive deterioration, a situation that reminds the pathology of iNPH. The involvement of AQP4 in this disease has been widely demonstrated but, AQP4 presence in the CSF of patients was not confirmed. On the other hand, we are currently studying the mechanism by which the deletion of AQP4 triggers a congenital hydrocephalus (HC) due to Silvio aqueduct stenosis in 9.6% of the colony of these animals (AQP4-/- or AQP4-KO). The analysis of gene expression in the periaqueductal tissue of Wt and AQP4-KO, hydrocephalic and non-hydrocephalic animals, at P11, indicated the overexpression of genes related to a subpopulation of microglial CD11c + in non-hydrocephalic AQP4-KO mice. The time course of expression of these CD11c+ cells showed a high magnitude and a large presence in the aqueduct of AQP4-KO animals compared to Wt. Similarly, an underexpression of genes related to ependyma maturation was determined in AQP4-KO mice at P11 compared to Wt. Depletion of microglia by the use of PLX5622 allowed us to determine that the apical area of the ependymal cells of the treated animals was reduced with respect to the untreated animals, and, in turn, in the latter it was smaller than in the Wt, indicating a low degree of ependymal maturation in the AQP4-KO mice. In conclusion, our results confirm a functional and structural role for AQP4 in the origin of hydrocephalus.

ROLE OF THE WATER CHANNEL AQP4 IN GLIAL CELLS HOMEOSTASIS IN PHYSIOLOGY AND DISEASE

Claudia Capurro

Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY) UBA-CONICET, Departamento de Ciencias Fisiológicas, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Aquaporin-4 (AQP4) is the most abundant water channel in the central nervous system. Within the retina, AQP4 is mainly expressed in glial Müller cells, largely involved in controlling extracellular homeostasis. Particularly, cell swelling occurring during neuronal activity is adjusted by a regulatory volume decrease response (RVD), which depends on the efflux of solutes and water through AQP4. Müller cells are also important for retinal integrity, as they respond to injury by a mechanism known as reactive gliosis, which involves cell cycle´s activation and dedifferentiation to other cell types. Interestedly, AQP4 is the target of autoantibody AQP4-IgG present in the sera of patients with Neuromyelitis Optica Spectrum Disorder (NMOSD), a severe demyelinating autoimmune disease. Previous studies demonstrated that AQP4-IgG binding to astrocytic AQP4 leads to cell-destructive lesions, and it is often associated with retinal abnormalities, but the early physiopathological events in Müller cells are poorly understood. Our physiological studies demonstrated that AQP4 inhibition decreased water permeability and RVD as expected, but also delayed swelling-induced changes in calcium kinetics, reinforcing the role of AQP4 as an osmosensor in retinal Müller cells. We also reveal an interplay between AQP4 and the calcium channel TRPV4 to control Müller cell migration. In addition, we present evidence that AQP4-IgG binding to AQP4 in Müller cells induces its partial internalization, reducing the water permeability, the swelling-induced increase of intracellular calcium levels and then, impairing RVD. The loss of AQP4 from the plasma membrane also delayed Müller cells' proliferation. Intravitreal injection to adult rat's retina of positive AQP4-IgG sera showed that, in fact, the antibody efficiently bound to AQP4 and produced complement-independent retinal injury. We propose that Müller cell dysfunction, after AQP4 inhibition or AQP4 removal from the plasma membrane by AQP4-IgG binding, could be a non-inflammatory mechanism of retinal injury *in vivo*, altering cell volume homeostasis and consequently cell proliferation and migration. This knowledge could be essential to understand the physiopathology of retinal diseases and the development of new therapeutic strategies.

ALTERED EXPRESSION OF AQUAPORIN 2 IN ANIMAL MODELS OF HYPERTENSION

M. Florencia Albertoni Borghese[1,2], M. del Carmen Ortiz[1], Lucas Oronel[1], Mónica Majowicz[1]

[1]Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina

The water channel aquaporin-2 (AQP2) plays a critical role in water reabsorption and in the regulation of extracellular fluid volume both in physiologic and pathophysiologic conditions. However, there was little information about AQP2 expression and/or activity in hypertension. In this presentation we will go through the findings in two hypertension models, chronic nitric oxide (NO) synthase inhibition and 1 kidney-1 clip (1K1C) renovascular model. In the chronic nitric oxide synthase inhibition model induced by L-NAME administration, AQP2 expression decreased in both outer (OM) and inner medulla (IM). This reduced AQP2 expression might partially account for the increased urinary volume and decreased urinary osmolality, since we obtained a strong correlation between AQP2 expression and these urinary parameters in both OM and IM. We proposed that AQP2 expression was modified in response to elevated arterial pressure in which case, other hypertension models would show similar results. However, another possibility was that the observed changes were due to the decrease in NO production itself, since it had been shown that AQP2 expression was regulated by the calcineurin/nuclear factor of activated T cells (NFATc) and NO was shown to regulate the activity of NFATc via JNK2. To test this hypothesis, we worked with mice IM and cells transfected with an AQP2 promoter luciferase reporter. Our results demonstrated that both Ca2+ and NO have a synergistic effect increasing AQP2 mRNA and protein in mouse IM and activation of the AQP2 promoter in kidney-derived cells. In addition, NO enhanced Ca2+-induced NFATc activation. We also investigated the regulation of AQP2 expression in another experimental model: the 1K1C Goldblatt model. We found that AQP2 expression, V2 receptor expression and cAMP concentration were decreased in the renal medulla of 1 K-1C rats, NFkB translocation was favoured towards the nucleus suggesting its activation while TonEBP translocation was not altered. In this model of hypertension, the decrease in AQP2 expression could be a mechanism that counteracts the high blood pressure promoting water excretion and this may be consequence of decreased vasopressin sensitivity and/or the increased activity of NFkB at renomedullary collecting duct level. Although historically, to explain the pathophysiology of hypertension the focus has been put on sodium transport, these results showed the importance of AQP2 water channel in different hypertension models.

DIVERSITY IN THE MIP FAMILY AND DESCRIPTION OF EXPANDED GROUPS IN KINETOPLASTID PARASITES

Karina Alleva

Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática; Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), UBA-CONICET.

Since the discovery of hAQP1, the paradigmatic exemplar of MIP family (*Membrane Intrinsic Proteins*) of channels, many homologous genes have been identified in archaea, bacteria, and different eukaryotic organisms. In most genomes there are multiple MIP genes with plants being the specimens where the greatest diversification has occurred. Understanding how such gene diversity emerged is still a challenge and an open question that entails the complexity of predicting the function of each sequence. In recent years, parasite aquaporins have been positioned as possible therapeutic targets based on the finding that MIP channels were able to internalize drugs of choice against *Trypanosoma brucei* and Leishmania spp. (i.e. pentamidine and antimonial compounds). This led us to carry out an exhaustive analysis of the diversity of the MIP family in trypanosomatids (Tesan et al., *Commun Biol* 4, 953 (2021)). Our phylogenetic analyzes revealed that trypanosomatid MIP channels are distributed exclusively in two groups: GLP or aquaglyceroporins, and a distant group that was not described until now, which we call AQPX. This group of aquaporins were already widespread in the Metakinetoplastina common ancestor before the origin of the parasitic order Trypanosomatida. Synteny studies show that African trypanosomes specifically lost the AQPX group, while American trypanosomes specifically lost GLP. GLP channels preferentially transport glycerol, while AQPX channels diverge from already described MIPs on crucial residues and present quite different selectivity filters that it is difficult to predict what transport function they may be fulfilling. The potential for AQPX channels to be, like GLPs, gateways for trypanocidal drugs makes studying them functionally and structurally highly relevant.

SAIC V - SAI IV SYMPOSIUM *Thursday, November 17, 16-18 hr*
EXTRACELLULAR VESICLES: FROM BASIC RESEARCH TO CLINIC
Chairs: Daniel Grasso - Matías Ostrowski

EXTRACELLULAR VESICLES: ANOTHER PIECE OF THE PUZZLE IN THYROID CANCER

Ana Carolina Donadio

Universidad Nacional de Córdoba. Facultad de Ciencias Químicas. Departamento de Bioquímica Clínica. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Córdoba, Argentina.

Thyroid cancer (TC) is the most prevalent malignant disease of the endocrine system. Although most patients with TC have an excellent prognosis, around 30% of cases evolve in an unfavorable way. From the first stages of tumor initiation, malignant cells interact with different populations of non-cancer cells and the extracellular matrix (ECM) in the tumor microenvironment (TME). These interactions include cell-to-cell contact, growth factor and cytokine signaling, and extracellular vesicles (EVs) exchanging bioactive molecules. Before their release, EVs are packed with bioactive molecules, including proteins, lipids, and nucleic acids. Importantly, interactions of EVs with recipient cells may regulate their pathophysiological state.The relevance of TME in TC is beginning to be clarified. In this sense, collagen deposition, ECM remodeling, and the concurrent presence of cancer activated fibroblasts have been described in association with more aggressive clinicopathological features. ECM remodeling creates a cancer permissive microenvironment and matrix metalloproteinases (MMPs) are among the main drivers of ECM degradation. Using a simulation of thyroid TME, based on the co-culture of TPC-1 cells, from Papillary TC; 8505c, from Anaplastic TC; or NThyOri, as thyroid non-tumor cells with human fibroblasts (Fb), we described that thyroid tumor cell-Fb interplay provides a permissive environment for the expression of MMP2 and MMP9 enzymes, also increasing the migratory phenotype of thyroid tumor cells. EVs, which express the classical exosome markers, are secreted from isolated and co-cultured cells. The over-representation analysis of enriched proteins from Fb-TPC-1-and Fb-8505c compared with Fb-NThyOri-derived EVs showed functional convergence in ECM remodeling. Moreover, only those vesicles derived from tumor cell-Fb interacting cells were able to modify the proteolytic performance of Fb, increasing the expression of MMP2. Uptake studies demonstrated a higher avidity of Fb to incorporate EVs from tumoral thyroid context, providing more efficient crosstalk between Fb and thyroid tumor cells. Interestingly, MMP2 interactors allow EVs from tumoral and non-tumoral contexts to be discriminated. The knowledge of the EV-cargo and a better the understanding of their biological function, could contribute to promoting novel treatment strategies, offering opportunities for minimally invasive procedures for the diagnosis and monitoring of cancer patients.

ROLE OF EXTRACELLULAR VESICLES IN PARASITE: PARASITE COMMUNICATION IN TRICHOMONAS VAGINALIS

Natalia de Miguel, Nehuen Salas, Manuela Blasco-Pedreros
Laboratorio de Parásitos Anaerobios, Instituto Tecnológico Chascomús (INTECH), CONICET-UNSAM, Chascomús B7130IWA, Argentina. Email: ndemiguel@intech.gov.ar

Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. Due to the great prevalence of T. vaginalis, mixed infections with several parasite strains are expected. An analysis of 211 T. vaginalis samples isolated in five different continents identified 10.9 % mixed infections. In these cases, the interaction of isolates with distinct phenotypic characteristics may have significant clinical repercussions. Although it is regarded as self-evident that parasites interact with their hosts to enhance their own survival and transmission, the extent to which unicellular parasites communicate with each has been severely underestimated. Here, we demonstrated that different T. vaginalis strains are able to communicate through the formation of cytoneme-like membranous cell-to-cell connections. We demonstrated that cytoneme formation of an adherent parasite strain (CDC1132) is affected in the presence of G3 and PA strains. Additionally, using a transwell assays, we demonstrated that the effect in cytoneme formation is contact independent and that extracellular vesicles (EVs) are responsible, at least in part, of the communication among T. vaginalis strains. Finally, we observed that parasite adherence to host cells is affected by these communication between parasites. Importanly, we observed that a poorly adherent parasite strain (G3) adhere more strongly to prostate cells in the presence if an adherent strain is loaded in the transwell compared to the presence of G3 itself. The study of signaling, sensing and cell communication in parasitic organisms will surely enhance our understanding of the basic biological characteristics of parasites and reveal new potential clinical outcomes.

EXTRACELLULAR VESICLES FROM HUMAN PLASMA DAMPEN INFLAMMATION AND PROMOTE A RESOLUTION PHENOTYPE IN MACROPHAGES AND NEUTROPHILS

Matías Ostrowski
Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS) - Facultad de Medicina -UBA- CONICET

Although inflammation is a vital defense response to infection, if left uncontrolled, it can lead to pathology. Macrophages and neutrophils are critical players in the inflammatory response but also in the resolution of inflammation. Extracellular vesicles are membrane-enclosed structures released by cells that mediate intercellular communication and are present in all biological fluids, including blood. Herein, we show that extracellular vesicles from plasma (pEV) play a relevant role in the control of inflammation by counteracting PAMP-induced macrophage and neutrophil activation. Macrophages simultaneously exposed to pEV and a PAMP exhibit reduced secretion of pro-inflammatory IL-6 and TNF and increased IL-10 response. This anti-inflammatory activity was associated with the promotion of a resolution phenotype in macrophages, characterized by augmented efferocytosis and a gene expression signature characteristic of resolution macrophages, including increased expression of VEGFa, CD300e, RGS2 and CD93. Mechanistically, we show that EVs trigger the production of PGE2 and that the anti-inflammatory activity of pEVs is abolished if the production of this prostaglandin is blocked by pharmacological inhibition of COX2, indicating that PGE2 and COX2 are critical for the pEV-mediated inhibition of inflammation. We also provide evidence showing that pEVs counteract the PAMP-mediated activation of neutrophils, as evidenced by reduced degranulation, oxidative burst, and IL-1 secretion. In conclusion, our results support that plasma EVs are endogenous homeostatic modulators of macrophage and neutrophil-driven acute inflammation and that, by activating the PGE2/CREB axis, they promote tissue repair.

THE ROLE OF EXTRACELLULAR VESICLES IN NEURODEGENERATIVE DISEASES

Andrew F. Hill
Institute for Health and Sport, Victoria University, Melbourne, Australia.
andy.hill@vu.edu.au

Neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and prion diseases are associated with proteins that misfold and deposit in the brain. Many cell types, including neurons, release extracellular vesicles (EVs) which include microvesicles and exosomes. Roles for these vesicles include cell-cell signalling, removal of unwanted proteins, and transfer of pathogens (including prion-like misfolded proteins) between cells. Our group has shown that EVs contain distinct processed forms of these proteins and that, in the case of prion disease, they

contain the transmissible form of the misfolded protein. In addition to their protein content these vesicles have recently been shown to contain genetic material in the form of protein coding (mRNA) and noncoding RNA species. We have analysed the protein and genetic cargo of EVs from a number of cell types and using deep sequencing, characterised the RNA cargo of these vesicles. As EVs can be isolated from circulating fluids such as serum, urine, and cerebrospinal fluid (CSF), they provide a potential source of biomarkers for neurological conditions. This talk will review the roles these vesicles play in neurodegenerative disease and highlight their potential in diagnosing these disorders through analysis of their RNA content.

SAI V SYMPOSIUM *Thursday, November 17, 16-18 hr*
AUTOIMMUNITY
Chairs: Marta Toscano - Rubén Motrich

FRONTIERS IN ORGAN TRANSPLANTATION AND AUTOIMMUNITY

Ignacio Anegon
INSERM-CR2TI Nantes, France.

We have recently described a tolerogenic therapy targeting with mAbs immune cells expressing CD45RC at high levels. These CD45RChigh cells are naïve T cells precursors of Th1 cells and TEMRA cells, as well as most of B cells. CD4+ and CD8+ Treg in rodents and humans are CD45RClow/neg and are not killed by anti-CD45RC mAbs. Furthermore, following depletion of CD45RChigh cells, Treg are activated, amplify, and become memory Treg with amplified suppressive functions in vitro and in vivo mAbs iwith anti-CD45RC mAbs imposing tolerance following a short treatment. The other isoforms of CD45, such as CD45RO, RA and RB are expressed by a fraction of Treg, CD45RC is the only isoform of CD45 not expressed by Treg. In humans and rodents, memory T cells responding to viruses, granulocytes, most monocytes and myeloid DCs, as well as tissue macrophages, parenchymal and epithelial cells are CD45RCneg. We demonstrated efficacy of anti-CD45RC mAb treatment in an organ transplantation rat model (Picarda et al 2017) and in acute GVHD allogeneic models in rats and mice as well as in immune humanized mouse models. We also showed efficacy in autoimmune diseases models in rodents, including in a genetic disease autoimmune disease, APECED, due to the deficiency of the *Aire* gene (Besnard et al. 2022). The expression of CD45RC in patients with organ grafts, APECED and autoimmune diseases under different conventional immunosuppression is like the one of healthy volunteers. Thus, this anti-CD45RC mAb strategy favors the balance of immune response towards tolerance and has potential for a clinical use in immune-mediated diseases.

WHAT CAUSES MULTIPLE SCLEROSIS?

Mauricio Farez, MD.
Centro para la Investigación de Enfermedades Neuroinmunológicas (CIEN). Buenos Aires, Argentina.

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, demyelinating disease affecting the central nervous system (CNS). It is the leading cause of non-traumatic neurological disability among young adults, and there is currently no definitive treatment for it. Among the risk factors that have been identified for the development of MS are: viral infections, smoking, levels of vitamin D and melatonin. The virus that has the most epidemiological evidence supporting its relationship with the risk of developing MS is the Epstein-Barr virus (EBV). EBV belongs to the herpesvirus family and is one of the most common viruses in humans. The virus is present worldwide and it is estimated that 95% of adults aged 35-40 years have been infected at some point in their lives. It is a double-stranded DNA virus that is transmitted primarily through saliva. EBV infection is usually asymptomatic when it occurs in early life. In contrast, infectious mononucleosis (IM) is usually the manifestation of infection acquired during adulthood. Late EBV infection is more frequent in developed countries, consistent with the incidence of IM. The main evidence connecting EBV infection with MS is that virtually 100% of MS patients had contact with EBV, reflected by positive blood serology, compared to 90% of controls. Furthermore, the titer of antibodies against EBV is increased in patients with MS and the risk of MS is doubled in patients with a history of mononucleosis. Finally, a large-scale prospective epidemiological study showed a zero risk of developing MS in patients who had not been exposed to EBV, although this risk increased sharply after EBV infection. Despite the strong evidence linking EBV to MS, the mechanism by which the virus contributes to the development of the disease is unknown. In this presentation we will review the evidence on EBV and other risk factors in the causation of MS.

PATHOPHYSIOLOGY AND THERAPEUTIC PERSPECTIVES IN AUTOIMMUNE NECROTIZING MYOPATHIES

Oliver Boyer

Univ of Rouen Normandie, Inserm, U1234, FOCIS Center of Excellence PAn'THER; Rouen University Hospital, Department of Immunology and Biotherapy, Rouen, France.

Immune-mediated necrotizing myopathy (IMNM) is a severe autoimmune disease pertaining to inflammatory myopathies (or myositis). IMNM is frequently rapidly progressive and debilitating, and failure to treat IMNM effectively may lead to life-threatening muscle impairment. IMNM are characterized by high creatine kinase levels, and necrosis of skeletal muscle fibers with deposition of C5b-9 membrane attack complex (MAC). Most IMNM patients have autoantibodies (aAbs) directed against signal recognition particle (SRP) or hydroxy-3-methylglutaryl-CoA reductase (HMGCR). In addition to their role as biomarkers, the relationship observed between aAb titers and disease severity has led to the suggestion that these aAbs could be pathogenic and therefore the central players in IMNM pathophysiology. *In vitro* experiments show that anti- SRP and anti- HMGCR aAbs cause muscle fiber atrophy and impair the fusion of myoblasts, therefore limiting their regenerative capacity. We show that passive transfer of IgG from anti-SRP+ or anti-HMGCR+ IMNM patients to mice induce a muscle deficiency accompanied with IgG and complement deposition on muscle fibers and some level of muscle necrosis. Disease is less pronounced in complement-deficient mice and augmented in mice supplemented with human complement. Together, these results establish the pathogenic role of aAbs in IMNM mouse model. Treatment of IMNM includes steroids and immunosuppressants such as methotrexate and azathioprine, that may be combined with intravenous immunoglobulins and/or rituximab. Although these treatments are effective, a high proportion of patients experience relapses, leading to irreversible muscle damage and disability. We used a humanized murine model of IMNM to evaluate preclinically the efficacy of candidate therapies. Inhibition of complement activation was effective in preventing IMNM but the therapeutic effect was less pronounced on overt disease. Consistently, the results of a recent therapeutical trial with the C5 inhibitor Zilucoplan (UCB pharma) has concluded to lack of clinical efficacy. An alternative strategy is blockade of FcRn-mediated IgG recycling to lower the level of aAb. Efgartigimod (Argenx) dramatically reduced circulating IgG levels and rapidly eliminated pathogenic anti-HMGCR+ Abs in mice, preventing further necrosis and allowing muscle fiber regeneration, resulting in regain of muscle performance. These results support investigating the therapeutic efficacy of efgartigimod through a clinical trial in IMNM patients.

ROLE OF EOMES IN COORDINATING CELLULAR METABOLISM AND LONG-TERM SURVIVAL OF EFFECTOR CD4 T CELLS DURING CHRONIC INFLAMMATION

Anne Dejean

INFINITY - Toulouse Institute for Infectious and Inflammatory Diseases. INSERM UMR1291 – CNRS UMR5051 – University Toulouse III, France.

The role of the transcription factor (TF) Eomes in the pathophysiology of inflammatory diseases is still elusive, especially in T helper (Th) subsets. Eomes is unlikely to define a specific CD4 T cell lineage, as it is expressed in nearly all Th subtypes in both mouse and human. However, it has been consistently reported that Eomes-expressing T cells accumulate in the inflamed tissues of patients with chronic inflammation or cancer. Yet, very little is known about the contribution of these infiltrating Eomes+ CD4 T cells to the pathophysiology of these diseases. We recently describe the molecular mechanisms by which Eomes promotes T cell accumulation in inflamed tissues and reveal a novel non-canonical role for this TF in mitochondrial metabolism. We show that Eomes is mainly dispensable for CD4 T cell initial priming and proliferation but is specifically required for the persistence of CD4 T cells infiltrating the tissues. We further demonstrate that Eomes sustain the expression of multiple genes necessary for the maintenance of mitochondrial architecture and function, thereby enhancing mitochondrial fitness and respiratory capacity. At the molecular level, Eomes expression confers to CD4+ T cell a unique chromatin accessibility landscape, Eomes binding resulting in chromatin opening at enhancer regions, in particular in gene loci involved in cellular metabolic regulation. Hence, we now describe a new mechanism for the control of effector CD4+ T cell longevity through the Eomes-dependent regulation of mitochondrial transcriptional modulators responsible for the long-term survival of effector CD4 T cells in inflamed tissues.

SAFIS III SYMPOSIUM *Thursday, November 17, 18-20 hr*
BETWEEN THE PHYSIOLOGY WE INVESTIGATE, THE ONE WE TEACH AND THE ONE THAT SOCIETY NEEDS
Chairs: Sebastián Caffera - Paola Boarelli

THINKING ABOUT POSSIBLE APPROACHES TO TEACH PHYSIOLOGY: ARE BASIC RESEARCHERS KEY PLAYERS IN THIS MATCH?

Irene Ennis
Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina.

Within the framework of the symposium organized by the teaching commission of SAFIS with the proposal to debate and exchange opinions about the different perspectives from which to approach the teaching of physiology to health sciences students, I would like to highlight the importance that, personally, I find in incorporating experimental work and the scientific method daily. I believe that none of us intends to "pass on" the enormous body of knowledge available on human physiology to our students. Encyclopedic teaching has lost its meaning. The amount of information available is endless and it is growing in an amazing way. Surely, by the time our students graduate, the "contents" we were able to transmit to them will be insufficient, and probably, partially obsolete. Therefore, I think it is necessary to carry out a review and hierarchy of the contents and use the selected ones to "teach to learn", "teach to generate knowledge". It is from this place that I emphasize the importance of scientific researchers participating in the teaching of physiology. That it be the students themselves who, guided by their teachers and using the scientific method, "discover" knowledge. That they incorporate observation, a critical attitude, the ability to infer from what they observe, to build their own knowledge. That they acquire solid tools to become generators of their own learning. In summary, I think it is extremely important that researchers be able to directly transmit their experiences of acquiring knowledge to those who are in the early stages of that same process. I believe that incorporating the perspective of those who do research in the field of physiology can only enrich the training process of those who must later use that knowledge for their professional development.

HOW SHOULD WE BE TEACHING PHYSIOLOGY?

Rosana Elesgaray
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias. Biológicas, Cátedra de Fisiología, Ciudad Autónoma de Buenos Aires, Argentina; Universidad de Buenos Aires-CONICET, Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), Ciudad Autónoma de Buenos Aires, Argentina.

Different factors have an influence on the teaching and learning of Physiology. Among them, some of the most important are the different teaching methods, the number of enrolled students, how many teachers are participating, the hours dedicated to class, the number of contents to be studied and the difficulty when it comes to understanding the concepts that will be key for future professionals. If we take a look to the different syllabi of this subject, no matter which university nor which course of studies we are talking about, we will find that all of them look like the index on any average Physiology book. And, whenever the teaching of Physiology is discussed, most teachers agree that the contents taught should be the same, whichever the course of studies is. However, is this really true? Should we actually be teaching everything? Or should we be selecting specific contents? And, in that case… how should we be teaching those contents? Along with globalization and the everchanging social demands come the shifts and adjustments in teaching. Simply discharging content into the students' brains is no use unless those contents are actually grasped and applied to daily life by students. What we should do is encourage their commitment to a learning method through which they can achieve critical thinking, so that they can later question what they have learned and acquire the skills that they will need in their professional lives. Our lessons should be stimulating their learning not as a passive process, but as a proactive exercise of building knowledge. We should be switching from the master lecture, with the teacher as its protagonist and the student as a mere recipient, to lessons in which the students are the main concern —along with all their needs and intricacies. And to achieve this we need to integrate different teaching strategies. Can we do it? There is no doubt it is a challenge. But we would not be standing here if we did not believe we can.

BASES OF PHYSIOLOGY FOR CURRICULA AND CLINICAL PRACTICE

Diego A. Rosso

Professor of Pharmacology, Faculty of Medicine, UBA. Independent Clinical Researcher of CONICET, Institute of Pharmacology, Faculty of Medicine, UBA. Hemato-oncologist Pediatrician of the Hospital Elizalde. Head of the Department of Pediatrics of the Hospital of Clinics.

SAFIS IV SYMPOSIUM *Friday, November 18, 9-11 hr*
MECHANICAL STRESS AS AN INTRACELLULAR SIGNALING PATHWAY
Chairs: Gustavo Pérez - Horacio Cantiello

THE ROLE OF THE MEDIAL OLIVOCOCHLEAR SYSTEM IN NOISE-INDUCED AND AGE-RELATED COCHLEAR SYNAPTOPATHY

María Eugenia Gómez-Casati

Instituto de Farmacología. Facultad de Medicina. Universidad de Buenos Aires

The main goal of our laboratory is to increase the knowledge on the consequences of different forms of hearing loss on the normal function of sensory hair cells in the mammalian organ of Corti and to study the role of the medial olivocochlear system (MOC). Noise and aging are the two most common causative factors among the defined etiologies of hearing loss. The clinical significance presented by noise-induced (NIHL) and age-related hearing loss (ARHL) has driven efforts to understand the underlying molecular, physiological, and biochemical mechanisms of the cochlear damage. Knowing how the physical structures in the inner ear are affected by noise and/or age is crucial in search for therapeutic agents that act as otoprotectants against hearing loss. Our lab has been trying to understand the role of the MOC system in protecting the inner ear from damage produced by overly loud sounds. We have shown an inverse correlation between the activity of the α9α10 nicotinic cholinergic receptor (nAChR) and noise-induced cochlear synaptopathy. Moreover, we have shown that the MOC system mediates resistance to ARHL – presbycusis, and that this occurs via the α9α10 nAChR complexes on outer hair cells. These results suggest that potentiation of the MOC feedback can trigger cellular and molecular mechanisms to protect and/or repair the inner ear sensory epithelium. These findings are beginning to bridge the gap from bench to clinics as they provide the first proof-of-principle supporting the enhancement of the MOC system as a viable approach for prevention or treatment of NIHL and/or ARHL.

IMPACT OF THE CYTOSKELETON ON BRAIN ELECTRICAL ACTIVITY

Horacio Cantiello

Laboratorio de Canales Iónicos. Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD), CONICET-UNSE, Santiago del Estero, Argentina.

Actin filaments (F-Actin) and microtubules (MTs) are long polyelectrolytes assembled from ATP-bound G-actin monomers and GTP-bound αβ-tubulin heterodimers, respectively. These essential components of the cytoskeleton drive a range of dynamic cellular processes. In neurons, the synergistic activity of F-Actin and MTs provides structural support during neuronal development, the establishment of cellular shape, and polarity during the transition from precursor cells into mature neurons. For the past thirty years, our laboratory has focused on the characterization of the electrical properties of F-actin and MTs. We demonstrated that F-actin functions as a biological “electrical wire” that can be conceptualized as a nonlinear transmission line that may have important implications in coupling intracellular electrical signals, particularly in actin-loaded organelles such as dendritic spines. On the other hand, MTs, whose wall is interspersed with nanopores, can amplify electrical signals, thus behaving as biopolymeric transistors. We have extensively applied the patch clamp technique to various preparations of brain MTs, including 2D sheets, bundles, permeabilized neurons, and brain tissue, to characterize their electrical properties. Voltage-clamped MT sheets and bundles generated cation-selective oscillatory currents whose magnitude depended on the holding potential, ionic strength, and composition. The most prominent fundamental frequency was observed at 29-39 Hz, although other fundamental frequencies were also present in the signals. This phenomenon is observed in permeabilized neurons. MTs show memristive capabilities, which enable voltage-driven neuromorphic circuits and architectures within neurons and support computational capabilities as binary classifiers. To further explore MTs’ role in brain function, we used the honeybee (*Apis mellifera*) as a helpful model to isolate brains and MTs. MT sheets from the honeybee brain sustained electrical oscillations that were also observed by local field potential recordings from the whole brain preparation. We were able to model the brain MT electrical oscillations by Empirical Mode Decomposition Analysis (EMD), demonstrating that their patterns resembled those observed in the human EEG. The encompassed evidence indicates that the electrical activity of cytoskeleton polymers in the brain may be a novel paradigm implicated in the brain’s electrical activity.

AUTOREGULATION OF EXCITATION-CA2+ SIGNALING-CONTRACTION IN CARDIOMYOCYTE UNDER MECHANICAL LOAD

Ye Chen-Izu, Zhong Jian, Bence Hegyi, Rafael Shimkunas, Mohammad A. Kazemi-Lari, John Shaw, Kit S. Lam, and Leighton T. Izu

Department of Pharmacology, University of California, Davis, California, USA.

The heart pumps blood into circulation against the mechanical load from vascular resistance and actively regulate the cardiac muscle contraction to compensate for load changes. To decipher the underlying mechanisms, we developed a Cell-in-Gel technology to investigate the mechano-chemo-transduction (MCT) mechanisms that transduce mechanical load to biochemical signals to regulate the cardiomyocyte contraction. Our experimental studies reveal that MCT regulates each of the dynamic systems involved in cardiac excitation-Ca2+ signaling-contraction (E-C) coupling. Mechanical load on cardiomyocyte during cardiac cycle leads to increased action potential (AP) duration by increasing the L-type Ca2+ current (ICaL), increasing the transient outward K+ current (Ito), and decreasing the inward rectifier K+ current (IK1). MCT also modulate the Ca2+ signaling by increasing the cytosolic Ca2+ transient to enhance contractility, and also increasing spontaneous Ca2+ sparks and waves that lead to arrhythmogenic activities. We conducted rigorous mechanical analyses of the 3D mechanical strain and stress in cardiomyocyte during beat-to-beat contraction. Based on experimental data, we developed mathematical models to incorporate the MCT feedback into the E-C coupling paradigm to study the nonlinear dynamic system's behavior. Our model predicted that cardiomyocytes can autoregulate contractility in compensatory response to load changes. Model predictions were further verified by experiments. In conclusion, our experimental and modeling studies reveal that autoregulation of contractility naturally arises from the MCT feedback in the cardiomyocyte E-C coupling dynamical system, which underlies the heart's intrinsic adaptive response to mechanical load changes in order to maintain cardiac output.

HYPEROSMOLARITY INDUCES CAVEOLAE DISRUPTION AND INTERNALIZATION IMPAIRING HUMAN EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION

Nora Alicia Martinez

Laboratorio de Biología de la Reproducción. IFIBIO-Houssay (UBA-CONICET). Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina.

The success of pregnancy depends on the normal placental development that involves the differentiation of the trophoblast cells in the different linages. A group of trophoblast cells acquires an invasive phenotype (extravillous trophoblast-EVT) which can migrate and invade the decidua and myometrium. The EVTs remodel the spiral uterine arteries and replace the endothelial cells, acquiring an "endothelial-like phenotype". Although these events are not entirely clear, it is known that they are tightly regulated in a spatiotemporal manner. Consequently, defects in these processes during the early first trimester of pregnancy can lead to pathologies associated with placental insufficiency such as stillbirth, fetal growth restriction (FGR), and preeclampsia (PE). Many of the signaling pathways involved in trophoblast differentiation have receptors located in the caveolae. These structures are small-specialized plasma membrane microdomains in which Caveolin-1 (Cav-1) is the major integral membrane protein required for their formation. We recently reported that an intact caveolar structure is necessary for adequate cell migration and tubulogenesis of the EVT cells. Emerging evidence supports that hyperosmolarity induces the internalization of caveolae into the cytoplasm and accelerates their turnover. We hypothesized that hyperosmolarity affects EVT differentiation and caveolae/Cav-1 participates in this process. Therefore, the aims of this study were to evaluate the effect of hyperosmolarity on 1) EVT cell migration, cell invasion and the formation of tube-like structures and, 2) caveolae structure. The EVT cells (Swan 71 cell line) were cultured in complete Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 and exposed to hyperosmolar condition (generated by the addition of 100 mM sucrose). Hyperosmolarity altered the EVT cell migration, the formation of tube-like structures and induces a reduction in the latent and active forms of matrix metalloproteinase-2 (MMP-2) secreted by these cells. On the other hand, hyperosmolarity enhanced Cav-1 degradation via the lysosomal pathway, increasing the number of vacuoles and the internalization of the caveolae into the cytoplasm. Taken together, these results suggest that hyperosmolarity may induce caveolae internalization and increase their turnover, compromising the normal differentiation of EVT cells.

SAIC VI SYMPOSIUM *Friday, November 18, 10:30-12:30 hr*
IMPACT OF ENVIRONMENTAL POLLUTANTS AND LIFESTYLE ON HEALTH
Chairs: Claudia Cocca - Andrea Randi

IMPACT OF THE UV FILTER BENZOPHENONE-3 ON FERTILITY: LESSONS FROM IN VIVO AND IN VITRO MODELS

Horacio A. Rodríguez[1,2]
[1] Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Santa Fe, Argentina; [2] Facultad de Bioquímica y Ciencias. Biológicas, Universidad Nacional del Litoral (UNL) Santa Fe, Argentina.

We are continuously exposed to chemicals included in personal care products (PCP) that may affect our health. Benzophenone 3 (BP3) is commonly used in sunscreens and other PCP due to its UV blocking efficacy. Several studies have evidenced that BP3 can act as an endocrine disrupting chemical (EDC). Our interest was to establish if some critical processes for fertility can be identified as targets of BP3 action. In first place we have shown that prenatal dermal exposure to BP3 affected fetal growth of the progeny in mice, inducing a fetal growth restriction (FGR) phenotype. In another experiment with a longer prenatal period of dermal exposure, we showed that pregnant mice exposed to BP3 have reduced the size of whole implantation sites (WIS) and fetus-placental index (FPI), leading to lower weights of fetuses compatible with FGR. Moreover, these growth abnormalities of fetuses were linked to an impaired spiral artery remodeling (SAR) in decidua of BP3-exposed mothers together with reduced presence of NK cells. Then, using an in vitro model of anchoring and implantation of murine blastocysts, we found that BP3-treated embryos displayed significant delayed hatching and attachment, demonstrating that BP3 can exert a direct action on early embryo development. This delay lead to a drastic reduction of implantation area at 6th day of culture. We also found that BP3 reduced the migration ability of human trophoblast cells (Swan 71), which restored to normal values when cells were exposed to BP3+flutamide, an AR inhibitor. On the other hand, we observed that prenatal exposure in two successive pregnancies to BP3 alone or in combination with bisphenol A (BPA), another common EDC, impaired the ability of the ovary to respond to gonadotropins stimulation. Then, we aimed to identify the effects on the offspring caused by a perinatal exposure to BP-3 comprising gestation and breastfeeding. Performing a forced-breeding protocol with the offspring born to mothers perinatally exposed to BP3, we observed a decrease of pups/mother and deliveries/mother, not linked to oocyte depletion. Taking together, these results showed that exposure to BP3 can affect different processes necessary for an optimal fertility. Some of these results were included by SCCS (European Commission's Scientific Committee on Consumer Safety) in their last opinion on BP3, recommending the reduction of the maximum percentage of BP3 allowed in sunscreens.

ASSOCIATIONS BETWEEN MIXTURES OF ENDOCRINE DISRUPTING CHEMICALS AND BREAST CANCER RISK IN OBSERVATIONAL STUDIES

German Cano-Sancho, Philippe Marchand, Bruno Le Bizec, Jean-Philippe Antignac
LABERCA, Oniris, INRAE, Nantes, France

The chemical industry revolution has brought a large list of technological advances of paramount relevance for the comfort and well-being of modern human society. Consequently, the large and diverse chemical production has resulted in a complex paradigm, where its benefits have been dramatically overtaken by the deleterious impacts on the environment and human health. Persistent organic pollutants (POP) are or special concern due to its toxicological properties and, despite being strongly regulated or banned during the 1980s, accumulates in body fat during the entire life. Among the multiple diseases related to POPs, growing evidence has been suggesting the role of POPs on breast cancer risk or hallmark of aggressiveness, especially for the carcinogen prototype 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, recent observational studies conducted in France has also suggested the role of more emerging compounds like perfluorooctane sulfonate, whose concentration in blood was linearly associated with receptor-positive tumor risk (e.g. 4th vs 1th quartile: OR = 2.33 [95%CI = 1.11–4.90]); p trend = 0.04). In turn, brominated flame retardants polybrominated diphenyl ethers (PBDEs) were not associated with breast cancer risk when assessed individually through biomarkers, but positive associations were found using dietary exposure modeling, strengthened by the consumption of vegetable oil. The impact of POPs on breast cancer aggressiveness has been also matter of concern. In a clinical-based case-control study conducted in France we found evidence that some POPs measured in peritumoral fat, were associated with metastasis among women with high body mass index (BMI >25 kg/m2), number of lymph nodes and tumor size. The results from multipollutant models supported the results that mixtures of PCDDs congeners were the main contributors of those associations. These results support the hypothesis that POPs contribute to the risk of breast can-

cer or its aggressiveness in specific sub-groups of population stressing the need of using tailored approaches to identify the most vulnerable women to these chemicals. Further epidemiological research in a larger population should validate these findings, and experimental studies considering realistic mixtures will be required to evaluate the joint effect of POPs on hallmarks of breast cancer and explore their underlying mechanisms.

PHARMACOTOXICOLOGICAL IMPACT OF THE INDUCTION OF MULTI-DRUG RESISTANCE TRANSPORTERS BY THE PHYTOESTROGEN GENISTEIN.

Maria Laura Ruiz[1], Mariana Semeniuk [1], Aldana Magalí Gola[1], María Bucci-Muñoz[1], Viviana Alicia Catania[1], Juan Pablo Rigalli[2]

[1]Instituto de Fisiología Experimental (IFISE-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. [2]Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Heidelberg, Germany.

ABC transporters play a central role in the pharmacotoxicology of drugs used in current therapies and other xenobiotics since they regulate their absorption and excretion. They also mediate multi-drug resistance in cancer cells. P-glycoprotein, also known as multi-drug resistance protein 1 (MDR1/ABCB1), multi-drug resistance-associated proteins 1, 2 and 3 (MRP1/ABCC1, MRP2/ABCC2 and MRP3/ABCC3) and breast cancer resistance protein (BCRP/ABCG2), are the most important transporters involved in drug and xenobiotic excretion. Phytoestrogens, such as genistein (GNT) belong to a class of naturally-occurring compounds found at high concentrations in commonly consumed soya based-foods and dietary supplements. We demonstrated that sustained exposition to low doses of GNT, usually associated with intake of dietary supplements or soy-rich diet,
results in induction of P-gp, MRP2, BCRP and MRP1 expression and activity in different experimental models (rat liver, HepG2, Huh7, Caco-2, MCF-7 cells), protecting cells from drug- and xenobiotic-induced toxicity. Conversely, a decrease in the therapeutic efficacy of different drugs was also observed. Drug efflux or cell viability were measured after treatment of the cells with GNT. In MCF-7, GNT (10 μM, 48 h) increased doxorubicin and mitoxantrone efflux concomitant with MRP1 and BCRP induction by GNT. In HepG2 cells, GNT (1 and 10 μM, 48 h) induced P-gp and consequently, decreased sorafenib cytotoxicity. We also studied the molecular mechanisms involved in the modulation of ABC transporters by GNT. GNT induced P-gp and MRP2 mRNA expression at 10 but not at 1 μM suggesting a concentration dependent regulation. Induction of both transporters by 1 μM GNT was prevented by cycloheximide, suggesting translational regulation. Downregulation of expression of the miR-379 by GNT could be associated with translational regulation of MRP2. Silencing of pregnane X receptor (PXR) abolished P-gp induction by GNT (at 1 and 10 μM) and MRP2 induction by GNT (only at 10 μM), suggesting partial mediation of GNT effects by PXR. In Caco-2 cells estrogen receptor β was involved in MRP2 and P-gp induction by GNT. Since the intake of nutritional supplements and even the diet itself are not always part of the anamnesis prior to the prescription, ABC transporters up-regulation could remain undetected. Consequently, a higher clearance and lower therapeutic efficacy of prescription drugs could take place unexpectedly. In addition, GNT may also decrease the systemic exposure to toxic compounds which are substrates of ABC transporters, probably accounting for the chemoprotective properties attributed to GNT.

EXPOSURE TO ENDOCRINE DISRUPTORS AND RISK OF ENDOMETRIOSIS

Florencia Chiappini

Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina.

Endometriosis is an estrogen dependent gynecologic disease, affecting 10-15% of reproductive age women with lasting implications for many women's fertility and overall quality of life. This disease is defined by the presence of stromal and/or endometrial glandular epithelium implants in extra-uterine locations,primarily the pelvic peritoneum, ovaries, and rectovaginal septum. The etiology and pathogenesis of endometriosis remains uncertain. Several studies support the fact that detached endometrial tissues of menstruation which include endometrial cells, glands and debris, reach the peritoneum by retrograde movement to get implanted, followed by acquisition of new blood supply through angiogenesis, and grow developing endometriotic lesions. Humans are daily exposed to chemical pollutants that could adversely influence physiological processes and potentially cause diseases, including endometriosis. Growing evidence suggests that endocrine disrupting chemicals (EDCs) may be etiologically involved in the development and severity of disease. EDCs can mimic hormones like estrogen and alter signaling pathways. Hexachlorobenzene (HCB), an organochlorine pesticide, is a weak ligand of the Aromatic Hydrocarbon Receptor (AhR), and in our laboratory we found that it acts as EDC in the uterus and mammary gland. Our results showed, in a rat endometriosis model, that HCB increases endometriotic like-lesions volume, microvessel density and the vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), AhR

and Aromatase expression levels. Moreover disrupts hormonal receptors expression and increases tumor necrosis factor-α (TNF-α) content in peritoneal fluid. In an in vitro model, we observed that HCB enhances cell migration and invasion, and matrix metalloproteinase 2 and 9 (MMP-2;-9) activities. Moreover the pesticide promotes prostaglandin E2 (PGE2) and VEGF secretion and c-SRC kinase activation. In addition, the pesticide enhances the angiogenesis process inducing endothelial cells proliferation, migration and their capacity to tube formation; increasing total tube length and the numbers of branching points. Our results provide experimental evidence that HCB induces alterations associated with endometriosis, suggesting that these mechanisms could contribute to EDCs exposure-induced endometriosis development.

SAI VI SYMPOSIUM *Friday, November 18, 11-13 hr*
NEW INSIGHTS IN CANCER IMMUNOTHERAPY
Chairs: Diego Croci - Eduardo Chuluyan

ROLE OF TUMOR- INFILTRATING CD4 T CELLS IN THE IMMUNE RESPONSE AGAINST TUMORS

Carolina Montes
Departamento de Bioquímica Clínica. CIBICI-CONICET, Facultad de Ciencias Químicas- Universidad Nacional de Córdoba, Argentina.

The role of the CD4+ T cells in the response against tumors is not completely elucidated. In this work, we aim to elucidate the functional and phenotype features of CD4 conventional T cells (FOXP3-) from experimental cancer model and breast cancer (BC) patients. We observed that compared to Healthy Donors (HD), BC patients exhibit an accumulation of senescent (KLRG-1+CD57+) CD4+ T cells in peripheral blood. These T cells infiltrate tumors and tumor-draining lymph nodes. KLRG-1+CD57+ CD4+ T cells from BC and HD exhibit features of senescence and, despite their high inhibitory receptor's expression, they produce more effector cytokines and exhibit higher expression of Perforin, Granzyme B and CD107a than non-senescent subsets. Additionally, we describe that CD39+ expressing CD4+ conventional T (T conv) cells infiltrate tumors from mouse cancer models and BC patients. This T cell population is nearly absent in mice´s lymphoid organs, and in non-invaded lymph nodes from patients, but it is present in metastatic lymph nodes. Furthermore, using different experimental models, we conclude that CD39 expression on tumor infiltrating CD4+ Tconv is a common feature across tumors of different histological origin and that the frequency of CD39+CD4+ Tconv increase with tumor growth. By using a phenotypic and transcriptional approach, we demonstrate that CD39+CD4+ Tconv represent a heterogeneous population with exhaustion features and transcriptional signature of cytotoxic T cells. By high dimensional flow cytometry, we evaluated inhibitory receptors (iRs) and transcription factors (TFs) associated with T cell activation and exhaustion. Tumor- infiltrating (TI) CD39+ CD4+ T conv from B16-OVA tumor-bearing mice exhibited higher frequency of iRs and TFs such as T-bet, Eomes, Blimp-1, cMaf, Tox, and Ki67 when compared with CD39- Tconv. TI-CD39+CD4+ exhibited higher percentage of IFNg+ cells, but an impairment in TNF production compared to CD39-CD4+Tconv. TI-CD39+Tconv exhibited the highest cytotoxic potential, evaluated by expression of Granzyme B, Perforin and CD107a. We defined a signature for CD39+CD4+ T conv and we studied the correlation of overall survival in a cohort of BC patients from the TCGA consortium. We observed that the lower expression of the signature genes positively correlated with a lower survival probability from patients from invasive breast carcinoma cohorts. Altogether, our results suggest that CD39+Tconv could acquire a transcriptional program associated with cytotoxic CD4+ T cells and they may contribute to the anti-tumoral immune response.

MONO- VERSUS COMBO- IMMUNOTHERAPY IN CANCER

Jose Cohen
Inserm U955, Mondor Institute for Biomedical Research, Paris Est Créteil University and Mondor University Hospital, Créteil, France.

Therapeutic approaches that modulate the immune response to cancer, such as immune checkpoint inhibitors (ICIs) have contributed to an unprecedented improvement of patient survival, transforming several life-threatening tumors into durably controlled diseases. However, the clinical benefit rate of ICIs, either as monotherapy or combined with chemotherapy is variable depending on the type of cancer. Moreover, adverse effects can be severe, and the treatment cost is high. Therefore, improving the current cancer immunotherapy is a significant unmet medical need. Multiple mechanisms can be responsible for tumor immune evasion, including local infiltration or systemic action of tolerogenic cells such as Tregs. We recently identified that TNFR2, a mostly restricted to immunosuppressive cells molecule (MDSCs and Tregs) at steady state was essential for Treg suppressive functions, both in human and mice. In a model of graft-versus-host disease prevention, we revealed a complete

in vivo inhibition of Treg suppressive activity when the TNF/TNFR2 pathway was blocked by an anti-TNFR2 antibody, thus unleashing a potent anti-leukemic effect by allowing reactivation of donor effector T cells. We also tested this approach in a mouse model of orthotopic pancreatic ductal adenocarcinoma (PDAC) where ICIs are also inefficient. Anti-TNFR2 treatment was able to decrease PDAC tumour growth but not to eradicate it. In both clinical settings, we have evaluated combined immune therapy associating anti-TNFR2 treatments with different ICIs. During this presentation, several protocols of combined approaches will be presented leading to very different results ranging from the observation of deleterious effects to the absence of beneficial effects or the observation of additive effects.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY MEDIATED BY NATURAL KILLER CELLS IN TUMOR IMMUNOTHERAPY WITH THERAPEUTIC *ANTIBODIES*

Estrella Levy
Centro De Investigaciones Oncológicas, Fundación Cancer (Cio-Fuca), CABA, Argentina

Breast cancer (BC) is one of the leading causes of death worldwide and usually remains incurable in advanced stages. Generally, BC is not considered an inflammatory tumor although triple-negative BC (TNBC) and HER2+ tumors are more immunogenic than the most common luminal A-like subtype. Two of the most important targeted cancer therapies today are based on monoclonal antibodies (mAbs). Among the mAbs directed to tumor antigens, cetuximab targets the epidermal growth factor receptor (EGFR) and trastuzumab the HER2. Whereas avelumab that targets PD-L1 belongs to a type of mAbs that block immune checkpoints. Since all these mAbs are IgG1, they could potentially mediate Ab-dependent cellular cytotoxicity (ADCC) against tumor cells. As EGFR and PD-L1 are overexpressed in TNBC, they represent potential therapeutic targets for certain subsets of patients. While for HER2+ tumors, trastuzumab together with pertuzumab constitute already a therapeutic reality. In recent years, our group has focused on the role of NK cells in therapy with these mAbs directed to TNBC or HER2+ cells. We determined that avelumab significantly improved NK cell-mediated cytotoxicity against TNBC cells expressing high levels of PD-L1. Furthermore, IL-2 and IL-15 stimulation of NK cells enhanced Ab-dependent cytokine release and degranulation along with increased lytic activity against tumor cells. We also examined the interaction between NK cells and dendritic cells (DCs) promoted by the anti-EGFR mAb. We found that opsonization of TNBC cells by cetuximab increased IFN-γ/TNF-α production and ADCC mediated by NK cells. Therefore, activated NK cells stimulated tumor material uptake and maturation of DCs. Furthermore, the addition of IL-15 increased both NK cell activation and DCs maturation. Nowadays, we are analyzing adaptive NK cells (aNK cells) that expand after human cytomegalovirus (HCMV) infection. aNK cells exhibit interesting properties that could be exploited for therapy with mAbs, including their longevity and their enhanced functional activity and proliferation in response to stimulation by CD16. We found that a high proportion of BC patients were HCMV+ and most of them had an aNK cell subpopulation. aNK cells produced more IFN-γ than their conventional (cNK) counterparts, against trastuzumab/pertuzumab-opsonized HER2+ cells. Besides, in a group of patients treated with chemotherapy, trastuzumab and pertuzumab, aNK cells retained greater IFN-γ than cNK cells after treatment. Our findings may have implications for therapy with mAbs directed to breast tumors. For this reason, our group continues working on understanding how NK cells could contribute to these treatments' efficacy.

THE IMMUNE SYNAPSE SHAPES THE FUNCTIONAL RESPONSE OF T LYMPHOCYTES

Claire Hivroz
Inserm U932, Institut Curie, Paris, France.

T cell activation is induced by the cognate recognition by the T-cell receptor (TCR) of antigenic peptides presented by the MHC molecules (pMHC) expressed at the surface of antigen presenting cells (APC). This leads to the dynamic formation of a cognate contact between the T cell and the APC: the immune synapse (IS). Formation of the IS is characterized by a strong remodeling of the T cell cytoskeleton and the patterning of receptors and signaling molecules in the contact zone. Our team was among the first to analyze the traffic to the IS of a key molecule of the TCR-induced signaling: the linker for activation of T cells (LAT). This transmembrane protein, present at the plasma membrane and in intracellular compartments, scaffolds numerous proteins involved in T cell activation, forming LAT signalosomes. We will discuss how the purification of LAT-containing vesicles performed by our team and their proteomic analysis revealed the presence of new players of T cell activation. I will also discuss recent data showing that engagement of the immune check point inhibitor PD-1 is regulating formation of the IS.

SAIC VII SYMPOSIUM *Friday, November 18, 14-16 hr*
CURRENT VISION ON DIABETES
Chair: Félix Puchulu

DIABETES MELLITUS: CHANGING THE FOCUS OF TREATMENT

Félix Miguel Puchulu
Jefe de la Sección Diabetología del Hospital de Clínicas "José de San Martín", Facultad de Medicina – UBA.

Diabetes mellitus is a chronic and progressive disease, whose presence is defined in relation to certain blood glucose values that have been shown to favor the appearance of certain complications in chronic form. The current diagnostic values of DM are based on those figures that showed a higher risk of developing microangiopathic complications. Classically, the value of glycosylated hemoglobin A1c (HbA1c) has been used as a reference value, that reflects the degree of hemoglobin glycosylation in the life span of the red blood cell. This very important beacon in the eyes of the doctor could be insufficient for the reduction in the development of micro and macroangiopathic complications. The American College of Cardiology developed an application in which the risk of presenting cardiovascular complications at 10 years can be calculated in patients with different risk factors, including the presence of diabetes, smoking, high blood pressure, dyslipidemia, and aspirin use. In this score, they do not give so much importance to the value of HbA1c. This score seeks to understand that to reduce cardiovascular risk, the goal of treatment for people with diabetes is not only to lower blood glucose, since other pathologies or associated habits are just as or more important in reducing that risk. The HbA1c, which has guided us in decision-making in the treatment of diabetes, has some limitations, such as not suggesting the variability of glycemia. On the other hand, the value found in the laboratory in certain situations may underestimate or overestimate the true degree of control. That is why a few years ago the possibility of performing continuous glucose monitoring with sensors appeared, being able to also make evaluations of how long the patient stays in the desired range, how long in hyperglycemia and how long in hypoglycemia. In these times, there is much evidence that guides us to the fact that the focus on the treatment of diabetes is no longer glucocentric, and that it is currently understood that, in addition to improving blood glucose levels, other cardiovascular risk factors such as overweight, smoking, sedentary lifestyle, dyslipidemia, and arterial hypertension. On the other hand, we must understand that glycosylated hemoglobin is very important in the management of diabetes, but that it has its limitations, and that in certain patients it is necessary to deepen the search for information, and that continuous glucose monitoring may be necessary to get better results.

THE ARTIFICIAL PANCREAS IN THE WORLD AND IN ARGENTINA

Luis A. Grosembacher
Jefe de la Sección Diabetes. Servicio de Endocrinología y Metabolismo. Hospital Italiano de Buenos Aires (HIBA).

ISLET TRANSPLANTATION FOR PANCREATIC BETA CELL REPLACEMENT

Sun Ho Hyon
Jefe de Trasplante Renopancreático y de Cirugía Miniinvasiva. Servicio de Cirugía General. Hospital Italiano de Buenos Aires (HIBA).

SAI VII SYMPOSIUM *Friday, November 18, 15:30-17:30 hr*
IMMUNE LANDSCAPE OF INFECTIOUS DISEASE
Chairs: Nora Goren - Claudia Sotomayor

NEUTROPHILS IN HUMAN TUBERCULOSIS: ARE THESE CELLS KEY PLAYERS IN THE FIGHT AGAINST MYCOBACTERIUM TUBERCULOSIS?

Verónica García
Departamento de Química Biológica-FCEN-UBA, CONICET, Buenos Aires Argentina.

Tuberculosis (TB) is one of the top ten causes of death worldwide. Then, it is crucial to elucidate the host immune mechanisms that operate during *Mycobacterium tuberculosis* (*Mtb*) infection. Neutrophils are the cells predominantly infected with *Mtb in patients' lungs.* Therefore, a deeper investigation into the biology of neutrophils in TB is crucial to identify specific targets for host-directed therapies. Previously, we demonstrated that autophagy collaborates with human immune responses against *Mtb* in close association with IFNG and that IL-17A augments autophagy in *Mtb*-infected monocytes from TB patients in association with the disease severity.

Besides, we showed that *Mtb*-Ag stimulation increased SLAMF1 expression on TB patients´ neutrophils Moreover, we demonstrated that neutrophil autophagy during human TB is modulated by SLAMF1. To investigate the importance of neutrophils in the fight against *Mtb*, we studied the functional phenotypes of these cells in patients with different immune responses to *Mtb*. TB patients were classified as high responder (HR) or low responder (LR) according to their T cell responses against *Mtb.* Significant higher *Mtb* autophagy was observed in HR compared to LR. Interestingly, autophagy levels were markedly decreased by IFN-α, IL-17, IFN-γ, IL-1β in HR but not in LR. Besides, LR patients' neutrophils secreted the highest IL-8 levels but generated the lowest ROS. *Mtb*-stimulated neutrophils from HR displayed the highest levels SLAMF1 and CD69, whereas PD-L1 expression was significantly augmented in LR. Together, we demonstrated that autophagy, ROS generation and innate receptors expression in *Mtb* stimulated neutrophils depend on the TB patient´s immune status and could be differentially modulated by cytokines. Our findings suggest that modulation of functional phenotypes of patients with different immune status to *Mtb* might contribute to the development of new immunotherapies to control active TB.

TRANS-SIALIDASE FRAGMENTS AS A POTENTIAL MUCOSAL VACCINE AGAINST CHAGAS DISEASE

Ana Rosa Pérez
Instituto de Inmunología Clínica y Experimental de Rosario IDICER - CONICET UNR, Argentina.

The Trans-sialidase (TS) superfamily of proteins has emerged as promising immunogens in experimental vaccines against *Trypanosoma cruzi (T. cruzi)* The new generation of vaccines for Chagas disease attempts to induce both humoral and cellular responses to control *T. cruzi* parasites. TSs from subgroup I were among the most tested. Previously, we reported that a vaccine based on a TS fragment from subgroup I protect mice against intraperitoneal or subcutaneous *T. cruzi* infection when vaccinated by subcutaneous route. The route of entry of *T. cruzi* in natural infection is through the skin or mucosa (oral, gastric, and conjunctival). Since vaccines administered at the mucosal level can induce both a mucosal and systemic specific response, we evaluated and compared the immunogenicity and prophylactic effectiveness of TS-based vaccines against the parasite administered orally and intranasally. Vaccines consisted of recombinant fragments of TS formulated in different adjuvants. TS plus c-di-AMP vaccine administered intranasally showed promissory results since induced an evident systemic humoral and cellular response, as judged by the increased plasma anti-TS IgG2a and mucosal anti-TS IgA levels, and also an enhanced cellular response against TS. Plasma-derived antibodies from TS plus c-di-AMP immunized mice also inhibit in vitro the invasion capacity of the parasite. Protective efficacy was also proved in vaccinated animals after oral *T. cruzi*-challenge, reflected by a significant reduction of parasite load during the acute and chronic phase, diminution of clinical manifestations, attenuation of cardiac tissue damage and diminution of electrocardiogram alterations. Therefore, TS + c-di-AMP formulation appears as a promising strategy for prophylaxis of Chagas disease.

MICROBIAL RNA: DIFFERENT FACES OF ITS IMMUNE-MODULATING CAPACITY

Paula Barrionuevo
IMEX-CONICET. Instituto de Medicina Experimental, Academia Nacional de Medicina CONICET, Argentina.

Traditionally, pathogen-associated molecular patterns (PAMPs) were described as structural molecular motifs shared by different classes of microorganisms. However, it was later discovered that the innate immune system is also capable of distinguishing metabolically active microbes through the detection of a special class of viability associated PAMPs (*vita*-PAMPs). Indeed, recognition of *vita*-PAMPs triggers an extra warning sign not provoked by dead bacteria. Bacterial RNA is classified as a *vita*-PAMP since it stops being synthesized once the microbes are eliminated. Most of the studies in the literature have focused on the pro-inflammatory capacity of bacterial RNA on macrophages, neutrophils, endothelial cells, among others. However, we, and other authors, have shown that microbial RNA also has down-modulatory properties. More specifically, we have shown that the RNA of an intracellular bacterium, *Brucella abortus*, reduces MHC class I and MHC class II surface expression on monocytes/macrophages evading immune surveillance of the T CD4+ and CD8+ cells respectively. This phenomenon has been described for several different bacteria and parasites, suggesting that microbial RNA plays a significant immunoregulatory role in the context of many infectious processes. Thus, beyond the pro-inflammatory capacity of microbial RNA, it seems to be a crucial component in the intricate collection of immune evasion strategies. This talk focuses on the different facets of the immune modulating capacity of microbial RNA.

RELEASE OF HIV-1 PARTICLES FROM THE VIRAL COMPARTMENT IN MACROPHAGES REQUIRES AN ASSOCIATED CYTOSKELETON AND IS DRIVEN BY MECHANICAL CONSTRAINTS

Philippe Benaroch
Inserm U932, Institut Curie, Paris, France.

Myeloid cells are highly plastic cells endowed with an ever-growing list of functions in numerous organs. Moreover, they are pivotal in innate immunity and probably represent the most ancient form of cellular immunity against pathogens and tumor cells. Our general goal is to decipher the molecular mechanisms that regulate the interplay between the myeloid cells, viruses, and tumors. I will review two recent HIV-1 studies from the lab. The first one concerns the unique relationship that Axl+DCs possess with HIV-1 among blood DC subsets. The second deals with the cytoskeleton's role in releasing HIV-1 from primary infected macrophages and how mechanical constraints can promote this release.

SAIC VIII SYMPOSIUM *Friday, November 18, 16-18 hr*
REPOSITIONING AND CANCER THERAPY. PARADIGMS THAT AGE AND OLD DRUGS THAT REJUVENATE
Chairs: Graciela Scharovsky

MUSCARINIC RECEPTORS AS NEW TARGETS IN BREAST CANCER ANTITUMOR THERAPY.

Alejandro Javier Español
Tumor Immunopharmacology Laboratory, Center of Pharmacological and Botanical Research. CONICET, University of Buenos Aires. Buenos Aires, Argentina.

The development of breast cancer is a complex process that involves the participation of different factors. Several authors have demonstrated the overexpression of muscarinic acetylcholine receptors (mAChRs) in different tumor tissues and their role in the modulation of tumor biology. Stimulation of these receptors with high agonist concentrations or during long periods of treatment induces cell death. Furthermore, non-tumorigenic human mammary cells or breast samples from patients with benign pathology lack the expression of mAChRs and they are not sensitive to the treatment with muscarinic agonists. Moreover, when transfected with mAChRs, normal cells acquire the ability to respond to muscarinic treatment. These facts position mAChRs as therapeutic targets in cancer. The conventional treatment for breast cancer involves, among others, chemotherapy, that presents disadvantages such as limited specificity, the appearance of resistance to treatment and other side effects. To prevent these ones, several schedules of drug administration, like metronomic therapy, have been developed. Metronomic therapy is a type of chemotherapy in which one or more drugs are administered at low concentrations repetitively. Many chemotherapeutic drugs are used in breast cancer treatment, but only paclitaxel and doxorubicin are able to bind to the active site of mAChRs. Both induce an inhibitory effect on cell proliferation similar to that observed with the cholinergic agonist carbachol. The combination of low concentrations of these chemotherapeutic agents with muscarinic agonists could be an effective therapy in breast cancer since it not only reduces tumor cell survival without affecting normal cells, but also decreases pathological neo-angiogenesis and the expression of drug extrusion protein. The research about new antitumor therapies with drugs that increase beneficial actions and reduce adverse effects is a challenge to improve breast cancer patients' lives. The usage of repurposing drugs, like the muscarinic agonist carbachol, which synergizes the action of traditional anti-tumor drugs might be an alternative schedule focused on mAChRs as new therapeutic targets. The presence of these receptors at high concentrations not only in breast tumors but also in other types of tumors could help to find a more specific and less aggressive manner to treat cancer patients.

REPOSITIONING OF LOSARTAN IN ANTI-TUMOR TREATMENT. RELEASING PRESSURE FROM CANCER

Leandro E. Mainetti[1,2], María José Rico[1,2], Cintia Daniela Kaufman[1,2]2, Monica Carolina Grillo[1], Julian Guercetti[1], María Virginia Baglioni[1,2], Antonela Del Giúdice[1,2], Maria Celeste Capitani[1], Matias Fusini[1], Viviana Rosa Rozados[1,2], and O. Graciela Scharovsky[1,2,3]
[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. [3]Metronomics Global Health Initiative, Marseille, France

Drug repurposing is a creative new approach to increase the number of therapies for different pathologies by exploiting available and already approved drugs. In oncology, drug repurposing is gathering momentum, mainly because revolutionary advances in pharmacology and genomics have demonstrated that many old drugs, designed for other indications, have antitumor activity. Metronomic chemotherapy (MCT) is characterized by the

chronic, equally spaced administration of (generally) low doses of chemotherapeutic drugs, without extended rest periods. Drug repurposing is frequently used with MCT, a combination of therapeutic approaches that has been defined as "Metronomics". Losartan (Los) is an angiotensin II receptor type 1 antagonist, approved to control hypertension in patients and, it is also an antifibrotic agent which has been shown to reduce the incidence of cardiac and renal fibrosis. On the other hand, it has been proved to have some direct and indirect therapeutic effect in cancer treatment. Losartan improves the delivery of therapeutic agents to the cancer cells by reducing extracellular matrix content and the associated "solid stress" in tumors. Losartan reduces stromal collagen, improving drug and oxygen delivery to tumors, thereby potentiating chemotherapy and reducing hypoxia in breast and pancreatic cancer models. In order to achieve an improvement of the therapeutic efficacy of MCT with cyclophosphamide (Cy) for triple negative breast cancers, we developed a Metronomics approach of the therapy, adding to the treatment the repurposed drug losartan. We showed that the combined treatment of Cy + Los has a noteworthy effectiveness on tumor growth, improving the therapeutic outcome when compared to individual treatments. Also, two important characteristics of the combined treatment are the oral administration, hence allowing a chronic drug delivery, and its low toxicity, which does not increase by the addition of losartan, without worsening the quality of life. Moreover, we elucidated some of the mechanisms of action involved that include, decrease of intratumor hypoxia, stimulation of the immune response and remodeling of the tumor microenvironment, especially decreasing the tumor associated fibroblast and collagen production. The remarkable therapeutic effect, the lack of toxicity, the oral administration and the low cost of the drugs involved in the combined treatment, strongly suggest its translation to the clinical setting in the near future.

DRUG REPOSITIONING FOR TREATMENT OF THERAPY-RESISTANT TUMORS

Menacho Márquez M[1-3]; Anselmino LE[1-3]; Baglioni MV4; Malizia F[1-3]; Cesatti Laluce N[1-3]; Rozados VR[3,4]; Scharovsky OG[3,4]; Rico MJ[3,4].

[1]Instituto de Inmunología Clínica y Experimental de Rosario. Universidad Nacional de Rosario (IDICER, UNR-CONICET). [2]Centro de Investigación y Producción de Reactivos Biológicos (CIPReB, Facultad de Ciencias Médicas-UNR). [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). [4]Instituto de Genética Experimental (IGE, Facultad de Ciencias Médicas-UNR)

Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer in men and the second in women. Most cases of CRC are diagnosed at advanced stages when the probability of developing resistance to treatment and distal or local recurrence of the tumor is higher. Since the 1950s, 5-fluorouracil (5-FU) has been the mainstay of chemotherapy for CRC treatment. However, epidemiological studies determined that almost half of patients develop resistance to 5-FU-based chemotherapies. On the other hand, triple negative breast cancer (TNBC) is the most aggressive type of breast cancer, highly metastatic and difficult to treat. For both types of tumors, the available treatments are generally associated to severe side effects. In order to counteract this situation, new treatment strategies are being implemented, such as the improvement of early diagnosis, the discovery of predictive biomarkers and the development of new drugs/drug combinations for treatment. In line with this, drug repositioning explores the reuse of non-cancer drugs to treat tumors, rather than using new drugs. In our work, we evaluated the effect of repurposing drugs on TNBC and 5-FU resistant CRC. By a combination of *in vitro* and *in vivo* studies, we demonstrated that treatment with drugs such as metformin and propranolol affect viability, epithelial-mesenchymal transition and migratory potential of both CRC and TNBC cells. Indeed, we showed that combined treatment affects different steps leading to metastasis in TNBC and is also effective preventing the development of 5-FU resistant CRC, suggesting that combination of metformin and propranolol could be useful as an adjuvant treatment for both type of tumors and an alternative for chemo-resistant CRC, providing a low-cost substitute therapy without associated toxicity. In a second approach, we used bioinformatics algorithms to identify genes associated with tumor recurrence after 5-FU-based chemotherapy in CRC patients. Then, we selected compounds with high probability to reverse the resistance-associated gene expression profile obtained. Combining this strategy with *in vitro* and *in vivo* studies, we demonstrated that *in silico* drug repositioning emerges as an interesting alternative to discover drugs potentially reverting resistance associated to cancer treatment.

DRUG REPURPOSING IN OSTEOSARCOMA: BETWEEN OPPORTUNITIES AND UNMET NEEDS

Garona, Juan[1,2,3], Solernó, Luisina M.[1,2], Sobol, Natasha T.[1,2], Llavona, Candela[1,2], Gottardo, M. Florencia[1,2,3], Vásquez, Liliana[4], Chantada, Guillermo[5], Daniel, F. Alonso[1,3].

[1]Center for Molecular and Translational Oncology (COMTra), Science and Technology Department, Quilmes National University, Argentina. [2]Center for Translational Medicine (Unit 6), El Cruce "Dr. Néstor Carlos Kirchner" S.A.M.I.C. Hospital, Argentina. [3]National Council for Scientific and Technical Research (CONICET), Argentina. [4]Center for Precision Medicine, Medical School, San Martín de Porres University, Perú. [5]Institute for Translational Research (IIMT), Austral University-CONICET, Argentina.

Osteosarcoma (OSA) is the most prevalent primary bone cancer, mainly affecting children and adolescents, and

represents a great clinical challenge given its limited response to therapy and early metastatic progression. The prognosis for these patients is even more dismal in low- and middle-income countries, probably linked to late diagnosis and inadequate access to therapeutic resources. In this context, the research and implementation of new cost-effective therapeutic tools is mandatory to improve the prognosis of patients with OSA. Since signaling associated with vasopressin receptors (AVPRs) and β-adrenergic receptors (ADRBs) regulates many cellular and microenvironmental processes linked to cancer initiation and progression, multiple efforts have been made by our group and others to reposition the hemostatic agent desmopressin (dDAVP) and the β-blocker propranolol (PPN) in oncology. Using different experimental models of OSA, as well as clinical samples, our group has begun to study its antineoplastic activity at the preclinical level, exploring associated mechanisms of action and the expression of the respective therapeutic targets in tumor tissue. Evaluated compounds displayed a potent direct and target-mediated cytostatic action, modulating key phenomena in malignant progression, such as apoptosis, cell cycle, 3D growth, cytoskeleton dynamics and chemotaxis, among others. Using dosage schemes with translational relevance, these drugs inhibited the growth of human OSA xenografts, modulating their aggressiveness and different histological parameters associated with therapeutic response, especially in combination with cytotoxic agents. As a result, the repositioned agents dDAVP and PPN could be postulated as coadjuvant agents for the management of OSA, in combination with chemotherapy or administered in the perioperative setting. Considering that developing countries concentrate 80% of all new cases of pediatric cancers worldwide, drug repurposing in oncopediatrics represents an extremely interesting paradigm that stands as an alternative to de novo development of antineoplastic agents. Interdisciplinary work and integration of Applied research in OSA with hospital groups, is key for deepening our studies and favoring the translation of results to the clinic.

SAIC IX-SAI VIII SYMPOSIUM *Friday, November 18, 16-18 hr*
GLYCANS: AT THE FRONTIERS OF HEALTH AND DISEASE
Chair: Gabriel Rabinovich

ROLES FOR GLYCANS IN NOTCH SIGNALING THAT REGULATES HEMATOPOIESIS

Pamela Stanley and Ankit Tanwar
Dept. Cell Biology, Albert Einstein College of Medicine, New Yor, NY, USA, 10461

Notch signaling is a conserved pathway in the metazoa that regulates cell fate decisions in hematopoiesis. Notch receptors in the plasma membrane are stimulated by Notch ligands in a neighboring cell to induce Notch signaling. The extracellular domain of Notch receptors and ligands contain epidermal growth factor-like (EGF) repeats that are modified by O-glycans that regulate Notch-ligand interactions and thereby, Notch signaling strength. Each O-glycan type is initiated at a particular EGF repeat consensus site by a distinct glycosyltransferase in the endoplasmic reticulum (ER). Extension of the initiating sugar for each O-glycan may occur during passage through the Golgi compartments. To determine specific functions of the different O-glycans, we are characterizing hematopoiesis in mice in which one or more of the glycosyltransferase genes is inactivated. We previously observed that deletion of all three Fringe genes (Lfng, Mfng and Rfng) which transfer GlcNAc to O-fucose on Notch receptors, leads to reduced Notch signaling and altered T, B and myeloid cell development. The triple Fng knockout phenotype was largely rescued by the presence of a single Fng allele. We have now found that mice lacking EOGT which cannot transfer O-GlcNAc to Notch EGF repeats, have altered development of T cells in thymus and B cells and myeloid cells in spleen. Their phenotype is partially transferred by bone marrow transplantation. When combined with conditional deletion of Pofut1 in hematopoietic cells via Vav1-Cre, mice lacking both O-GlcNAc and O-fucose glycans have more severe T, B and myeloid cell developmental defects than deletion of Pofut1 alone. Thus, O-GlcNAc glycans are also required for optimal Notch signaling during hematopoiesis.

DIVERSE ROLES OF HOST GALECTINS IN VIRAL INFECTION OF MUCOSAL EPITHELIA

Gerardo R. Vasta
Department of Microbiology and Immunology, University of Maryland School of Medicine, UMB, and Institute of Marine and Environmental Technology (IMET), Baltimore, Maryland, USA. E-mail: gvasta@som.umaryland.edu

Carbohydrate structures on the cell surface encode complex information that through specific recognition by carbohydrate-binding proteins (lectins), modulates interactions between cells, cells and the extracellular matrix, or mediates reciprocal recognition of microbial or viral pathogens and the host. Galectins are a family of ß-galactoside-binding lectins, which are structurally conserved and have been identified in most organisms, from fungi to invertebrates and vertebrates, including mammals. Their biological roles were initially understood

as limited to recognition of endogenous carbohydrate ligands in development, tissue repair, and regulation of immune homeostasis. Most recently, the realization that galectins can bind exogenous ("non-self") glycans on the surface of viruses, bacteria, protista, and fungi, has led to a new paradigm about their potential roles as pattern recognition receptors and effector factors in innate immunity. In particular, through mechanisms initiated by recognition of the glycosylated viral envelope, the host's galectins can exert direct protective activity against virus infections. Some viruses, however, may have adapted to "subvert" the recognition roles of the host galectins for successful attachment and host entry. Furthermore, downstream effects of the host's galectins can enhance the pathogenic effects of the primary viral infection. These recent findings have revealed a striking functional diversification in this structurally conserved lectin family, as well as evolutionary adaptations of viruses to enter the host. [Supported by grants IOS-1656720 from NSF, and R01GM070589 from NIH].

RELEVANCE OF POLYPEPTIDE GALNAC-TRANSFERASE 3 IN THE HUMAN HEALTH

Irazoqui FJ, Garay GC, Parodi P, Ferrero FA, Angeloni A

Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, and CIQUIBIC, CONICET.

Polypeptide GalNAc-transferase 3 (ppGalNAc-T3) is an enzyme involved in the initiation of O-GalNAc glycan biosynthesis. Acting as a writer of frequent post-translational modification (PTM) on human proteins, ppGalNAc-T3 has key functions in the homeostasis of human cells and tissues. This molecule has relevant roles in the biosynthesis of O-GalNAc glycans, as well as in biological functions related to human physiological and pathological conditions. With main emphasis in ppGalNAc-T3, we draw attention to the different ways involved in the modulation of ppGalNAc-Ts enzymatic activity. In addition, different subcellular localizations of ppGalNAc-T3 are represented, highlighting critical intrinsic and extrinsic functions in cellular physiology that are exerted by ppGalNAc-T3-synthesized PTMs. Moreover, we update on several human pathologies associated with dysfunctional ppGalNAc-T3. Finally, biotechnological tools as new therapeutic options for the treatment of pathologies related to altered ppGalNAc-T3 are considered.

NUTRIENT REGULATION OF SIGNALING AND GENE EXPRESSION BY O-LINKED N-ACETYLGLUCOSAMINE

Gerald W. Hart*

**GRA Eminent Scholar, Complex Carbohydrate Research Center & Biochemistry Department, University of Georgia, Athens, GA USA 30602-4712*

Email: gerald.hart@uga.edu, Mob = +1-443-921-6484

O-GlcNAcylation cycles on over nine thousand human nucleocytoplasmic and mitochondrial proteins, and it has extensive crosstalk with phosphorylation. O-GlcNAc is abundant on nearly all proteins involved in transcription, where it regulates gene expression in response to nutrients. For example, O-GlcNAc regulates the cycling of the TATA-binding (TBP) protein on DNA during the transcription cycle, and O-GlcNAc on RNA polymerase II is required for assembly of the pre-initiation complex. O-GlcNAc modifies at-least 34/80 ribosome proteins and many translation factors, and the sugar also regulates translation and mRNA selection. Targeted deletion of the O-GlcNAc Transferase (OGT) in excitatory neurons of adult mice results in a morbidly obese mouse with a satiety defect. Thus, O-GlcNAcylation not only serves as a nutrient sensor in all cells, but also directly regulates appetite. O-GlcNAcylation also regulates the trafficking of AMPA receptors in neurons and the development of functional synaptic spines. There are nearly two-thousand O-GlcNAcylated proteins at neuronal synapses. More than two-thirds of human protein kinases are O-GlcNAcylated and all kinases that have been tested are regulated by the sugar. Abnormal O-GlcNAcylation of CAMKII contributes directly to diabetic cardiomyopathy and to arrhythmias associated with diabetes. Prolonged elevation of O-GlcNAc, as occurs in diabetes, contributes directly to diabetic complications and is a major mechanism of glucose toxicity. Targeted over-expression of OGT to the heart causes severe heart failure in mice, which is reversed when they are crossed with mice having O-GlcNAcase over-expressed in their hearts. Drugs that elevate O-GlcNAcylation in the brain, which prevents hyperphosphorylation, appear to be of benefit for the treatment of Alzheimer's and Parkinson's disease in animal models. To date, all cancers have elevated O-GlcNAc cycling. Supported by NIH P01HL107153, R01GM116891, R01DK61671. Dr. Hart receives a share of royalty received on sales of the CTD 110.6 antibody, managed by JHU.

SAFIS V SYMPOSIUM *Friday, November 18, 16-18 hr*
TRANSIENT RECEPTOR POTENTIAL CHANNELS: MULTIPLE FUNCTIONS FOR A LARGE FAMILY
Chairs: Emiliano Diez - Paula Ford

STRUCTURE-FUNCTION CORRELATIONS IN TRP CHANNELS. LESSONS FROM POLYCYSTIN-2 AND PARTNERS.

Rocio Cantero
Laboratorio de Canales Iónicos. Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD), CONICET-UNSE, Santiago del Estero, Argentina.

Polycystin-2 (TRPP2, PC2) is the product of the *PKD2* gene, whose mutations cause autosomal dominant polycystic kidney disease. PC2 belongs to the superfamily of TRP (Transient Receptor Potential) which are tetrameric proteins complexes that function as Ca2+-permeable nonselective cation channels. In the last twenty years, our group identified and characterized PC2 ion channel properties and various forms of regulation. We explored the structural and functional properties of PC2 and whether the conductance substates represent monomeric contributions to the channel complex. A kinetic analysis of spontaneous PC2 channel currents showed four intrinsic, non-stochastic subconductance states, which followed a staircase behavior. To confirm the oligomeric contributions to PC2 channel function, heteromeric PC2/TRPC1 channel complexes were functionally assessed by single channel current analysis. Low pH inhibited PC2 currents in PC2 homomeric complexes but failed to affect PC2 currents in PC2/TRPC1 heteromeric complexes. Amiloride, in contrast, abolished PC2 currents in both the homomeric PC2 complexes and the heteromeric PC2/TRPC1 complexes, thus PC2/TRPC1 complexes have distinct functional properties from the homomeric complexes. To assess the topological features of the homomeric PC2-, TRPC1- and heteromeric PC2/TRPC1 channel complexes we used atomic force microscopy (AFM). TRPC1 and PC2 channels had different average diameter and protruding height. The contribution of individual monomers to the PC2/TRPC1 hetero complexes was made evident. More recently, we designed and constructed a BLM reconstitution microchamber that supports the simultaneous recording of electrical currents and AFM imaging from single channel complexes. This platform is unique in our country. To date practically no data exist on direct correlations between electrical features and topological parameters from functional single channel complexes. The experimental setup provided direct structural-functional correlates from the PC2 channel, disclosing novel topological changes between the closed and open sub-conductance states of the functional channel. We demonstrated an inverse correlation between open state conductance conductance and height of the channel. The platform provides a suitable means of accessing topological information to correlate with ion channel electrical parameters essential to understand the physiology of these transmembrane proteins.

FUNCTIONAL AND PHYSICAL INTERACTION BETWEEN THE CALCIUM CHANNEL TRPV4 AND THE WATER CHANNEL AQP2 IN RENAL CELLS

Paula Ford
Universidad de Buenos Aires, Facultad de Medicina, Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Laboratorio de Biomembranas, Buenos Aires, Argentina

Aquaporins (AQPs) are a family of proteins whose essential function is the transport of water through the cellular membranes. However, today there is evidence indicating that these channels would be involved in numerous processes not directly related to water transport. Several studies have indicated that different AQPs exert an influence on cell signaling through interaction with the Ca 2+ channel TRPV4 (Transient Potential Vanilloid Channel type 4). We have been pioneers in proposing an interaction between AQP2 and TRPV4 in the regulation of cell volume in renal cells. Our current hypothesis is that the AQP2-TRPV4 interaction would not only have consequences on the volume regulation mechanisms that are set in motion in the face of hypotonic shock but could also modulate mechanisms secondary to the entry of Ca 2+ related to cellular homeostasis, even in isotonic conditions. We have evaluated the impact of the AQP2-TRPV4 interaction on the changes in the Vm; the release of Ca 2+ from intracellular stores; the influx of Ca 2+ triggered by the emptying of intracellular stores (SOCE) and the release of ATP to the extracellular medium (ATPe). Using fluorescent probes, we studied the signals of intracellular Ca 2+ and the membrane potential, in response to the activation of TRPV4, in two cell lines derived from the rat cortical collecting tubule, one that does not express AQPs (WT-RCCD 1) and the other transfected with AQP2 (AQP2-RCCD 1). We found that AQP2 interacts physically with TRPV4 and with the K + SK3 channel and that this interaction is crucial for the activation of SK3 by TRPV4, generating hyperpolarization of the plasma membrane and modulating the magnitude of SOCE. On the other hand, using a highly sensitive luminescence

detection system we found that AQP2 is critical for the release of ATP induced by TRPV4activation. This ATP release occurs by an exocytic and a conductive route. ATPe, in turn, stimulates purinergic receptors leading to ATPe-induced ATP release by a Ca 2+ -dependent mechanism. Finally, we found that the addition of ATP eliminates the differences previously reported in the migration of RCCD 1 cells, depending on the expression of AQP2. The increased migration of AQP2-RCCD 1 is likely related to the ability to modulate the levels of Ca 2+ and ATP in microdomains close to the focal adhesions. Elucidating the joint function of AQPs with TRPs channels is essential to improve our understanding of mammalian physiology in health and disease.

CELL DAMAGE AND APOPTOSIS INDUCED BY TRPV4 ACTIVATION IN HUMAN MELANOMA CELLS AND HACAT KERATINOCYTES

Aida Oliván-Viguera[1], Ángel Luis García-Otín[2], Javier Lozano-Gerona[2], Edgar Abarca-Lachen[3], Ana J. García-Malinis[4], Kirk L. Hamilton[5], Yolanda Gilaberte[6], Esther Pueyo[1], Ralf Köhler[7]

[1]Biosignal Interpretation and Computational Simulation (BSICoS), Aragón Institute of Engineering Research (I3A), University of Zaragoza, Zaragoza, Spain, Instituto de Investigación Sanitaria (IIS) Aragón, Zaragoza, Spain, Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 50018-Zaragoza, Spain. [2]Instituto de Investigación Sanitaria (IIS) Aragón, Zaragoza, Spain, Aragón Institute of Health Sciences (IACS), Zaragoza, Spain. [3]Universidad San Jorge, Faculty of Health Sciences, Villanueva de Gállego, Spain. [4]Dept. of Dermatology, Hospital San Jorge, Huesca, Spain. [5]Dept. of Physiology, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand. [6]Dept. of Dermatology, Hospital San Jorge, Huesca, Spain, Dept. of Dermatology, University Hospital Miguel Servet, Zaragoza, Spain. [7]Instituto de Investigación Sanitaria (IIS) Aragón, Zaragoza, Spain, Aragón Institute of Health Sciences (IACS), Zaragoza, Spain, Aragón Agency for Research and Development (ARAID), Zaragoza, Spain.

The transient receptor potential channels subtype 4, TRPV4 channels, are calcium-permeable cation channels that are activated by a broad variety of physicochemical stimuli. TRPV4 channels have been suggested to serve as physiological osmosensors, mechanosensors, thermosensors, and implicated also in epithelial/endothelial barrier functions in several tissues, such as arteries, lungs, kidneys, and skin. TRPV4 are promising drug targets to treat disease, like bladder dysfunction, sepsis, and pulmonary edema caused by heart failure, giving rise to novel selective small molecule modulators, such as the activator, GSK1016790A, and several selective inhibitors, such as HC067047. Whether TRPV4 were also mechanistically implicated in melanoma cell proliferation was not clear. Here, we hypothesized that TRPV4 is expressed in human melanoma and that pharmacological activation interferes with cell proliferation. TRPV4 functions were studied in melanoma cell lines (A375, SK-MEL-28, MKTBR), immortalized non-cancer keratinocytes (HaCaT), and murine 3T3 fibroblasts by patch-clamp, qRT-PCR, optical mapping of intracellular calcium measurements, cell proliferation, and flow cytometric assays of apoptosis and cell cycle. GSK1016790A elicited TRPV4 currents in all cell lines. GSK1016790A-induced currents were blocked by HC067047. TRPV4 mRNA expression was demonstrated by qRT-PCR. In A375 cells, TRPV4 activation was frequently paralleled by co-activation of calcium/calmodulin-regulated KCa3.1 channels. Light microscopy showed that TRPV4-activation produced rapid cellular disarrangement, nuclear densification, and detachment of a large fraction of all melanoma cell lines and HaCaT cells. TRPV4-activation induced apoptosis and drastically inhibited A375 and HaCaT proliferation that could be partially prevented by HC067047. Our study showed that human melanoma cell lines expressed functional TRPV4 channels and that the TRPV4-activator, GSK1016790A, caused a strong calcium-overload and cellular disarrangement, increased the rate of apoptosis, and strongly inhibited cell proliferation/survival. Similarly, GSK1016790A induced apoptosis and impeded proliferation of HaCaT keratinocytes, a spontaneously immortalized aneuploid keratinocyte cell line from human skin. Pharmacological targeting of TRPV4 could be an alternative or adjuvant therapeutic strategy to treat melanoma progression and other proliferative skin disorders.

STRUCTURE-BASED VIRTUAL SCREENING IDENTIFIES NOVOBIOCIN, MONTELUKAST, AND CINNARIZINE AS TRPV1 MODULATORS WITH ANTICONVULSANT ACTIVITY IN-VIVO.

Pedro Martín[1], Manuel Llanos[2], Nicolás Enrique[1], María L. Sbaraglini[2], Federico M. Garofalo[2], Alan Talevi[2], Luciana Gavernet[2]

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Universidad Nacional de La Plata - CICPBA - CONICET, Buenos Aires, Argentina, [2]Laboratorio de Investigación y Desarrollo de Bioactivos (LIDeB) - Universidad Nacional de La Plata, Buenos Aires, Argentina.

Epilepsy is a disease characterized by the recurrent presence of seizures. It affects more than 50 million people worldwide. Pharmacotherapy is the first-line treatment for this pathology. However, approximately 30% of patients do not respond to existing pharmacological therapies. Transient Receptor Potential Vanilloid 1 (TRPV1) is a nonselective cation channel modulated by ligands, pH, temperature, and voltage. It has been proposed as

a promising target to develop novel anticonvulsant drugs (ACDs). However, thermoregulatory side effects associated with channel inhibition have hindered the way toward TRPV1 antagonists becoming marketed drugs. In this work, we conducted a virtual screening (VS) campaign to repurpose TRPV1 inhibitors among approved drugs, which are known to be thermally-neutral. To this end, three homology models of the hTRPV1 were constructed and refined with Rosetta. The experimental structures of the *Rattus norvegicus* channel (rTRPV1) were used as templates (PDB IDs: 5IRZ, 5IRX, and 5IS0 for the apo, fully-open, and closed conformation, respectively). Two different docking programs were evaluated, QuickVina-2 and Autodock4-GPU. All docking complexes were re-scored using three functions, Vina, Vinardo, and AD4_scoring. The performance of the docking protocols was evaluated in terms of sampling power and scoring power, in order to find the best docking mode able to identify compounds interacting with the capsaicin (CAP) binding site. The best protocol was applied in a prospective VS over the DrugBank database, and three hits were selected for biological testing. Novobiocin, Montelukast, and Cinnarizine were evaluated *in vitro* by the patch-clamp technique and *in vivo* on the maximal electroshock seizure (MES), the 6 Hz psychomotor (6 Hz), and pentylenetetrazole (PTZ) mice tests. The interaction between the selected compounds and the molecular target was evaluated by their ability to reduce TRPV1 currents induced by 250 nM CAP using the patch-clamp technique on HEK293 cells expressing the channel. All tested compounds (100 nM) showed inhibitory effects on CAP-induced TRPV1 currents measured at -100 mV (% inhibition: 53,4±7,1 (n=7), 56,3±6,7 (n=9), and 50,4±9,2 (n=7) for Montelukast, Novobiocin, and Cinnarizine, respectively). Finally, their *in vivo* anticonvulsant profile was completed, showing good activity mainly in the MES test. Our results further support the modulation of TRPV1 channels as a promising strategy to develop novel ACDs.

SAIC X SYMPOSIUM *Friday, November 18, 18-20 hr*
LIQUID BIOPSY
Chairs: Hernán García Rivello

LIQUID BIOPSY IN PEDIATRIC TUMORS

Dr. Guillermo Chantada
Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral-CONICET

ONCOLIQ: NEW TECHNOLOGIES FOR CANCER DETECTION BASED ON LIQUID BIOPSIES AND MICRORNAS

Adriana De Siervi
Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, IBYME.
Oncoliq.

Half of people will get cancer at some point in their life. If the cancer diagnosis is late, mortality within 5 years after detection is 11% and if the diagnosis is early, mortality can decrease to 11%. Furthermore, 70% of cancers develop in organs that do not have a routine screening method. Since 2014, our group has been investigating microRNAs (miRNAs), short non-coding RNA molecules that mostly silence the expression of target genes. miRNAs are considered master regulators because a single one can regulate the expression of hundreds of genes. That is why they are considered good biomarkers. Also in 2008 it was discovered that they can circulate in body fluids, like blood. Our group developed an early breast and prostate cancer detection assay called Oncoliq, based on liquid biopsies that detect miRNAs. Both assays have around 90% sensitivity and 70% specificity. We are currently running a pilot study in several institutions (Hospital Posadas, Hospital Bernardo Houssay, Hospital Militar Central and Instituto Alexander Fleming) to establish the positive predictive value of Oncoliq together with conventional early detection methods including gold standard for these cancer types. Additionally, we are enrolling patients with urologic (kidney, bladder) and gynecologic (ovarian, endometrial, cervix) cancers and planning to add new clinical trial protocols to detect up to 50 cancer types with one single blood sample in the future. Moreover, we are also developing an Oncoliq assay for patient management and predict oncological course disease improving patient prognosis. We are convinced that by using Oncoliq we will reduce cancer mortality in the future.

USE OF LIQUID BIOPSY IN EARLY DETECTION AND MINIMAL RESIDUAL DISEASE IN LUNG CANCER

Dr. Christian Rolfo

Associate Director Clinical Research Center of Thoracic Oncology at The Tisch Cancer Institute at Mount Sinai Health System. Presidente de la Sociedad Internacional de Biopsia Líquida (ISLB - International Society of Liquid Biopsy). Nueva York, Estados Unidos.

CIRCULATING TUMOR DNA OF CLASSIC HODGKIN LYMPHOMA

Dr. Davide Rossi

Vicejefe de Servicio. Hematología. Instituto Oncológico de la Suiza Italiana (IOSI - Istituto Oncologico della Svizzera Italiana). Bellinzona, Suiza. Líder de grupo Hematología Experimental en el Instituto de Investigación Oncológica (IOR - Istituto Oncologico di Ricerca). Bellinzona, Suiza.

SAIC XI SYMPOSIUM *Saturday, November 19, 9-11 hr*
HUMAN MICROBIOTA: A NEW SOURCE OF BIOMARKERS
Chairs: Julieta Trinks

SEARCH FOR DIAGNOSTIC AND PROGNOSTIC BIOMARKERS FOR METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD): INTEGRATED ANALYSIS OF THE GUT TRANSCRIPTOME AND METABOLOME IN ARGENTINA

Julieta Trinks

Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) – CONICET - Instituto Universitario del Hospital Italiano (IUHI) - Hospital Italiano de Buenos Aires (HIBA), Ciudad Autónoma de Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

In the last decade, the prevalence of MAFLD has risen around the world in parallel with the changes in obesity, physical inactivity, and type 2 diabetes. In particular, Latin American population has one of the highest prevalence and severity rates of this disease. Although liver biopsy is the "gold standard" for MAFLD diagnosis and prognosis, it remains a costly and invasive procedure with inherent risks. These limitations have driven the search for non-invasive MAFLD screening and risk stratification methods. Consequently, and in order to early prevent liver damage and improve clinical outcomes, the current worldwide challenge is to identify steatohepatitis, as it increases the likelihood of liver disease progression. This seems to be imperative in Latin America, where the high burden of MAFLD reinforces the need for innovative approaches for the practical and effective identification of this disease, particularly for those at high risk of complications, in the context of local health-care environments and available resources. This growing medical need to discover novel non-invasive biomarkers has focused its efforts on gut microbiota research. The human gut microbiota is a diverse microbial ecosystem dominated by bacteria, but also includes commensal populations of fungi, viruses, archaea, and protozoa. Recent advancements in next-generation sequencing and computational technologies have provided opportunities to investigate the genetic material within the gut microbial communities (microbiome) in MAFLD patients. However, there is a lack of reproducible results as each human gut microbiota differs due to enterotypes, body mass index, exercise frequency, lifestyle and cultural and dietary habits. In addition, the gut microbiota varies among ethnicities and thus, it would be of interest to study the presence of specific gut microbiota biomarkers that may contribute to a higher MAFLD prevalence or different disease severity in Latin America, where data is still scarce.

THE ROLE OF THE GUT MICROBIOME IN IMMUNOTHERAPY RESPONSE: LESSONS FROM AN URUGUAYAN COHORT

Riera, Nadia[1], Parada, Andrés[1], Elgul, Nabila[1], Peñalba, Florencia[1], Florez, Valeria[1], Pittini, Álvaro[2], Carlos, Meyer[3], Cawen, María Laura[3], Ferrari, Aracely[3], Laureiro, Elena[3], Alonso, Maria Isabel[3], Malvasio, Silvina[3], Berois, Nora[2], Osinaga, Eduardo[2], Iraola, Gregorio[1,4]

[1]Laboratorio de Genómica Microbiana, Institut Pasteur de Montevideo. [2]Laboratorio de Glicobiología e Inmunología Tumoral, Institut Pasteur de Montevideo. [3]Departamento de Oncología, Centro Asistencial del Sindicato Médico del Uruguay. [4]Wellcome Sanger Institute

Monoclonal antibodies that target immune checkpoints have revolutionized cancer medicine and therapeutics. Drugs targeting the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and the programmed death 1/programmed death ligand 1 (PD-1/PD-L1) axis have transformed the management of a variety of advanced cancers. Recently, a role of the gut microbiome in modulating immunotherapy response has been identified. Human microbiome is highly influenced by environmental factors, the host lifestyle and the geographic region. Efforts to understand the role of the microbiome in cancer immunotherapy on a local scale are needed to incorpo-

rate this knowledge in the clinic. We recruited 25 patients (melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma (HNSCC)) and we aimed to characterize the microbial population before and after starting immunotherapy. Using shotgun metagenomics and culture based tools we profiled the microbial composition of our uruguayan cohort. Using a healthy cohort as a reference (n = 68) we compared the relative abundance of different microbial populations, gene families and metabolic pathways in both cohorts. The microbial composition of the oncologic cohort differs from that of the healthy control. Our results show higher relative abundance of Enterococcus faecium and Klebsiella pneumoniae in the gut of oncologic patients as compared to healthy individuals. In addition, data from several studies previously obtained in other parts of the world were used to identify putative microbial biomarkers associated with immunotherapy response. We found no single microbial feature associated with immunotherapy response. Using culture-based techniques, we began the first Uruguayan microbial biobank from the gut microbiome. We speculate that these results can be used in the future to fuel the development of new clinical interventions.

GUT BACTERIAL METABOLISM AS A MODULATOR OF CARDIOMETABOLIC DISEASE

Federico E. Rey

Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA.

The gut microbiome influences cardiometabolic disease in part by transforming dietary and host-derived substrates into molecules that enter circulation, reaching specific tissues where they act on dedicated host receptors. A major focus of my group is to understand how bacterial metabolism modulates the effects of diet and host's susceptibility to cardiometabolic disease. To address these questions, we use a combination of hypothesis-generating, sequencing-centered analyses of microbiomes from human and mouse populations, followed by proof-of-principle/proof-of-mechanism studies in gnotobiotic mouse models of disease and classic bacteriology experiments. During my talk I will describe efforts to examine causal connections between gut bacteria, diet and cardiometabolic disease, with a focus on our recent and ongoing studies aimed at dissecting the role of bacterial metabolism of dietary choline, flavonoids and purines on host health.

A TWO-TIME POINT ANALYSIS OF GUT MICROBIOTA IN THE GENERAL POPULATION OF BUENOS AIRES AND ITS VARIATION DUE TO PREVENTIVE AND COMPULSORY SOCIAL ISOLATION DURING THE COVID-19 PANDEMIC

Alberto Penas-Steinhardt[1,2,3], Pablo Aguilera[1], María Florencia Mascardi[2,4], Fiorella Sabrina Belforte[1,2,5], Ayelén Daiana Rosso[1,2,5], Sofía Quesada[1,2], Ignacio Llovet[6], Gregorio Iraola[7,8,9], Julieta Trinks[2,4]

[1]Laboratorio de Genómica Computacional (GEC-UNLu), Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [3]Fundación H.A. Barceló, Instituto Universitario de Ciencias de la Salud, Buenos Aires, Argentina. [4]Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB), CONICET, Instituto Universitario del Hospital Italiano (IUHI), Hospital Italiano de Buenos Aires (HIBA), Buenos Aires, Argentina. [5]Departamento de Ciencias Básicas, Instituto de Ecología y Desarrollo Sustentable (INEDES) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-UNLu, Universidad Nacional de Luján, Luján, Argentina. [6]Departamento de Ciencias Sociales, Universidad Nacional de Luján, Luján, Argentina

[7]Microbial Genomics Laboratory, Institut Pasteur de Montevideo, Montevideo, Uruguay. [8]Wellcome Sanger Institute, Hinxton, United Kingdom. 9Center for Integrative Biology, Universidad Mayor, Santiago de Chile, Chile.

The COVID-19 pandemic poses a great challenge to global public health. The extraordinary daily use of household disinfectants and cleaning products, social distancing and the loss of everyday situations that allow contact between individuals, have a direct impact on the transfer of microorganisms within the population. Together, these changes, in addition to those that occur in eating habits, can affect the composition and diversity of the gut microbiota. We carried out a two-time point analysis of the fecal microbiota of 23 Metropolitan Buenos Aires (BA) inhabitants to compare pre-pandemic data and its variation during preventive and compulsory social isolation (PCSI) in 2020. To this end, 23 healthy subjects, who were previously studied by our group in 2016, were recruited for a second time during the COVID-19 pandemic, and stool samples were collected from each subject at each time point (n = 46). The hypervariable region V3-V4 of the 16S rRNA gene was high-throughput sequenced. We found significant differences in the estimated number of observed features ($p < 0.001$), Shannon entropy index ($p = 0.026$) and in Faith phylogenetic diversity ($p < 0.001$) between pre-pandemic group (PPG) vs. pandemic group (PG), being significantly lower in the PG. Although no strong change was observed in the core microbiota between the groups in this study, a significant decrease was observed during PCSI in the phylum Verrucomicrobia, which contributes to intestinal health and glucose homeostasis. Microbial community structure (beta diversity) was also compared between PPG and PG. The differences observed in the microbiota structure by unweighted UniFrac PCoA could be explained by six differential abundant genera that were absent during PCSI.

Furthermore, putative functional genes prediction using PICRUSt infers a smaller predicted prevalence of genes in the intestinal tryptophan, glycine-betaine, taurine, benzoate degradation, as well as in the synthesis of vitamin B12 during PCSI. This data supports the hypothesis that the microbiome of the inhabitants of BA changed in the context of isolation during PCSI. Therefore, these results could increase the knowledge necessary to propose strategic nutraceutical, functional food, probiotics or similar interventions that contribute to improving public health in the post-pandemic era.

MICROBIOME, METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD)/ NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND CARDIOVASCULAR RISK

Mario Reis Alvares-da-Silva
Universidade Federal do Rio Grande do Sul, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil

As the liver is the first natural target of the blood arising from the bowels, it is quite understandable that gut microbiome is associated with several liver injury mechanisms, which include translocation of viable bacteria and PAMPs as well as bile acids' abnormalities and systemic inflammation, all of them driving the hepatic injury. Non-alcoholic fatty liver disease (NAFLD), recently renamed as metabolic associated fatty liver disease (MAFLD), has been related to gut dysbiosis in the last years. Liver steatosis and atherosclerotic cardiovascular disease have similar pathogenetic features, such as chronic inflammation, oxidative stress, angiogenesis, lipid profile, paracrine signalization, as well as hepatokine and adipokine disturbances, and genetic polymorphisms of risk related to genes as PNPLA3, TM6SF2 and MBOAT7. Moreover, studies in NAFLD population consistently demonstrated that cardiovascular disease (CVD) is its main cause of death, depending on the severity of liver fibrosis. Recently, some studies showed that cardiovascular risk (CVR) and CVD mortality is higher in MAFLD than in NAFLD patients. Altered gut microbiota is another important NAFLD/MAFLD and CVD link to be considered. So, the relationship between microbiome and CVR in MAFLD/NAFLD experimental models and humans will be addressed, emphasizing cardiomyocytes' abnormalities, CVR screening and available and future therapeutic measures.

SAI IX SYMPOSIUM *Saturday, November 19, 9-11 hr*
ARGENTINIAN RESEARCHERS AGAINST COVID-19
Chair: Ana Ceballos - Marisa Fernández

DISTINCT IMMUNE SIGNATURES DISCRIMINATE SARS-CoV-2 VACCINE COMBINATIONS

Mariana Maccioni
Departamento de Bioquímica Clínica. CIBICI-CONICET, Facultad de Ciencias Químicas- Universidad Nacional de Córdoba, Argentina.

Several vaccines have been found effective against COVID-19, usually administered in homologous regimens, with the same vaccine used for the prime and boost doses. However, recent studies have demonstrated improved protection via heterologous mix-and-match COVID-19 vaccine combinations, and a direct comparison among these regimens is needed to identify the best employment strategies. Here, we show a single-cohort comparison of changes to the humoral and cellular immune compartments following five different COVID-19 vaccines spanning three technologies (adenoviral, mRNA and inactivated vaccines). These vaccines were administered in a combinatorial fashion, resulting in sixteen different homologous and heterologous regimens. SARS-CoV-2-targeting antibody titres were highest when the boost dose consisted of mRNA-1273, independent of the vaccine used for priming. Priming with BBIBP-CorV induced less class-switching among spike-binding memory B cells and the highest antigen-specific T cell responses in heterologous combinations. These were generally more immunogenic in terms of specific antibodies and cellular responses compared to homologous regimens. Finally, single-cell analysis of 754 samples revealed specific B and T cell signatures of the vaccination regimens, indicating distinctive differences in the immune responses. These data provide new insights on the immunological effects of COVID-19 vaccine combinations and a framework for the design of improved vaccination strategies for other pathogens and cancer.

ARVAC CECILIA GRIERSON VACCINE: AN ADAPTED RECOMBINANT BOOSTER VACCINE AGAINST SARS-COV-2 THAT CAN BE PRODUCED IN ARGENTINA

Juliana Cassataro

Instituto de Investigaciones Biotecnológicas, Escuela de Bio y Nanotecnologías- EByN- Universidad Nacional de San Martin, Buenos Aires, Argentina.

Our group has been working on the development of adjuvants for vaccines against infectious diseases. When pandemic started, in May 2020 we focused on the development of a vaccine against SARS-CoV-2 that can be produced in Argentina, can be adapted for new emerging variants of concern (VOC) of the SARS-CoV-2 virus and that can be used as primary or booster vaccine. The project is currently being developed in Argentina by our group in the University of San Martin together with the Pablo Cassará Foundation and the pharmaceutical company Cassará. To date we have performed preclinical studies in different animal species to evaluate the toxicity and immunogenicity of the developed vaccine. The vaccine formulation elicited high levels of neutralizing antibodies of the virus and induced a specific T cell response in line with the current requirements for vaccines against COVID19. Vaccine-induced antibodies can neutralize different VOCs. In addition, in an animal model of severe disease, the vaccine induced protection against the experimental challenge with SARS-CoV-2. Preclinical toxicological studies of the prototype vaccine have been completed in December 2021. In March 2022 we received the regulatory approval to start a phase I clinical trial in 80 vaccinated individuals. Preliminary results of this ongoing phase I study showed that the vaccine was safe and was able to significantly boost the neutralizing antibody response against different VOCs including Omicron irrespective of the primary vaccination platform of the study participants. Moreover, ARVAC was able to boost antigen specific IgG and IFN-γ cellular immune responses in volunteers that have different primary vaccine platforms. Based on these results we are preparing to start a phase II/III trial.

COVID-19 IN CHILDREN: ANTIBODY RESPONSE INDUCED BY INFECTION AND VACCINATION, HYBRID IMMUNITY AND LONG-TERM SYMPTOMS

Lourdes Arruvito

Institute for Biomedical Research in Retroviruses and AIDS (INBIRS) School of Medicine, UBA-CONICET, CABA, Argentina.

Children and youth infected with SARS-CoV-2 frequently show an asymptomatic or mild disease contrasting with other viral respiratory infections which can induce a severe disease. It has been suggested that children develop an earlier and more robust innate immune response upon SARS-CoV-2 infection compared with adults. The possible contribution of a more efficient adaptive immune response to the favorable outcome of pediatric COVID-19 has not yet been determined**.** Here, we show that in response to SARS-CoV-2 infection, children developed a higher and more sustained antibody response compared with adults. When measured at 7-17 months after infection, both the titers of IgG antibodies directed to the spike protein of SARS-CoV-2, and the plasma neutralizing activity against the Wuhan original variant and the VOCs Delta and Omicron were higher in children compared with adults. We also found that vaccination with two-doses of the inactivated BBIBP-CorV vaccine or the mRNA vaccines BNT162b2 and mRNA-1273 further increased the plasma neutralizing activity against Delta and Omicron in previously infected children. The special signature of the antibody response in children upon SARS-CoV-2 infection may provide some degree of immune advantage compared to adults in terms of adaptive immunity. This observation together with the ability of the current anti-SARS-CoV2 vaccines to further enhance the antibody response against VOCs including Delta and Omicron in previously infected children, may inform vaccination strategies in the pediatric population. While long COVID is widely recognized in adults, its existence in children is more controversial. We also studied the long-term symptoms and associated risk factors for persistent symptoms beyond 3 months of COVID-19 in pediatric population. Almost 30% of children that suffered COVID-19 reported at least one persistent symptom. Compared with the control group, SARS-CoV-2 infection in children increased 3 to 6 folds the risk of having headache, dizziness, loss of taste, dyspnea, cough, fatigue, muscle pain and loss of weight. Loss of smell was only reported in infected children. Finally, older age, symptomatic COVID-19 and comorbidities were predictor variables significantly associated with a higher risk of developing long-term symptoms. Pediatric long COVID is a new condition that requires urgent public health policies.

SAIC XII SYMPOSIUM *Saturday, November 19, 11-13 hr*
TRANSLATIONAL RESEARCH IN THE AREAS OF HUMAN HEALTH
CHAIR: RODOLFO REY

THE TRADEOFFS BETWEEN CLINICAL AND LABORATORY BIOMEDICAL RESEARCH

Oscar Bottasso1
1Instituto de Inmunología Clínica y Experimental de Rosario (IDICER, UNR-CONICET)

The need to achieve a greater understanding of the diverse array of medical problems imposes a kind of conducting force capable of pushing this continuously shifting exploration border towards areas of greater confidence. The science and the medical practice digging their backgrounds through hypotheses, experimental verifications, and subsequent interpretations are presently getting the input of Translational Medicine viewed as a goal of biomedical research aimed at offering better control tools. Something that is closely linked to the origins of the very medicine. The transition between basic and clinical research is complex requiring appropriate crosstalk helpful enough for the input/output relationship to become fruitful. Partly because the in vitro experiments and preclinical studies do not necessarily mirror the clinical situation to sensibly translate
the "truth" emerging from laboratory data. This is particularly relevant when trying to develop new biomarkers, potentially predictive scoring systems, biostatistical procedures for combined endpoints, and mostly, the efforts towards the availability of novel therapeutic approaches. Regardless of the precise situation, intermingled communication between players from different fields is necessary, if we are about to establish new conceptual and methodological frameworks likely to facilitate a better theoretical/practical approach. To some extent, it constitutes a sort of burst of the biomedical sciences into the clinical scenario aimed at validating the knowledge arising in the laboratory to become part of some clinical practice or guidelines for health policies, with all the implications therein. Conversely, many clinical observations raise questions that can be elucidated from the experimental ground, as a valuable way of affording some feedback on this interactive process. The way ahead appears as long as challenging, given that the bulk of the investigative work carried out in our setting has been mostly performed in self-confined compartments (biomedical, clinical, and public health). Purportedly, we have lastly realized that field-specific speeches concentrated on themselves end up hindering the portrayal of problems in their different facets, as well as the way of formulating investigative designs addressed to provide improved alternatives.

EARLY DETECTION OF THE MOSQUITO AEDES AEGYPTI: CORNERSTONE OF DENGUE PREVENTION IN THE CENTRE OF BUENOS AIRES PROVINCE.

Darío Vezzani
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto Multidisciplinario sobre Ecosistemas y Desarrollo Sustentable, UNCPBA-CICPBA, Tandil, Provincia de Buenos Aires, Argentina; dvezzani@gmail.com

The main vector of dengue, the mosquito Aedes aegypti, has been expanding in Argentina since its reintroduction in the north of the country in 1986. During the 1990's it reached Greater Buenos Aires and from there a slow and constant dispersion to the centre and south of the province has been observed. Currently, established populations of Ae. aegypti have been reported in some localities of the region as Olavarría, Tandil and Bahía Blanca, but for many others there is no available information and lack adequate vector surveillance for its detection. In addition, there are some isolated records in northern Patagonia at Neuquén and Río Negro provinces. Like the mosquito vector, dengue burden has increased in our national territory, both in intensity and geographic extension. The number of localities with autochthonous dengue tripled from 167 in the first national outbreak (with 27,000 cases in 2009) to 496 localities during the most recent one (60,000 cases in 2020). In association with the increase of positive localities and reported cases, a shift of the disease transmission limit towards the south has been recorded, from Greater Buenos Aires to the locality of Saladillo in the centre of the province. Besides the mentioned dengue expansion, there has been a marked increase in the co-circulation of different serotypes (DEN1 in 2009, DEN1-4 in 2016, DEN1-2-4 in 2020), worsening the epidemiological situation. During non-epidemic years, in which alerts and prevention actions are relaxed, there is also transmission in some regions of the country. For example, during the last two years a total of 2,300 autochthonous cases were registered in 12 provinces. Unfortunately, these numbers seem to be insufficient to the media interest and to worry health authorities. In the search for dengue prevention, updated data about Ae. aegypti is a key piece to increase the awareness of the local health system and to make this threat visible in the limits of the vector distribution. In other words, in localities without previous dengue experience, the result of the entomological surveillance is a cornerstone to produce behavioral changes in the responsible authorities as well as in the community as a whole.

TRANSLATIONAL MEDICINE IN ARGENTINA: PENDING SUBJECTS

Jorge Geffner
Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS). Facultad de Medicina. UBA. CONICET.

The SARS-CoV-2 pandemic posed enormous challenges to the scientific community as a whole. Thousands of researchers had to rethink their activities in order to respond to the urgent needs imposed by the pandemic in various fields: the diagnosis and treatment of infected patients, the local manufacture of diagnostic kits, an outstanding participation in clinical trials that validated the use of different vaccines, the local development of new vaccines, the study of the social problems associated with the pandemic, the participation in social networks and the media aimed at providing truthful and rigorous information on the application of individual and social protection measures as well as on the reliability of the different vaccines approved in different international and national scenarios, among many other activities. Yet, a rigorous balance should be made on what has been done by the scientific community in the face of a pandemic that continues to threaten us. However, to a first approximation, I think we have given a satisfactory response. I also believe that we should take advantage of this experience in order to rethink the best way to build an effective bridge between basic research and healthcare medicine, an issue that has been discussed so often and never addressed with due force, rigor and conviction. Advancing in this direction requires, in a first stage, the adoption of a set of measures aimed, among other things, at avoiding moonlighting in health personnel and researchers driven by really very poor salaries, setting up research groups/centers in large national and provincial hospitals, define priority areas of research in line with national and regional needs, encouraging research projects that address central issues in the field of translational research and create particular areas where researchers and health personnel can share ideas and projects. Now is the right time to move in this direction.

CHALLENGES FOR IMPLEMENTATION SCIENCE IN LOW RESOURCE SETTINGS

Esandi ME[1,2], Ortiz Z[1,3]
[1]Instituto de Investigaciones Epidemiológicas, Academia Nacional de Medicina de Buenos Aires. [2]Departamento de Economía, Universidad Nacional del Sur. [3]Cátedra de Salud Pública, Universidad Nacional de Cuyo

Dissemination and Implementation Science (D&I-Sc) is a relatively novel research field that proposes the scientific study of methods to promote the systematic uptake of research findings and other evidence-based practices into routine practice. This research is mainly characterized for being embedded in healthcare organizations and community settings and thus, it is strongly influenced by context. By using a systemic thinking, D&I-Sc addresses the implementation gap through a rigorous, multidisciplinary and participatory approach. In the context of low-resource settings, this gap deepens, what it is traduced in unmet population needs, a wide variation in the use of evidence-based treatments in routine practice, low quality of care and thus, a high burden of disease. D&I-Sc can contribute to the reduction of this implementation gap; even when its development in high-income countries is well-stablished, conducting this type of research in low- and middle-income countries faces many challenges. Recently, it was highlighted the need of rigorous and systematic documented examples of implementation research in these settings as a valuable resource for building research capacity. We present different D&I-Sc case- studies in Argentina upon implementation research, including one that was performed during the COVID-19 pandemic. Key components of conducting implementation research in these settings, including how to select, adapt, and apply implementation science models, theories, and frameworks to these settings; develop and test implementation strategies; and evaluate implementation processes and outcomes are described. Challenges and lessons learned throughout these experiences are presented.

SAI X SYMPOSIUM *Saturday, November 19, 11-13 hr*
CLINIC IMMUNOLOGY: PRIMARY PEDIATRIC IMMUNODEFICIENCIES
Chair: Liliana Berodznik - Gabriela Mora

IFRS AND PREDISPOSITION TO INTRACELLULAR PATHOGENS

Jacinta Bustamante
Paris Cité University, Imagine Institut. Paris, France.

The discovery of inborn errors of immunity (IEI) underlying severe infectious diseases delineates the essential vs. redundant functions of the corresponding human genes in host defense *in natura,* while clarifying the pathogenesis of these infections. Inborn errors of human IFN-γ immunity underlie mycobacterial diseases, while inborn errors of IFN-α/β immunity underlie viral diseases. Both IFNs induce the transcription factor IRF1, the

role of which in either pathway is unknown. We recently identified two unrelated children with inherited complete IRF1 deficiency and early-onset, multiple, life-threatening diseases caused by weakly virulent mycobacteria. They have no history of severe viral disease, despite exposure to a great many viruses, including SARS-CoV-2 that is life-threatening in individuals with even slightly deficient type I IFN. In fibroblasts, we find a much greater number of IRF1-dependent target genes in response to IFN-γ than IFN-α/β. The patient's fibroblasts, monocytes derived macrophages, or iPSC-derived macrophages do not upregulate the numerous IRF1-dependent target genes in response to IFN-γ. In contrast, cell-intrinsic IFN-α/β immunity to a wide range of viruses, including HIV and SARS-CoV-2, is maintained. Human IRF1 is thus largely redundant for antiviral IFN-α/β immunity. By contrast, human IRF1 is essential for IFN-γ immunity to mycobacteria in mononuclear myeloid cells.

NEW INBORN ERRORS OF IMMUNITY DUE TO RELOPATHIES, CASE REPORT

Gisela Seminario
Hospital de Niños, Gutierrez. CABA, Argentina.

Introduction: Inborn Errors of Immunity (IEI), are caused by germline variants in single genes. IEI present increased susceptibility to infections. *Staphylococcus aureus* (sa)is a major bacterial pathogen with a global impact on human health. Inherited or acquired disorders affecting innate immunity predispose to sa diseases but account for only a small proportion of cases. Most cases of severe staphylococcal disease remain unexplained. Objectives: Report clinical case of a 71 years old (yo)man, with history of severe lung and skin infection. Results: He present history of recurrent otitis media in childhood. From his teens, he presented several episodes of skin abscesses, requiring hospitalization, intravenous antibiotics, and surgical treatment for resolution with necrosis of the affected tissues and suffered septic shock due to Staphylococcus aureus. Subsequently had elbow bursitis and necrotizing cellulitis. In adulthood, he presented at 47 yo Pneumonia, the next year suffered cellulitis in the pubic region and abdomen with bad evolution and involvement of the left thigh and at 63 yo severe pneumonia and pneumococcal bacteriemia. Because his infectious background Immunology consult was done with absence of manifest immunological compromise. We continued his study with J.L. Casanova and heterozygous variant in *OTULIN* gene was found. We could demonstrate that haploinsufficiency of *OTULIN* leads to cellular accumulation of M1-UB and affects the ubiquitination status of caveolin-1 in dermal fibroblasts. Causing an intrinsic susceptibility of non-hemopoietic cells to the main virulence factor of sa. Naturally neutralizing antibodies to toxin α rescue haploinsufficiency of *OTULIN* and thus contribute to incomplete penetrance. Conclusion: Serious staphylococcal diseases, due to new PID. OTULIN haploinsufficiency, previously genetically unrecognized IEI of severe sa disease. 1st episode of illness, during adolescence. Necrosis is a characteristic of the disease in patients. Clinically, it is expressed in a tissue-specific manner because the skin and/or lungs are the affected organs in all patients.

FUNCTIONAL STUDIES IN NOVEL MUTATION EMERGED BY NGS IN PATIENTS WITH INBORN ERRORS OF IMMUNITY IN ARGENTINA

Belen Almejum
IQUIBICEN Instituto de Quimica Biologica de la Facultad de Ciencias Exactas y Naturales -CONICET, CABA, Argentina.

Inborn Errors of Immunity (IEI) are a heterogeneous immune disease with alteration in different components of the immune system. Next Generation Sequencing (NGS) has revolutionized the diagnosis of genetic diseases making it possible to improve diagnostic possibilities in patients with IEI, especially in those cases where the clinical presentation is complex, and the candidate gene strategy has not been successful. We have evaluated different novel mutations emerged by NGS in IEI patients in Argentina through functional studies. We have evaluated a novel heterozygous variant R129P in STX11 in a pediatric patient diagnosed with Evans syndrome. We observed a reduction of NK-cells and T-cells functionality in the R129P-cells and a lesser protein expression. TLR4 re-localization was impaired in the patient's monocytes. Structural analysis showed an impact in helix stability and in the protein-protein interaction. We demonstrated that the novel R129P-STX11-mutation can play a pathogenic role. This novel mutation may explain the clinical patient Evans Syndrome phenotype. We assessed novel variants in CARD11 by functional analysis using JPM50.6, Jurkat and HEK293T cells transfected with a wild type CARD11 construct and/or with mutated constructs. Genetic defects in CARD11 can present as a loss of function (LOF) or contrarily, a gain of function (GOF) of the affected gene product. A total of 6 novel heterozygous CARD11 variants were identified. Two variants resulted in LOF/Dominant Negative by ex vivo evaluation, defining them as causative of CARD11-associated atopy with dominant interference of NF-kB signaling (CADINS). Two variants showed a GOF effect as well a spontaneous aggregation in the cytoplasm, leading to B cell expansion with NF-κB and T cell anergy (BENTA) diagnosis. The remaining variants showed neutral functional assays. Our results, underlines the importance of studying novel mutations, not only for the early and accurate diagnosis of IEI patients, but also to understand a protein function.

GENETIC PREDISPOSITION TO EARLY-ONSET AUTOIMMUNE DISEASES HIGHLIGHTS KEY PATHWAYS CONTROLING IMMUNE TOLERANCE IN HUMANS

Frederic Rieux Laucat
INSERM-UMR1163, Université Paris Cité, Imagine Institut. Paris, France.

Primary Immunodeficiencies (PIDs) gather more than 500 rare monogenic diseases affecting the development, the function, or the regulation of the immune response. From a register of >6000 PID patients, our center showed that autoimmunity or inflammation occurs in more than 25% of PID cases. A combination of autoimmune, inflammatory, lymphoproliferative, and infectious manifestations characterizes a group of rare inborn errors of immunity that we summarize as autoimmune-lymphoproliferative primary immunodeficiencies (AL-PID). AL-PID is characterized by repeated hospitalizations and reduced life expectancy. Moreover, a given monogenic cause could be associated with variable expressions ranging from no symptom (non-penetrance) to a broad spectrum of severe and debilitating manifestations. Using Whole exome sequencing, in-depth Immune phenotyping, and single cell RNAseq approaches, combined with classical in vitro functional approaches, we and others have defined the genetic landscape of AL-PID, the underlying pathophysiological mechanisms, paving the way to targeted treatments.

SAI XI SYMPOSIUM *Saturday, November 19, 14-16 hr*
TRASLATIONAL MEDICINE IN THE AREA OF HUMAN IMMUNOLOGY
Chairs: Guillermo Docena - Guillermo Giambartolomei

CONTROL OF T CELL EXCLUSION BY CANCER-ASSOCIATED FIBROBLASTS IN HUMAN LUNG TUMORS

Hélène Salmon
Inserm U932, Institut Curie (Paris), France.

T cell exclusion from the tumor bed can restrict response to immune checkpoint blockade, yet the cellular and molecular mechanisms underlying T cell marginalization in solid tumors are poorly understood. I will discuss our recent findings pairing molecular and spatial analysis to study how cancer-associated fibroblasts (CAF) contribute to T-cell localization in the tumor microenvironment of human non-small cell lung carcinoma. We identified four main CAF populations, of which two are associated with T cell exclusion: (i) MYH11+ CAF, which are present in early-stage tumors and form a single-cell layer lining cancer aggregates, and (ii) FAP+aSMA+ CAF, which appear in more advanced tumors and organize in patches within the stroma or in multiple layers around tumor nests. Both populations orchestrate a particular structural tissue organization through dense and aligned fiber deposition compared to T cell permissive CAF. Yet they produce distinct matrix molecules, including collagen IV (MYH11+ CAF) and collagen XI/XII (FAP+aSMA+ CAF). Importantly, our study characterizes intra-tumor organization, revealing molecular factors behind inter-tumor CAF and T cell heterogeneity.

MASTOCYTOSIS A RARE DISEASE THAT SHED LIGHT ON THE PHYSIOPATHOLOGY OF CANCER, IMMUNE RESPONSE, INFLAMMATION AND NEURODEGENERATIVE DISEASES IN HUMAN AND VETERINARIAN MEDICINE

Olivier Hermine
Laboratory of physiopathology of hematological disorders and therapeutic implications
National reference center of mastocytosis. Department of Adult Hematology
Imagine institute, INSERM-UMR1163, Université Paris Cité, Paris, France

Mastocytosis is a rare disease characterized by the accumulation of mast cell in various tissues. It is an heterogenous disease with indolent or aggressive features, associated with various skin, GI tract, cardiovascular, neurological, and psychiatric symptoms due to mast cell activation and/or organ failure due to invasion and tissue destruction particularly the liver and the bone marrow. It may occur early in life and in this case may regress spontaneously at teenagerhood or alternatively may start in adult but in this case does not regress. It is also the most frequent cutaneous neoplastic disorders in dogs. At the molecular level the disease is associated to *KIT* mutations mainly in extracellular and in the kinase domains in pediatric forms and in adult forms, respectively. The regression of the disease might be associated to oncogenic senescence occurring in the case of extracellular domain *KIT* mutations. Aggressiveness in adult is associated to additional mutations involving epigenetic regulators associated to hematological malignancies, which may explain the frequent association of mastocytosis with myelodysplastic and myeloproliferative disorders. These findings have led to the development of treatment with kinase inhibitors in dogs and humans with some significant successes both in indolent and aggressive forms

of the disease.
Symptoms of mastocytosis may occur also without accumulation of abnormal mast cells in what it is called Mast cell activation syndrome. Mast cell activation may be idiopathic or associated with various diseases including developmental and inflammatory disorders, neurodegenerative, psychiatric and sickle cell diseases among others. This observation led to the discover of new pathway involved in mast cell activation and their unexpected roles in some of these disorders suggesting their broad activities beyond allergic reactions and to the use of kinase inhibitors in these diseases.

AZITHROMYCIN PROMOTES RELAPSE BY DISRUPTING IMMUNE AND METABOLIC NETWORKS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

David Michonneau
INSERM U976, Hôpital Saint-Louis. Paris, France.

Azithromycin (AZM) is a second-generation macrolide. In the context of allogeneic hematopoietic stem cell transplant (allo-HSCT), AZM was blindly compared to a placebo to prevent chronic pulmonary graft-versus-host disease in the ALLOZITHRO study. Unexpectedly, azithromycin was associated with higher risk of relapse of the initial disease (hazard ratio=1.7, p=0.002). We hypothesize that AZM could inhibit anti-tumor immune response following allo-HSCT. The aim of this study was to determine if AZM may favor relapse by a direct inhibitory effect on immune response or indirect through modifications of circulating metabolites or microbiota specific skewing. We studied 240 blood samples from patients included in ALLOZITHRO trial. Mass cytometry allowed to study 55 immune subsets. We found that AZM treated patients were associated with (i) lower frequency of T cells, (ii) skewed CD4+ differentiation toward Th2 cells, (iii) higher T regulatory cells, (iv) higher CD8+ T cells. T cells from patients treated with AZM exhibited exhausted profiles characterized by the expression of Tox, PD1 and TIGIT. Plasma and cells metabolomic revealed changes in metabolites involved in lipid and cell energy metabolism pathways. Multi-omics integration highlighted that Th2 and cell energy metabolism were also associated with relapse. Single cell transcriptomic analysis (scRNA-Seq) confirmed enrichment in metabolism pathways, glycolysis and mitochondrial respiration metabolism. In vitro, AZM dampened T cells proliferation, anti-tumor cytotoxicity and synthesis of pro-inflammatory cytokines. This loss of function was associated with the inhibition of TCR signaling and of glycolysis following T cells activation. Two days following the activation, scRNA-Seq on T cells revealed a down-regulation of pro inflammatory and mitochondrial genes whereas genes involved in inhibition of immune response were up-regulated including SOCS1. We next confirmed that the downregulation of mitochondrial genes was associated with mitochondrial respiration chain loss of function in activated and resting T cells. Altogether these results show that AZM directly dampens anti-tumor immune response by inhibiting T cells energy metabolism. This effect might also be reinforced through microbiota and metabolome changes associated with AZM intake.

THE MICA-NKG2D AXIS AND THE VALIDATION OF A NOVEL TARGET IN IMMUNO-ONCOLOGY

Norberto Zwirner
Laboratorio de Fisiopatología de la Inmunidad Innata Instituto de Biología y Medicina Experimental (IBYME-CONICET). Argentina.

NK cells are currently at the forefront in immuno-oncology. To exploit their antitumor potential, several monoclonal antibodies (mAb) that target immune checkpoints on NK cells are under investigation, while other mAb that promote ADCC (antibody-dependent cell-mediated cytotoxicity) constitute the current standard of care for some cancer patients. NK cells express NKG2D, an activating receptor that promotes NK cell effector functions and tumor elimination through recognition of several ligands (NKG2DLs) such as MICA, MICB and the ULBPs 1 to 6. Although NKG2DLs are overexpressed on a wide variety of tumors (making them attractive targets for immunotherapy modalities), tumors can shed NKG2DLs and such soluble forms can subvert the NKG2D-NKG2DL axis and mediate tumor-immune escape. To validate MICA as a novel target in immuno-oncology, we explored whether deliberately induced anti-MICA (Ab) elicited by vaccination with a chimeric protein harboring the ectodomain of MICA can promote the restoration of antitumor immunity. Indeed, such Ab delayed the growth of MICA-expressing tumors, and their mechanism of action involved the induction of ADCC by NK cells and scavenging of sMICA, which resulted in a remodeling of the tumor microenvironment. Also, studies in cell renal cell carcinoma patients demonstrated that MICA is overexpressed in this type of tumor and associated with diminished overall survival. Expression of MICA was detected on tumor cells, including cancer stem cells, and tumor-infiltrating leukocytes, but not on peripheral blood lymphoid cells. Also, tumor-infiltrating NK cells exhibited impaired effector functions, features of exhaustion and an altered metabolic fitness. Thus, expression in primary tumor cells and druggability with Ab validate MICA as a leading-edge target for innovative immunotherapy strategies based on the use of anti-MICA mAb that may reinstate antitumor immunity unleashing NK cell effector functions to treat a wide range of cancer patients.

SAFIS VI SYMPOSIUM *Saturday, November 19, 16-18 hr*
USE OF GENE TRANSFER AS A THERAPEUTIC STRATEGY.
Chairs: Julieta Marrone - Alberto Crottogini.

EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS: DELIVERY OF THERAPEUTICS GENES FOR LIVER CIRRHOSIS

Esteban Fiore[1], Luciana M. Domínguez[1]; Milagros Albornoz[1]; Juan Bayo[1]; Ma. José Cantero[1]; Bárbara Bueloni[1]; Catalina Atorrasagasti[1]; Mariana García[1]; Gustavo Yannarelli[2]; Guillermo Mazzolini[1]

[1]Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral - CONICET, Buenos Aires, Argentina. [2]Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro-CONICET, Buenos Aires, Argentina

Liver cirrhosis involves chronic damage, wound healing and fibrogenic processes. Mesenchymal stem cells (MSC)-derived extracellular vesicles (EVs) are an interesting therapeutic option for regenerative medicine. We previously demonstrated that EVs mediates the therapeutic effect of human umbilical cord perivascular cells (a type of MSC) over-expressing IGF1 (AdIGF-I-HUCPVC) on liver fibrosis. Our aim is to establish a new tool employing EVs derived from MSCs to deliver therapeutic factors for liver fibrosis therapy. First, we compared a scalable method by ion exchange chromatography to isolate EVs from HUCPVC conditioned media with classic ultracentrifugation method. By the chromatograph, EVs was collected in 3 elution fractions, showed typical and homogeneous morphology, and CD9, CD63 and CD81 markers expression. Second, we evaluated the therapeutic potential on liver fibrosis of EVs derived from different clinically relevant sources of MSC; adipose tissue (ASC-EVs), HUCPVC-EVs and induced pluripotent stem cells-derived MSC (iMSC-EV). *In vitro* EVs antifibrotic effect was tested on hepatic stellate cells (HSC) line. After incubation, EVs from different MSC sources down-regulated pro-fibrogenic genes Col1a2 and α-SMA expression in similar levels. Then, the therapeutic potential of EVs was compared on experimental mice model of liver fibrosis (thioacetamide for 8 weeks in BALB/c mice). On week 6, ASC-EV, iMSC-EV and HUCPVC-EV were i.v. injected every 5 days for a total of 3 doses. At week 8, animals were sacrificed, and liver samples analyzed. The treatment with the three different EVs decreased collagen deposit and αSMA levels in liver tissue. In addition, EVs from different sources induce the hepatocellular proliferation compared with vehicle. Third, we confirmed by ELISA that chromatograph isolated' EVs derived from AdIGF-I-HUCPVC transport IGF1. Incubation of HSCs with EV-IGF1 resulted in downregulation of Col1a2 and αSMA expression demonstrating a reduction of its activation status. Finally, *in vivo* treatment with EV-IGF1 resulted in a further amelioration of collagen deposition and αSMA levels in liver tissue in comparison with controls. Consistently, an increase of PCNA+ cells after EV-IGF1 application show the induction of liver regeneration. Conclusion: EVs derived from HUCPVCs load and transport therapeutic factors, enhancing its anti-fibrotic and pro-regenerative potential. The scalable chromatographic method retained the therapeutics potential of engineered EV, emerging as an alternative for the treatment of liver disease.

GENE THERAPY WITH THE YAMANAKA GENES IN THE HIPPOCAMPUS OF SENILE RATS TO RESTORE COGNITIVE FUNCTION

Martina Canatelli Mallat

Instituto de Investigaciones Bioquímicas de la Plata "Prof. Dr. Rodolfo R. Brenner", Universidad Nacional de La Plata-CONICET, Argentina.

Impaired performance in spatial learning and memory during aging in rats is associated with morphological and molecular changes in the brain, particularly in the hippocampus. In this study, we assessed the cognitive performance of young (4 mo.) untreated rats and old (27 mo.) treated and control rats. Treatment was carried out by intracerebroventricular administration of an adenovector constructed in our laboratory that carries the green fluorescent protein (GFP) reporter gene as well as a gene tandem, termed STEMCCA, which harbors the 4 Yamanaka genes (*oct4*, *sox2*, *klf4*, and *c-myc*), both under the control of a Tet-Off bidirectional promoter. Control rats received an adenovector that only carries the gene for GFP. Learning and spatial memory performance were assessed by means of the Barnes maze test, which consists of a circular platform with twenty holes around the periphery. Only one hole is connected to a removable escape box (hole 0). This hole is in a fixed position relative to visual cues. The test involves three days of acquisition trials, in which rats are expected to learn the location of the escape box, followed by a probe trial, in which the escape box is removed. To measure learning we assessed latency and errors to escape box. To assess consolidation, we measured the permanence of animals in goal sector 1 (hole 0) and goal sector 3 (holes -1, 0, and 1) when the escape box was removed. We observed that the learning performance of the treated rats was significantly improved compared to that of the untreated counterparts. However, no significant improvement in spatial memory retention was observed in the treated rats versus controls.

USE OF ADENO-ASSOCIATED VIRAL VECTORS FOR SILENCING AND OVEREXPRESSION OF THE CARDIAC ELECTROGENIC BICARBONATE SODIUM COTRANSPORTER

Romina Di Mattia

Centro de Investigaciones Cardiovasculares "Dr. Horacio E. Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.

Cardiac cells depend on specific sarcolemmal ion transporters to assure the correct intracellular pH regulation. Two alkalinizing mechanisms coexist in cardiac myocytes: sodium/bicarbonate cotransporter (electroneutral isoform NBCn1 and electrogenic isoform NBCe1) and sodium/proton exchanger (NHE1). NBCe1 generates an anionic repolarizing current that modulates the action potential duration (APD). In addition to regulating the pH, the NBC is a source of sodium influx. It has been postulated that NBC could play a role in the development of hypertrophy. The aim of this research was to study the contribution of NBCe1 in heart electrophysiology and in the development of heart hypertrophy in an *in vivo* model. We used recombinant cardiotropic adenoassociated virus (AAV9) to achieve NBCe1 overexpression (AAV9-NBCe1) and NBCe1 silencing (AAV9-shNBCe1) in mice and rats, respectively. Patch clamp and electrocardiogram were performed. In both cases, we observed APD and electrocardiogram QT interval corrected by cardiac rate significant changes, emphasizing for the first time NBCe1 relevance for the electrical activity of the heart. Furthermore, the downregulation of NBCe1 caused significant hypertrophic heart growth and increased the frequency of Ca2+ waves without any significant changes in Ca2+ transients. An increased compensatory expression of NBCn1 and NHE1 was also found. We conclude that the reduction of NBCe1 is sufficient to induce cardiac hypertrophy without changes in blood pressure.

BACULOVIRAL VECTORS AS A GENE THERAPY TOOL FOR THE TREATMENT OF BRAIN DISORDERS

Matías Garcia Fallit[1,2], Matías L. Pidre[3], Antonela S. Asad[1], Jorge A. Peña Agudelo[1], Sofia Sagripanti[1], Alejandro J. Nicola Candia[1], Melanie Pérez Kuper[1], Abril Marchesini[3], Leslie C. Amorós Morales[3], Nazareno Gonzalez[1], Adriana Seilicovich[1,4], Mariana B. Vera[6], Guillermo Videla Richardson[6], Flavia Zanetti[5], Marianela Candolfi[1*]

[1]Instituto de Investigaciones Biomédicas (INBIOMED, CONICET-UBA), Facultad de Medicina, Universidad de Buenos Aires, Argentina. [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. [3]Instituto de Biotecnología y Biología Molecular (IBBM, UNLP-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata. [4]Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad de Buenos Aires, Argentina. Instituto César Milstein (CONICET). [5]Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina.

Glioblastomas (GBM) are the most frequent and aggressive primary malignant brain tumors in adults for which there are no effective therapeutic alternatives. Recombinant adenoviral (AdV) vectors are the gold standard for gene therapy in neuro-oncology. However, they are highly immunogenic and virtually the entire population has pre-existing anti-AdV immunity, leading to transient transgene expression. Baculoviral (BV) vectors are pathogens of insects but can also transduce cells from other species. Its advantage is that pre-existing immunity against BV in humans has not been reported so far. In order to develop therapeutic strategies for the treatment of GBM, we evaluated the transduction efficiency of BV as well as its possible neurotoxicity and compared them with those of AdV. Our hypothesis is that BVs constitute a useful tool for the persistent expression of therapeutic transgenes in the brain for the treatment of GBM. We constructed AdV and BV encoding fluorescent proteins under the control of the cytomegalovirus (CMV) promoter. The transduction efficiency of these vectors was evaluated in astrocyte cultures, GBM cell lines and short cultures derived from GBM biopsies by microscopy and flow cytometry. To assess in vivo transduction efficiency and neuropathology, vectors were injected by stereotactic surgery into the brain of naïve and GBM-bearing mice. We observed that even though the transduction efficiency of AdV is higher in rat and mouse cells, both vectors exhibit very similar efficiencies in human GBM cells. Both vectors showed high capacity to transduce normal rat and mouse astrocyte cultures. The transduction efficiency in the brain of naïve mice was comparable with both vectors, as was the immune infiltration at the injection site, with no apparent signs of neurotoxicity. We observed that BV transduce GBM cells and astrocytes in vivo. Brain reporter protein expression was stable for 21 days in naïve mice but was significantly reduced in mice systemically preimmunized against BV. We conclude that BV may be a useful tool for the expression of therapeutic transgenes in the treatment of GBM.

SAIC I ROUNDTABLE *Thursday, November 17, 10-12 hr*
HEALTH SCIENCE EDUCATION
Chair: Alberto D´Ottavio - Daniel Gómez

DEBATABLE REFLECTIONS ON UNDERGRADUATE CURRICULAR DESIGN IN HEALTH SCIENCES

Alberto Enrique D'Ottavio
Facultad de Ciencias Médicas y Consejo de Investigaciones, Universidad Nacional de Rosario, Rosario (Santa Fe), Argentina

Introduction: The undergraduate and postgraduate degrees (research, teaching and/or assistance) constitute an educational continuum in health sciences. As both degrees have curricular designs with common and specific issues, some reflections on their undergraduate facet are needed. Background: Status of educational levels; Laws and resolutions; Population health needs; Current and future conditions in the health care system and practice models; Health as a right; Number and heterogeneity of incoming students, Availability of health care centers, human, material, and financial resources. Curricular design: Appropriate model selection; Specific goals in competencies (knowledge, attitudes, habits, values, skills) focused on health promotion, prevention, diagnose and disease cure; Rational use of learning theories; Structure in cycles, areas, modules, mandatory, elective and correlative subjects; instrumental areas; evaluation and promotion systems supported in Departments or Areas; Acquisition of biological and scientific skills in increasing complexity with psycho-socio-anthropological, historical-philosophical and humanistic-artistic counterparts; Main strategies (primary health care, basic subjects, active students, individual and teamwork, abductive, deductive, inductive and analogical reasoning, concrete, abstract, critical and complex thinking, integration, Interdisciplinarity, accurate use of formal and technical language, comprehensive reading and writing, balanced simulations and practice, ICTs); Professional teachers; Evaluation of curriculum, courses and competencies of students and teachers; Ethical and bioethical support; Hidden curriculum. Obstructions: Dogmatism, false reductionisms, fads, unviable models, easy postures, academic, economic and/or political conveniences, discard of manageable proposals with improper arguments, and seemingly revolutionary but authoritarian ventures. Conclusions: An admissible undergraduate curriculum requires rigorous, valid, and reliable planning, implementation, and evaluation with singular competencies in each of them, rescuing the best of the past, applying for the current advances, and anticipating future needs. Its design should be guided by what, when, where, why, who, and how, and be oriented towards the feasible on the way to the desirable, considering debatable topics as the PAHO Institutional Self-Assessment of Social Responsibility Tool, along with the social mission of education in health equity.

CHAGAS' DISEASE: TEACHING AND LEARNING OF A COMPLEX ENDEMICAL PROBLEM

Edgardo Moretti, Beatriz Basso
School of Medical Sciences, National University of Córdoba, Argentina

Teaching about Chagas, although a punctual topic, can be a reflection of the orientation given to health sciences in university education. Chagas is a complex problem, included by OMS among the "neglected diseases", which requires the contribution of both biomedical and social disciplines. In elective subjects that we thought in Medical Sciences, National University of Córdoba (UNC) we detected a high lack of knowledge of this disease in advanced students. On such basis, we organized at UNC the First Meeting for the reinforcement of education on Chagas disease in Health Sciences. In such meeting, it was confirmed that this problem was widespread in Medicine and Biochemistry around the country. One of the main conclusions was: "Lack of information among health professionals is an additional risk factor for Chagas disease, since it has negative effects on prevention, it complicates diagnosis and prevents the possibility of adequate therapy". In order to analyze possible causes of the failure and advance in overcoming it, the Meetings continued biannually. In subsequent editions, held in Córdoba, Rosario, Santa Fe, Mendoza and Corrientes, other biomedical, biological and social careers were included. The proposal is to educate in order to help health professionals to "think about Chagas", a previously rural endemic and currently urbanized and globalized by human transmission: congenital, by transfusion and by transplants, and also by oral route. Finally, in cases of reactivations in immunocompromised patients, diagnosis and early treatment can dramatically change the course of the disease. Impacts of the Meetings; Improvements on teaching in some academic units; Inclusion of the topic in the career's Final Practice; Courses for instructors of Final Practice; Inclusion of the subject in non-traditional careers; Workshops in Brazil and Peru, organized by OPS and DNDi, with which the topic took regional dimension; Beyond university field, inclusion of university education as a tool in the National Chagas Program; Creation of a permanent forum on teaching Chagas. Despite the hereby-listed achievements, we believe that the impact is not enough and should continue with efforts to optimize teaching of Chagas and other pathologies of regional impact, in order to contribute to a more effective control of this endemic that affects mainly people who are below the poverty line.

POSTGRADUATE EDUCATIONAL CHALLENGES: HOW TO REACH MILLENNIALS.

Dr. Lucio Criado.
Ex-presidente Soc. Arg. de Medicina. Magister en Farmacopolitica.

Post-pandemic undergraduate and graduate medical education. Changes that were insinuated and came to stay: how to do this educational reengineering? New educational paradigms in medical residences under critical scrutiny with the challenge of deconstructing the old model and looking for the new model while the work continues. What are the requirements of 21st century physicians? How "to learn to learn" in a reality of continuous changes?

SAFIS ROUNDTABLE *Thursday, November 17, 16-18 hr*
Chair: Martín Vila Petroff

SCIENTIFIC DEBATE: DOES BAUMAN'S NEW MODERNITY INFLUENCE THE WAY WE DO SCIENCE?

Martín Vila-Petroff[1], Alicia Mattiazzi[1], Oscar Botasso[2]
[1]Centro de Investigaciones Cardiovasculares, "Dr. Horacio E. Cingolani", La Plata, Argentina. [2]Instituto de Inmunología Clínica y Experimental de Rosario, Rosario, Argentina.

The concept of liquid modernity was coined by the sociologist and philosopher Zygmunt Bauman as a metaphor to describe the condition of constant mobility and change that is observed in most aspects of contemporary society. Bauman visualizes a transition from a solid modernity to a new liquid modernity ("…incapable of keeping a certain form or a given course for long…" "…prone to change…" "…light…" (2)) and proposes that it influences all aspects of life. This round table will discuss the possibility that some practices or behaviors originated by the new modernity in which we are immersed, influence our scientific work, and conspire against what should be the main (only) objective of science: the enthusiastic search of the truth.

SAIC II ROUNDTABLE *Thursday, November 17, 18-20 hr*
WHAT THE PANDEMIC LEFT IN HEALTH AND SCIENCE ÁREAS
Chairs: Alejandro Curino - Cristina Carrillo

THE ROLE OF SCIENTIFIC ORGANIZATIONS IN THE FACE OF THE SPREAD OF FALSE AND UNSCIENTIFIC DATA ON VACCINATION AND THE PANDEMIC

Dr. Alejandro Curino
Instituto de Investigaciones Bioquímicas de Bahía Blanca. Bahía Blanca.

A CONGRESS IN THE PANDEMIC

Dra. Cristina Carrillo
Instituto de Fisiología Experimental. Centro Científico Tecnológico CONICET – Rosario. Rosario, Santa Fe.

THE SCIENTIFIC COMMUNITY IN THE FACE OF THE PANDEMIC: A BEFORE AND AFTER?

Dra. Ana Franchi
Presidenta del CONICET.

CHALLENGES

Dra. Alejandra Sánchez Cabezas
Asesora del Ministerio de Salud de la Nación. Vicepresidenta de la Asociación Argentina de Salud Pública.

SAIC MINICOURSE *Thursday, November 17, 8-9 hr*

INTRODUCTION TO THE WORLD OF EXTRACELLULAR VESICLES

[1]Maria Noé Garcia, [2]Matías Ostrowski, [1]Daniel Grasso, [1]Daniela L Papademetrio
1 Instituto de Estudios de la Inmunidad Humoral (IDEHU, UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.
2 Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS, UBA-CONICET), Cátedra de Inmunología, Facultad de Medicina, Universidad de Buenos Aires.

The term extracellular vesicles (EVs) comprisesall those vesicles naturally released from cells and which are delimited by a lipidic bilayer without replicative capabilities. Based on their characteristics, such as size or subce-

llular origin, EVs represent an entire family of different extracellular entities including exosomes, ectosomes/ microvesicles and others, with a remarkable relevance in human health and diseases. All cell types release these tiny vesicles, which are found in most human fluids . Importantly, EVs bear molecules capable of activating cellular pathways that eventually initiate effector responses in distant recipient tissue. Since EVs' cargo reflects an unique profile of its originating cell which is efficiently delivered to its target, the potential of these vesicles as human diseases biomarkers or target-specific delivery of therapeutics drugs is being intensively explored. Having in mind its definition it is not a surprise that the importance of EVs has increased exponentially in the last years. The course aims to introduce the attendants in the most relevant concepts, definitions and classifications of EVs. Additionally, an overview of the principal scientific milestones, technical challenges and perspectives about EVs will be discussed.

SAFIS - CAMILIÓN DE HURTADO AWARD *Friday, November 18, 11-13 hr*
Juries: Alicia Mattiazzi, Andrea Fellet, Emiliano Diez
Chair: Ernesto A. Aiello.

ACUTE ANTIOXIDANT EFFECT OF GLUCOCORTICOIDS IMPROVE CARDIAC FUNCTION FOLLOWING ISCHEMIA/REPERFUSION

Daiana S. Escudero, Juliana C. Fantinelli, Valeria R. Martínez, Néstor G. Pérez and Romina G. Díaz
Centro de Investigaciones Cardiovasculares "Dr. Horacio E. Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina.

Glucocorticoids (GCs) are steroid hormones involved in several physiological processes including cardiovascular function. GCs like cortisol bind and activate both the GC receptor (GR), and the mineralocorticoid receptor (MR), highly expressed in cardiac tissue. While MR was reported to trigger deleterious effects in the myocardium by activating the sodium-hydrogen exchanger (NHE1), little is known about the effects of GR activation in cardiac tissue. This study was aimed to evaluate whether acute GCs may protect the myocardium against ischemia/reperfusion injury (I/R). Acute myocardial infarction (I) was performed in isolated hearts from 5-month-old Wistar rats, by ligation of left anterior descending coronary artery (40min I/60min R) in absence (ischemic control "I", n=4) or presence of Hydrocortisone (HC, 10nM n=4). HC reduced infarct size (in % of risk area: 37±3 I, vs. 9±3 HC, $p<0.05$), and improve post-ischemic mechanical recovery: developed pressure (LVDP in % of preischemic control: 28±4 I, vs. 60±5 HC, $p<0.05$), and diastolic pressure (LVEDP in mmHg: 55±4 I, vs. 28±5 HC, $p<0.05$). These effects were reversed in the presence of the GR inhibitor Mifepristone (M, 10mM, n=5): infarct size 24±4%; LVDP 34±10%; LVEDP 10±2 mmHg. Also, preliminary results showed an antioxidant action by reducing reactive oxygen species, thiobarbituric acid reactive substances and increasing the reduced/oxidized glutathione ratio. In isolated papillary muscles subjected to transient acidosis (TA) NHE1 activity was reduced by HC (1 or 10nM), JH+ in mmol/ml/min: 1.22±0.19TA; 0.68±0.11HC1; 0.56±0.15HC10, $p<0.05$. Importantly, the inhibitory Ser648 NHE1-phosphorylation site significant increased after HC treatment (in % of control): 102.1±7TA, 111.3±15.7HC1, 155.0±23.3HC10, $p<0.05$. Although preliminary, the results suggest that HC protect the heart against the deleterious effects of I/R through an antioxidant effect probably related to NHE1 inhibition.

PLACENTAL ANGIOGENESIS DEPENDS ON AQP1 AND AQP4, AND THE PROPER INTERACTION WITH CAV-1

Julieta Reppetti[1], Marcus V. Reis[1], Juan J. Casal[2], Germán Gornalusse[3], Alicia E. Damiano[1,4], Nora Martínez[1]
[1]Laboratorio de Biología de la Reproducción. IFIBIO-Houssay (UBA-CONICET). Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. [2]IFIBIO Houssay (UBA-CONICET). Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. [3]Department of Medicine, University of Washington, Seattle, Washington, USA. [4]Cátedra de Biología Celular y Molecular. Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Placental vasculature development involves the proliferation, migration, and differentiation of endothelial cells (EC). Caveolin-1 (Cav-1) is a constitutive protein of the caveola that interacts with numerous proteins. Aquaporins (AQPs) are transmembrane water channels, which move water in response to osmotic gradients. Emerging evidence shows that AQPs and Cav-1 are required for cell migration. We hypothesized that placental angiogenesis depends on the normal expression and function of AQPs that interact with Cav-1 in EC. Objective: To study the role of AQP1 and AQP4 in placental angiogenesis and their interaction with Cav-1. Materials and Methods: The macrovasculature cell line, EA.hy926 (ATCC®CRL-2922™), was used. AQP1, AQP4, and Cav-1 expressions were explored. *In silico* analysis was performed to investigate putative Cav-1 binding motifs in AQP1 and AQP4 proteins. Co-localization of these proteins was assessed by immunoprecipitation. AQPs were blocked with tetramethylammonium chloride (TEA), and Cav-1 and AQP1 were silenced by specific siRNA. Cell migration was evaluated by wound healing assay. Angiogenesis was evaluated by tube formation assay. Results: EA.hy926 cells expressed AQP1, AQP4, and Cav-1. *In silico* analysis identified a putative Cav-1-binding site in AQP1 and AQP4. Immunoprecipitation assay confirmed these results. Cav-1 silencing produced a significant re-

duction in cell migration (n=4; p<0.05) and tube-like formation (n=3, p<0.05). On the other hand, the blocking of both AQPs was required to significantly reduce cell migration (n=4; p<0.01), while only the inhibition of AQP1 was sufficient to negatively affect tube-like formation (n=3, p<0.05). Conclusions: These results suggest that AQP1 and AQP4 interact with Cav-1 in the caveolae of EC. Therefore, an intact caveolar structure is necessary for correct EC migration, although this process would not depend on the AQP isoforms. In contrast, AQP1 may be crucial for the proper tube formation of the EC.

EFFECT OF ADIPOSE MUSE CELL IMPLANTATION ON HOMING AND ANGIOGENESIS IN A SHEEP MODEL OF ACUTE MYOCARDIAL INFARCTION

Giovanna Castillo[1], Tomás M. Peralta1, Paola Locatelli[1], Alberto J. Crottogini[1], Fernanda D. Olea[1], Luis A. Cuniberti[1]
[1]Laboratorio de Regeneración Cardiovascular. Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB) Universidad Favaloro – CONICET. Buenos Aires, CABA, C1078AAI, Argentina.

Objective Muse cells have been studied in a variety of diseases. However, the potential of Muse cells isolated from adipose tissue (AT) to promote new blood vessel formation in acute myocardial infarct (AMI) has not been entirely evaluated. The current study sought to investigate the neovascularization ability of AT-Muse cells in vitro and in vivo, as well as their differentiation potential in a translational ovine model of AMI. Methods Adipose-derived mesenchymal stromal cells (ASCs) and Adipose Muse cells were isolated from donor sheep. AMI was induced by thirty minutes of permanent ligation of one or more diagonal branches of the left anterior descending coronary artery. One hour after AMI, animals were randomized in two groups: 4 sheep received 10 intramyocardial injections of 2x107 AT-Muse cells labeled with PKH26 Red Fluorescent Dye and 4 sheep received PBS (Vehicle group). Results In vitro assay showed that the gene expression of angiogenic genes ANG, VEGF and PGF was higher in AT-Muse cells group in comparison with ASCs group, (p<0.05). Seven days after AMI, the group treated with AT-Muse cells showed a significant increase in the number of capillaries and arterioles in comparison with the Vehicle group (p<0.05). PKH26+ cells showed co-localization with sarcomeric actin, troponin T, desmin and also with lectin marker suggesting an incipient differentiation to cardiac and vascular lineage of Muse cells after homing. Conclusion Intramyocardially injected AT-Muse cells induced significant neovascularization potential and homing capacity in an ovine model of AMI.

SAFIS - SOCIEDAD ARGENTINA DE FISIOLOGÍA AWARD *Saturday, November 19, 11-13 hr*
Juries: Irene Ennis, Gisela Di Giusto, Enrique S§nchez Pozzi
Chair: Cecilia Larocca.

PREVENTION OF HEMOLYTIC UREMIC SYNDROME (HUS) USING DRUGS AGAINST SHIGA TOXIN TYPE 2 (STX2): CANDIDATE SELECTION BY MOLECULAR DYNAMICS (MD)

Gioia D.S., Casal J.J., Toriano R.
Instituto de Fisiología y Biofísica Bernardo Houssay, UBA-CONICET
gioiadaiana@gmail.com

Hemolytic uremic syndrome (HUS) is the clinical triad of thrombocytopenia, anemia, and acute kidney injury. Is an endemic disease in Argentina and the main cause of pediatric renal failure, with about 300 new cases per year. HUS is classically associated with Shiga toxin–producing E. coli (STEC) enterocolitis. Shiga toxins exist as two immunologically distinct types, Stx1 and Stx2. Epidemiological data show that Stx2 are more likely to cause HUS. Although several therapies were designed for prevention and/or treatment, none have advanced beyond the early clinical stages. Reverse pharmacology, through rational drug design with *in silico* strategies, allows cost and time saving, compared to classical pharmacology. The aim of this work was to obtain molecules with anti-Stx2 activity that can be transformed into drugs. We started from the structure of Stx2 (id:1R4P, PDB) solved by Rx spectroscopy at 1.77 Å. For all steps, open-source software was used (DataWarrior, OBabel, AutoDock VINA, GROMACS, VMD, KNIME). The workflow was automated using Python scripts. Stx2 1R4P was completed by sequence homology and 14,269 non-toxic molecules were selected by molecular docking. These molecules, that showing a binding free energy of (-10 < ΔGu < +10) kcal/mol, were divided into 4 groups and classified according to ΔGu: Group A: molecules with a known synthesis route (N=88, Me=-8.1kcal/mol); Group B: FDA-approved drugs (N=948, Me=-7.3kcal/mol); Group C: “lead-like” drugs (N=3110, Me=-5.3kcal/mol) and Group D: all other molecules (N=10123, Me=-4.3kcal/mol). With the heads of each group, 57 DMs were performed, and the calculation of ΔGu was refined. The number of DM performed and the maximum (ΔGu±sd) kcal/mol obtained were A: 24, (-20.09±0.65); B: 25, (-23.41±1.05), C: 3, (-16.35±0.88) and D: 6, (-27.75±0.67). To evaluate the stability of systems and the level of non-covalent interaction, RMSD and H-bonds (drug-Stx2) were measured.

EFFECTS OF STRIATAL SOMATOSTATINERGIC INTERNEURON INHIBITION ON THE DEVELOPMENT OF L-DOPA INDUCED DYSKINESIA

Agostina M. Stahl, Cecilia Tubert, Lucía Garbini, Juan E. Belforte, Lorena Rela, M. Gustavo Murer
Grupo de Neurociencia de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Bernardo Houssay, UBA-CONICET, Buenos Aires, Argentina.

Parkinson's disease (PD) is a neurodegenerative disorder whose prevalence has increased over the past years. The main symptoms of PD are caused by the loss of mesencephalic dopaminergic neurons and the resulting functional disbalance between striatal projection pathways that control voluntary movement. Chronic administration of L-dopa remains the gold standard therapy. However, in advanced stages of the disease, it frequently produces abnormal involuntary movements known as L-dopa induced dyskinesias (LID), which have also been related to a functional disbalance between the striatal output pathways. Besides projection neurons, the striatum is composed of different interneurons such as somatostatinergic interneurons (iSOM). Since iSOM regulate the activity of the striatal projection pathways, we asked if chemogenetic inhibition of iSOM in a mouse model of PD modifies the expression of LID, once they are already established, and if it could prevent the development of LID. To this aim, we used the 6-OHDA mouse model of PD, and the expression in iSOM of an inhibitory Designer Receptor Exclusively Activated by Designer Drugs -DREADD- named hM4D, which is activated only after the administration of its synthetic ligand: CNO (clozapine N-oxide). PD mice were treated with daily injections of L-dopa (3 or 6 mg/kg) for 4 days, and CNO (3mg/kg) or vehicle were coadministered with L-dopa on day 3 or 4, and LID were quantified. We found a small increase in LID expression after CNO administration, only with 6mg/kg of L-dopa (paired t test, $p=0.0429$, $n=12$ per group). To evaluate if inhibition of iSOM prevents the development of LID, L-dopa and CNO were coadministered for 4 consecutive days, and LID were quantified on day 5 in the absence of CNO. Preliminary results showed a reduction of LID in animals that express hM4D in comparison with control animals ($n=5$ and $n=6$, respectively), which needs to be confirmed in another cohort of mice. The data suggest that iSOM inhibition modulates LID.

MECHANISMS MEDIATING INTERNALIZATION AND TRANSPORT OF GHRELIN IN HYPOTHALAMIC TANYCYTES

Ivana María Gómez, Daniel Castrogiovanni, Maia Uriarte, Mario Perelló, Pablo Nicolás De Francesco
Laboratorio de Neurofisiología, Instituto Multidisciplinario de Biología Celular (IMBICE, CIC-CONICET-UNLP).

Hypothalamic tanycytes are polarized glial cells that line the base of the third ventricle. Their somas contact the cerebrospinal fluid (CSF), while their terminal side (endfeet) contact the capillaries of the blood-brain barrier (BBB) or the fenestrated capillaries of the median eminence, forming a relevant anatomical interface for the transport of molecules between blood and CSF. Using mice and rats, we recently described that tanycytes internalize the orexigenic hormone ghrelin *in vivo* and *in vitro*. Here, we study the cellular mechanisms of ghrelin uptake by hypothalamic tanycytes and its transport direction. Specifically, we incubated primary cultures of rat hypothalamic tanycytes with a fluorescent variant of ghrelin (Fr-ghrelin) in basal conditions or after pharmacological blockage of either clathrin-mediated internalization using Pitstop, or intracellular transport using colchicine. We also coincubated tanycytes with Fr-ghrelin and native ghrelin. We then quantified fluorescence intensity in soma, process and endfeet of each cell. Data were compared using Kruskal Wallis test with Dunn's post hoc test. We found that intracellular fluorescence: 1) is found predominantly in the soma of tanycytes after a 5 min incubation (K-W $p<0.0001$; $p<0.0001$); 2) increased in somas (K-W $p<0.0001$; $p= 0.003$), processes (K-W $p<0.0001$; $p< 0.0001$) and terminals (K-W $p<0.0001$; $p< 0.0001$) after 30 min incubation as compared to 5 min incubation; 3) was reduced only in terminals in the presence of colchicine (K-W $p<0.0001$; $p< 0.0001$); 4) was decreased in all compartments in the presence of Pitstop (K-W $p<0.0001$; $p<0.0001$); and 5) was reduced in somas (K-W $p<0.0001$; $p< 0.0001$), processes (K-W $p<0.0001$; $p= 0.0018$) and endfeet (K-W $p<0.0001$; $p= 0.0027$) after coincubation with native ghrelin. This evidence shows that tanycytes are able to specifically internalize ghrelin through clathrin-mediated endocytosis in the somas and presumably to transport it from the apical to the terminal side.

GHSR DEFICIENCY INCREASE LIRAGLUTIDE EFFECTS IN FEMALE MICE

Daniela Cassano[1], Gimena Fernandez[1], María Paula Cornejo[1], Abdella M Habib2, Mario Perelló[1]
[1]Instituto Multidisciplinario de Biología Celular (IMBICE), [CONICET – CIC – UNLP], Laboratorio de Neurofisiología, La Plata, Argentina, [2]Qatar University – College of Medicine, Basic Medical Science Department, Qatar, Qatar.

Liraglutide is a GLP-1 receptor agonist that reduces glycemia and appetite and is used as an anti-diabetic medi-

cation to treat type 2 diabetes, obesity, and chronic weight management. Ghrelin is an orexigenic peptide hormone that acts via the growth hormone secretagogue receptor (GHSR) and upregulates mechanisms that increase glycemia. Aim: we tested here if the genetic deficiency of GHSR signaling in mice affects the anorexigenic and hypoglycemic effects of Liraglutide. Methods: we treated WT and GHSR-deficient (GHSR-def) female mice with vehicle or Liraglutide (400 microg/Kg) under different experimental conditions and assessed glycemia and food intake. Results: In *ad libitum* fed condition, Liraglutide did not acutely (0-90 min) affect glycemia of GHSR-def vs. WT mice, but induced a stronger reduction of overnight food intake in GHSR-def mice than WT mice (Two-way ANOVA detected significant treatment x genotype interaction: P<0,01, F (1, 23)=8,748). In fasted condition, Liraglutide did not acutely affect glycemia of GHSR-def vs. WT mice. After refeeding, Liraglutide reduced both fasting-induced hyperphagia (Two-way ANOVA detected significant treatment effect (P<0,0001, F (1, 34)=52.34) and genotype effect) P=0,0425, F(1, 34)=4.443)) and post-prandial increase of glycemia (Tukey's post-test comparison P=0.034) in GHSR-def mice and in WT mice but both effects were significantly more pronounced in GHSR-def mice. In vehicle-treated WT mice that were refed after fasting with the same amount of food eaten by Liraglutide-treated WT mice, postprandial glycemia was similar as detected in unrestrictedly refed WT mice suggesting that the liraglutide-induced reduction of postprandial glycemia was independent of food intake. After glucose infusion, Liraglutide similarly reduced hyperglycemia in GHSR-def vs. WT mice. Conclusion: The genetic deficiency of GHSR enhances the anorexigenic and hypoglycemic effects of liraglutide in mice via independent mechanisms.

OMEGA 3 MITIGATION ON DEFICIENT HYPOXIC-VENTILATORY RESPONSE (HVR) INDUCED BY MODERATE ETHANOL DOSES IN AN ANIMAL MODEL EQUIVALENT TO THE THIRD TRIMESTER OF HUMAN PREGNANCY

Marisol Magalí Segovia[1], Verónica Balaszczuk[1-2] & Ana Fabiola Macchione[1-2]

[1]Laboratorio de Psicología Experimental, Instituto de Investigaciones Psicológicas IIPsi-CONICETUNC. Córdoba, Argentina.
[2]Facultad de Psicología-UNC. Córdoba, Argentina. marisol.segovia@mi.unc.edu.ar

Maternal ethanol (EtOH) intake during pregnancy and lactation is a highly frequent "social" behaviour in Argentine, exposing fetus or neonates to moderate EtOH intoxication through amniotic fluid and maternal milk. Early exposure to EtOH triggers in the organism a spectrum of neurobehavioral dysfunctions affecting, also, the breathing response. Hypoxia acts as an environmental stressor eliciting breathing adaptations, like Hypoxic Ventilatory Responses-HVR, that may be altered by the EtOH exposure. One of the detrimental effects of early EtOH exposure is the reduction of the Omega3-O3 levels in the CNS. Experiment 1: Pups were intoxicated with 2.0g/kg or 0.0g/kg of EtOH, ig at postnatal days (PDs) 3-5-7-9. At PD9, also were subjected to a hypoxic event [5 min of initial normoxia, followed by 3 episodes of hypoxia (O2 8%) of 5 min, separated by periods of recovery-normoxia of the same duration]. Breathing frequencies and apneas were recorded in a whole-body plethysmograph at PDs 3-5-7 x 5 min and at PD9 x 35 min of test. Experiment 2: The same experimental protocol of EtOH exposure at PDs 3-5-7-9 was employed. However, at PDs 3-5-7, pups received 0.0 or 720 mg/kg O3 (ig), 20 min after EtOH administration. At PDs 5 and 7, EtOH-intoxicated pups elicited a breathing depression that was not affected by the O3 administration. During the hypoxic challenge, EtOH-exposed pups expressed a significant breathing depression during initial normoxia and also at each hypoxic event (i.e. a reduced HVR) relative to it expressed in vehicle-exposed pups ($p<0.05$). Preliminary results indicated a restoration of the HVR in pups exposed to EtOH with O3. A significant increase in the number of apneas in EtOH intoxicated pups was observed after the 2nd and 3rd hypoxic events. At these periods, a protective O3 effect was observed by a reduction in the number of apneas in EtOH-intoxicated pups. These results seem to indicate a mitigation due to O3 upon deleterious EtOH effects in the neonatal HVR.

SAIC - FUNDACIÓN LUCIO CHERNY AWARD *Saturday, November 19, 14-16 hr*
Juries: Alejandro de Nicola, Paula Heller, Ana María Buzaleh, Carlos Davio, Alejandro Curino

BIOLOGICAL AND MOLECULAR STUDIES IN MICRODEVICES BASED ON CANCER STEM CELLS FOR THE DEVELOPMENT OF A PERSONALIZED MEDICINE PLATFORM

Eduardo Imanol Agüero[1,2], Denise Belgorosky[1], Ana Belén Peñaherrera-Pazmiño[2,3], María Eugenia Azar[4], Valeria Cáceres[5], Betiana Lerner[2,3], Maximiliano Sebastiá, Pérez[2,3], Ana María Eiján[1,2]

[1]Universidad de Buenos Aires (UBA), Facultad de Medicina, Instituto de Oncología "Ángel H. Roffo", Área de investigación, Buenos Aires, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [3]Universidad Tecnológica Nacional (UTN), Facultad Regional de Haedo, Buenos Aires, Argentina. [4]Universidad de Buenos Aires (UBA), Facultad de Ciencias Médicas, Instituto de Oncología "Ángel H. Roffo", Área Quirúrgica, Departamento de Mastología, Buenos Aires, Argentina. [5]Universidad de Buenos Aires (UBA), Facultad de Ciencias Médicas, Instituto de Oncología "Ángel H. Roffo", Área Médica, Departamento de Oncología Clínica.

The development of microfluidic devices (MD) for cancer cell research is present as new highly versatile strategies of clinical and biological research around the world. Microfluidic-based strategies for cancer stem cells (CSC) analysis have translational relevance facilitating drug screening, disease prognostic and other clinical applications using minimal samples and optimizing the use of reagents. In this sense, we have developed two MDs to study CSCs by sphere forming to evaluate CSC growth, chemotherapeutic treatment (CT) response, pluripotency markers expression and to isolate CSCs derived from cancer cell lines and from bladder (BLC) and breast (BRC) cancer primary cultures. First, we analysed murine BLC MB49-I and BRC LM38-LP cancer cell lines. Then, to validate the results, human lines J82 and BRP6 (established from a patient of A. H. Roffo Institute) were used. Finally, primary cultures of murine tumors were studied. CSC studies in MD involved the analysis of the sphere formation efficiency (SFE), their size and their growth slope in basal condition or under CT. A significant reduction of between 30-55% in SFE (***) and 20-40% in sphere size (***) was observed with all treatments in all cell lines tested. In addition, the expression of pluripotency markers was evaluated by qPCR and immunofluorescence (IF) within MD and more than 200% increase of expression was recorded in most of the analysed genes (**). As a proof of concept for the development of a platform for patient-derived CSC isolation, primary cultures of mouse tumors were grown in the MD. It was possible to demonstrate the CSC growth and its response to CT. The MD is useful to evaluate treatment response, to study specific markers by IF and to isolate cells and nucleic acids for molecular studies. The development of this technology is projected as a useful, versatile, and economic tool for estimating clinical response and as a new strategy for personalized medicine (** $p<0,005$, *** $p<0,0005$).

COMPREHENSIVE MOLECULAR-GENETIC ANALYSIS OF A HEARING LOSS COHORT FROM ARGENTINA. FUNCTIONAL VALIDATION OF NOVEL VARIANTS IDENTIFIED

Paula Buonfiglio[1], Vanesa Loterzstein[2], Sebastián Menazzi[3], Paola Plazas[4], Ana Belén Elgoyhen[1], Viviana Dalamón[1]

[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor Torres"- INGEBI /CONICET. [2]Centro Nacional de Genética Medica- CENAGEM. [3]Servicio de Genética, Hospital de Clínica "José de San Martín". [4]Instituto de Farmacología, Facultad de Medicina, UBA.

Hearing loss (HL) is the most prevalent sensorineural deficit, affecting about 20% of the global population, with nearly half due to genetic causes. About 1 in 500 newborns present congenital HL and more than 100 genes are involved. Most of the patients are non-syndromic with an autosomal recessive mode of inheritance (80%), caused most frequently by *GJB*2 and *GJB*6 genes. In this work, we aimed to identify the genetic cause of HL designing a multistep approach to analyze target genes. Besides, we performed *in silico* and *in vivo* analyses in order to further study some of the identified variants in the zebrafish model. A total of 650 patients were studied by Sanger Sequencing and Gap-PCR in *GJB*2 and *GJB*6 genes respectively, diagnosing 15.5% of sporadic cases and 36% of familial ones. Overall, 50 different sequence variants were detected. Next, 50 patients with moderate HL were tested for deletions in *STRC* gene by MLPA technique. After initial screening, 38 families were selected to be analyzed by Whole Exome Sequencing, achieving diagnosis in 40%. Half of the identified variants were novel. One of them was a missense variant detected in a familial case in *MYO*6 gene. To further analyze the functional implication of this variant, a protein modeling with I-Tasser software was performed revealing its pathogenic effect. In order to functional validate this candidate variant a knockdown phenotype rescue assay using an antisense oligonucleotide (morpholino) in zebrafish was carried out demonstrating the deleterious effect on the auditory system. In the present study, we showcased that our algorithm is suitable for the sequential multigenic approach to HL in our cohort. This involves both everyday routine molecular biology and next generation

sequencing techniques to identify the genetic etiology of HL. Furthermore, *in silico* and *in vivo* analyses provide not only evidence to validate novel variants but also new knowledge to better understand the genetic basis of HL.

ABLATION OF NKX2.1 DERIVED STRIATAL INTERNEURONS RESULTS IN TOURETTE – LIKE PHENOTYPES

Camila Coll, Juan Pablo Beccaria, Bárbara Yael Braz, Analía López Díaz, Juan Emilio Belforte, Mario Gustavo Murer
Grupo de Neurociencia de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Bernardo Houssay; UBA-CONICET, Facultad de Medicina, Buenos Aires, Argentina.

Tourette Syndrome (TS) is a neurodevelopmental disorder characterized by motor and vocal tics, impulsivity and repetitive behaviors. Although TS pathophysiology remains poorly understood, postmortem studies show a reduce number of PV+, NOS+ and ChAT+ striatal interneurons (SIs) in TS patients. Previous studies in mice failed to induce tic-like movements by ablating specific subtypes of SI. Notably, all the SI subtypes affected in TS derive from cell precursors expressing the transcription factor Nkx2.1. In order to reproduce more closely the striatal changes reported in patients, we performed a combined ablation of SIs using a Cre/loxP transgenic system to express human diphtheria toxin receptor (hDTR) in the Nkx2.1+ cell lineage. Intrastriatal diphtheria toxin administration produced a specific ablation of Nkx2.1+ SIs, as shown by IHC, in mice expressing hDTR only but not in control Cre- mice. We developed a Mouse Tic Severity Scale (MTSS) based on the Yale Tics Severity Scale used in clinical settings. Lesioned mice developed abnormal involuntary movements resembling motor tics and had a higher MTSS score than controls as determined by blind observers (Median and IQR: 9, 6-15, n=22; 5, 2.4-8, n=21, respectively; Mann-Whitney; p=0,001). Lesioned mice developed repetitive behaviors reminiscent of those present in TS, including an 80% increase of head pokes (Mann-Whitney; p=0,017), and a trend to hyperlocomotion (t-test; p=0,068) and increased stress-induced stereotypic behaviors (grooming; Mann-Whitney; p=0,067) compared to controls. To determine if acute SI inhibition also induces a TS-like phenotype, we expressed the hM4Di DREADD selectively in Nkx2.1+ SI and found a subtle but significant increase of the MTSS score (Two Way RM-ANOVA, significant interaction, p=0.049 Tukey post hoc test) under hM4Di stimulation with CNO (Mean ± SEM: CNO=4.5 ± 0.7; vehicle=3.3 ± 0.5). In summary, ablation, or inhibition of Nkx2.1 derived SIs induce TS-like phenotypes.

INHIBITION OF AUTOPHAGOSOMAL DEGRADATION IN MELANOMA CELLS PROMOTES THE RELEASE OF MEDIATORS THAT INCREASE GLOBAL RESISTANCE TO VEMURAFENIB

Cristian R. Falcón[1], Adolfo R. Zurita[1], Federico Cuello-Orlandi[2], Mercedes L Sangiacomo Ruiz[2] Itatí Ibáñez[3], Johinna Mons[1], Celia N Pérez[1], Sergio E Alvarez[1]
[1]IMIBIO-SL CONICET; [2]Universidad Nacional De San Luis; [3]Instituto De Ciencia Y Tecnología "DR. CESAR MILSTEIN"

Vemurafenib (Vem) is used in the treatment of melanomas that have the BRAFV600E mutation but resistance to this drug is rapidly induced. Autophagy has been implicated in resistance to Vem. Objective: to study the influence of autophagosomal degradation inhibition on resistance to Vem in human melanoma cells. Results: Lu1205 cells cultured in hypoxia (Hx) have higher resistance to Vem than in normoxia (Nx). We show by western blot that Hx produces accumulation of LC3 and p62, indicating decreased autophagic flux. Although autophagy inhibition with NH4Cl or Chloroquine (Cq) in Nx and Hx increased sensitivity to Vem (MTT viability and apoptosis), only conditioned media (CM) from cultures in Hx transferred Vem resistance to sensitive cells and this ability was not significantly modified by autophagy inhibitors. Surprisingly, CM from Lu1205 cultured in Nx with NH4Cl or Cq increased the resistance to Vem of sensitive cells, similarly to CM-Hx. In addition, we developed a novel cell line derived from Lu1205 cells expressing the M2 protein of the influenza virus A/PR8 strain, which inhibits the autophagosome-lysosome fusion. These cells (Lu1205-M2iv) showed increased resistance to Vem both in Nx and Hx and augmented migratory capacity with respect to the parental line. In addition, CM obtained from Lu1205-M2iv cells enhanced chemoattraction of PMA-differentiated THP-1 cells and transferred resistance to sensitive cells. Finally, Lu1205 cells with acquired resistance to Vem (Lu1205R) showed strongly decreased autophagic flux and increased expression of mRNA of RAB27a and RAB27b involved in excretory pathways. These cells subtly decreased their viability when cultured with Vem in the presence of the autophagy inhibitors and showed an increased ability to induce Vem resistance in sensitive cells. Conclusion: Therapy with inhibitors of autophagosomal degradation in melanoma cells increases initial sensitivity to Vem but could promote the general tumor resistance to treatment.

TNF-INDUCED UP-REGULATION OF RAC3 EXPRESSION ELICITS A PRO-INFLAMMATORY PROFILE IN BREAST CANCER-ASSOCIATED ADIPOCYTES

María Cecilia Lira[1], Francisco Damián Rosa[1], Ignacio Aiello[2], Mileni Soares Machado[1], Iara Castellanos[1], Juliana Lourdes Bernacchia[1], Alejandra Graciela Palma[1], María Cecilia Salazar Güemes[3], Natalia Paladino[2], Mónica Alejandra Costas[1] and María Fernanda Rubio[1]

[1]Instituto de Investigaciones Médicas A. Lanari, UBA, Lab. Biología Molecular y Apoptosis, IDIM UBA-CONICET, [2]Universidad Nacional de Quilmes, Lab. Cronobiología, Dpto CyT, UNQ, [3]Instituto de Investigaciones Médicas A. Lanari, UBA, Dpto Oncología.

The molecular mechanisms that control adipocyte behavior in breast cancer (BrCA) context remain unclear. We previously reported that the expression of RAC3 coactivator in adipose tissue (AT) adjacent to tumors from BrCA patients, was high in 59% of the samples, showing active NF-kB in nucleus and elevated levels of pro-inflammatory cytokines (TNF and MCP1). Moreover, 3T3-L1 adipocytes (3T3-L1ad) exposed to conditioned medium (CM) from T-47D BrCA cells, exhibited higher RAC3 levels compared to CM from other tumoral and non-tumoral cells. Therefore, the aim of this work was to elucidate the role of RAC3 and the mechanism by which it is modulated in BrCA-associated adipocytes. First, we evaluated TNF and MCP1 levels by dot plot and qPCR, respectively, in 3T3-L1ad stimulated with T-47D CM or serum-free medium. Both molecules showed higher levels with T-47D CM ($p<0.05$ vs basal). This effect was reversed by the NF-kB inhibitor, Sulfasalazine (SSZ). Of interest, 3T3-L1ad transfected with short hairpin RAC3 RNA plasmid also showed diminished levels of the cytokines, as the basal condition. Due to T-47D cells secrete TNF, and this cytokine up-regulates RAC3 expression in HEK293 cells, we sought to investigate if TNF is one of the tumoral factors responsible for the increase of RAC3 levels. We confirmed TNF up-regulates RAC3 expression in 3T3-L1ad ($p<0.05$ vs basal). This effect was mediated by NF-kB, as SSZ reversed RAC3 expression, and ChIP assay showed NF-kB was recruited to RAC3 promoter. In mammary AT from female C57-BL/6J mice, T-47D CM and TNF increased RAC3 mRNA and protein expression (measured by qPCR and IHQ, respectively), as well as, nuclear active NF-kB, TNF and MCP1 levels. Importantly, in AT from TNF receptor 1 knock-out mice in basal condition or stimulated with T-47D CM or TNF, RAC3 levels did not show significant differences. Altogether, we suggest RAC3 is a key molecule to understand BrCA-associated adipocyte behavior and the inflammatory setting.

SAIC - FUNDACIÓN GADOR AWARD *Saturday, November 19, 13-14 hr*
Juries: Claudia Lanari, María Marta Facchinetti, Graciela Cremaschi

TUMOR INTRINSIC EFFECTS OF FOXP3 IN BREAST CANCER CELLS

Alejandro Nicola Candia[1], Nazareno Gonzalez[1], Jorge A. Peña Agudelo[1], Matías Garcia Fallit[1,2], Antonela S. Asad[1], Sofia Sagripanti[1], Araceli Abt[1], Melanie Pérez Küper[1], Mariela A. Moreno Ayala[1], Adriana Seilicovich[1], Flavia Zanetti[3], Marianela Candolfi[1]

[1]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires; [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina; [3]Instituto Cesar Milstein (CONICET)

Foxp3 is a transcription factor required for Treg function. In the last years, it has been detected in breast tumor and other cancer cells, but its role in tumor pathogenesis is controversial. Our previous results suggest that P60, a cell-penetrating peptide that impairs Foxp3 function, improves the efficacy of antitumor vaccines and inhibits tumor progression in experimental breast cancer. Here we aimed to assess the tumor intrinsic role of Foxp3 in breast cancer. Meta-analysis of Foxp3 expression in TCGA and METABRIC data bases indicated that Foxp3 is over-expressed in HER2+ and triple negative (TNBC) breast tumors compared to hormone-dependent tumors. In addition, upregulation of Foxp3 correlated with lower OS in HER2+ tumors and TNBC. Foxp3 expression positively correlated to PDL1, IDO and IL-10 expression and EMT markers. Considering that Foxp3 may affect cell survival, we next evaluated the effect of chemotherapy on its expression in HER2+ and TNBC cells. Cisplatin upregulated Foxp3 in both cell lines ($p<0.05$). Inhibition of Foxp3 with P60 reduced cell survival in these cell lines and enhanced chemosensitivity. We next developed an Adenoviral vector (Ad.P60) that encodes P60 and the reporter gene dTomato under the control of the CMV promoter. Ad.P60 efficiently transduced breast tumor cells in vitro and in vivo. Foxp3-GFP C57Bl/6 mice bearing EO771 TNBC were i.t. injected with Ad.P60 or a control vector. After 8 days, Ad.P60 reduced PD-1+/CD8+ lymphocytes in spleen and lowered tumor infiltrating Tregs ($p< 0.05$). Then, we assess the direct effects of Ad.P60 in tumor cells, Transduction of breast tumor cells with Ad.P60 reduced cell viability and improved the response to cisplatin ($p<0.05$). Our results add evidence to support a protumoral role of Foxp3 in HER2+ breast cancer and TNBC. Considering that Foxp3 expression is associated with a poorer prognosis in these patients, Foxp3 could constitute a valuable target for the treatment and diagnosis of these tumors.

CIRCULATING GALECTIN-1 DELINEATES RESPONSE TO BEVACIZUMAB IN MELANOMA PATIENTS AND REPROGRAMS ENDOTHELIAL CELL BIOLOGY

Nadia Bannoud[1], Alejandro J. Cagnoni[2], Juan C. Stupirski[2], Pablo F. Hockl[2], Juan M. Pérez Saez[2], Pablo A. García[1], Yamil D. Mahmoud[2], Julián Gambarte Tudela[1], Marco Scheidegger[2], Karina V. Mariño[2], M. Romina Girotti[2], Diego O. Croci[1] and Gabriel A. Rabinovich[2,3]

[1]IHEM-CONICET-UNCUYO, Mendoza, Argentina. [2]IBYME-CONICET, Buenos Aires, Argentina. [3]Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

Blockade of vascular endothelial growth factor (VEGF) signaling with bevacizumab, a humanized anti-VEGF monoclonal antibody (mAb), has improved progression-free survival and, in some indications, overall survival across several types of cancers by interrupting tumor angiogenesis. However, the clinical benefit conferred by this therapy is variable, and tumors from treated patients eventually reinitiate growth. Previously we demonstrated, in mouse tumor models, that galectin-1 (Gal1), an endogenous glycan-binding protein, preserves angiogenesis in anti-VEGF resistant tumors by co-opting VEGFR2 signaling. However, the relevance of these findings in clinical settings is uncertain. Here we explored in a cohort of melanoma patients from AVAST-M [a multicentre, open-label, randomized controlled phase 3 trial of adjuvant bevacizumab (N=94) versus standard surveillance (N=95)], the role of circulating Gal1 as part of a compensatory mechanism that orchestrates endothelial cell programs in bevacizumab-treated melanoma patients. We found that increasing Gal1 levels over time in patients on the bevacizumab, but not on the observation arm, significantly increased their risks of recurrence and death ($p<0.0001$). Remarkably, plasma Gal1 was functionally active as it was able to reprogram endothelial cell biology, promoting migration ($p<0.05$) and tubulogenesis ($p=0.029$) in vitro. Interestingly, blockade of Gal1, using a newly developed fully human anti-Gal1 neutralizing mAb (mAb42), prevented these effects but only in patients under bevacizumab arm. Notably, exposure to mAb42 resulted in both anti-angiogenic and immunostimulatory effects, highlighting the dual benefits of Gal1 blockade. Thus, using samples from the larger-scale clinical trial from grade II-III melanoma patients, we validated the clinical relevance of Gal1, an endogenous lectin widely associated with angiogenesis and immunomodulation, as a potential resistance mechanism to bevacizumab treatment.

SAIC - IRENE FARYNA & ROBERTO RAVEGLIA AWARD *Saturday, November 19, 16-18 hr*
Juries: Edith Kordon, María Cristina Carrillo, Patricia Pennisi

PIN1 AS A MOLECULAR TARGET FOR GLIOBLASTOMA TREATMENT: TARGET VALIDATION, INHIBITORS RATIONAL DESIGN AND BIOLOGICAL ACTIVITY DETERMINATION

Julián Maggio[1;2], Georgina Alexandra Cardama[1;2], Lara Balcone[1], Román Nicolás Vilarullo[1;3], Romina Gabriela Armando[1], Daniel Eduardo Gomez[1;2], Diego Luis Mengual Gomez[1;2].

[1]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes, [2]CONICET, [3]CIN.

The Peptidyl-prolyl isomerase PIN1 controls diverse cellular functions and participates in all the biological processes linked to tumor development and progression in several cancer types, including glioblastoma (GBM). GBM is a lethal disease with poor therapeutic resources. Thus, this research aims to assess PIN1 as a molecular target for a new therapeutic alternative. First, we developed a PIN1 knockout GBM cell line model (LN PIN1 KO) using CRISPR/Cas9 on LN229 cells. This new generated cell model was evaluated in diverse cellular processes such as migration, cell cycle progression, cell immortality and tumoral progression. Results showed a significant decrease in LN229 oncogenic features both in *vitro* and in vivo assays due to PIN1 absence.

Once PIN1 was confirmed as a relevant molecular target, we proceeded to search for specific inhibitors by a docking-based virtual screening. We obtained a ranking of compounds from which we pre-selected 4 candidates that demonstrated the ability to bind to PIN1 in *vitro* in a Thermal Shift Assay. Following this, we evaluated the antiproliferative effect of selected compounds both in LN229 and LN PIN1 KO cells, to ensure that the effect was PIN1 dependent. Among all the candidates tested, compound 7 presented the greatest effect on LN229. This growth inhibition was also significantly reduced in absence of PIN1. Finally, we evaluated compound 7 on a panel of GBM cell lines which consistently inhibited proliferation among all the cell lines tested after 72hs exposure ($p<0.05$, T-test or ANOVA, GraphPad Prism). In conclusion, we demonstrated the key role of PIN1 in LN229 tumoral behavior, validating this protein as a relevant molecular target in GBM. Simultaneously, a list of candidate compounds was obtained with *in silico* and in *vitro* tests. From these candidates, a PIN1 inhibitor with specific biological activity was identified. This novel PIN1 inhibitor sets the bases for a promising therapeutic alternative for GBM treatment.

TARGETING OF MITOCHONDRIAL PEPTIDE HUMANIN TO IMPROVE CHEMOSENSITIVITY IN GLIOBLASTOMA CELLS

Jorge Armando Peña Agudelo[1], Matías Pidre[4], Antonela S. Asad[1], Matías García Fallit[1,2], Melanie Pérez Küper[1], Alejandro J. Nicola Candia[1], Leilane Glienke1, Sofía Sagripanti[1], Abril Marchesini[4], Leslie C. Amorós[4], Mariana Belén Vera[5], Nazareno González[1], Guillermo Videla Richardson[5], Adriana Seilicovich[1,3], Marianela Candolfi[1]

[1]nstituto de Investigaciones Biomédicas (INBIOMED, CONICET-UBA), Facultad de Medicina, Universidad de Buenos Aires, Argentina. [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. [3]Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad de Buenos Aires, Argentina. [4]Instituto de Biotecnología y Biología Molecular, Universidad Nacional de la Plata, Argentina. [5]Fundación para la lucha contra las enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina.

Humanin (HN) is a mitochondrial peptide with a robust cytoprotective many cell types. HN can interact with proteins of the bcl-2 family or be released and bind with two membrane receptors: a trimetric receptor, and the FPR-2 receptor. HN protects normal tissues from chemotherapy, and the administration of HN analogs has been proposed as a therapeutic approach for degenerative diseases. However, its role on the pathogenesis of cancer is poorly understood. Here we aimed to evaluate whether HN affects chemo-resistance of glioblastoma (GBM) cells. We first assessed the effect of chemotherapy on HN expression in murine (GL26) and human (U251) GBM cell lines, as well as in primary cultures from GBM biopsies. By immunofluorescence we observed that cisplatin upregulates HN in all the cells evaluated. To analyze the effect of HN on chemotherapeutic cytotoxicity, we used a HN analog peptide (HNG). In human GBM cells we observed that HNG abolished the cytotoxic and antiproliferative effect of cisplatin, restoring viability and clonogenic capacity (Two-way ANOVA $p<0.05$). Blockade HN interaction with the FPR-2 receptor, using a specific antagonist (WRW4), limited the cytoprotective function of both endogenous and exogenous HN in human GBM cells exposed to cisplatin, as assessed by MTT assay and BrdU incorporation (Two-way ANOVA $p<0.05$). To explore the effect of endogenous HN on GBM cell chemosensitivity, we developed a baculoviral vector encoding a HN-specific shRNA for the transcriptional silencing of its expression. These vectors showed excellent transduction efficiency in these cells. We observed that the inhibition of endogenous HN exerts an inhibitory effect on the viability of GBM cells and increases their sensitivity to cisplatin. Our study suggests that HN favors chemoresistance in GBM cells and that it could hold value as a therapeutic target to improve their response to conventional treatment.

4-METHYLUMBELLIFERONE AS A CHEMOSENSITIZER IN A GLIOBLASTOMA MODEL

Daniela Poodts[1], Martín Ledesma2, Magalí Ferreira[3], Carlos Vay[4], Silvia Hajos[1], Matías Pibuel[1], Silvina Lompardía[1].

[1]Instituto de Estudios de la Inmunidad Humoral (IDEHU, UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]Unidad de Conocimiento Traslacional, Hospital de Alta Complejidad del Bicentenario de Esteban Echeverría [3]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes. [4]Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires

4-Methylumbelliferone (4MU) is a coumarin derivative used in Europe and Asia as choleretic drug with no adverse effects. Many groups have reported its antitumor effects but little is known about its effects on glioblastoma (GBM). GBM is the most frequent malignant primary tumor in the CNS with a median survival of 14.6 months after diagnosis. Standard treatment includes tumor resection, radiotherapy and temozolomide (TMZ) as adjuvant chemotherapy. TMZ causes severe adverse effects and even 50% of patients are resistant. In these cases, there are little options, such as some cocktails that include vincristine (VCR). Therefore, it is imperative to find new therapies. Previously, we showed that 4MU inhibits cell proliferation, cell migration and MMP-2 activity, causing senescence on U251 and LN229 human GBM cell lines. In this work, we aimed to study the antitumor effect of 4MU on a chemoresistant model. We evaluated metabolic activity by XTT assay, cell proliferation by BrdU incorporation, lectin binding by flow cytometry and proteic profile analysis by MALDI-TOF-MS. We established two TMZ-resistant cell lines, U251-R and LN229-R, which exhibit distinct glycan and proteic profiles respect to their *wt* counterparts ($p<0.05$). 4MU decreased metabolic activity and cell proliferation on both resistant cells ($p<0.001$), similarly to the *wt* counterparts. Remarkably, 4MU sensitized both cell lines to TMZ effects ($p<0.0001$). On U251-R cells we observed the acquisition of resistance to VCR as well, which is also reduced when co-treating with 4MU ($p<0.05$). Finally, we observed that several proteins seem to be distinctly modulated on U251-R cells with respect to U251 *wt,* which could be involved in the resistant mechanisms, whereas treatment with 4MU seems to be able to revert such modulation back to the *wt* levels, in accordance with its chemosensitizing effects. Overall, our results highlight 4MU as a potential adjuvant chemotherapeutic alternative, even for TMZ-refractory patients.

TISSUE PROTEOMICS AND CUSTOM-MADE TRANSCRIPTOMIC ANALYSIS TOOL TO IDENTIFY PROGNOSTIC BIOMARKERS IN PROSTATE CANCER

Juan Bizzotto[1,2], Agustina Sabater[1,2,3], Pablo Sanchis[1,2], Sofia Lage-Vickers[1,2], Rosario Lavignolle[1,2], Pía Valacco[1,2], Elba Vazquez[1,2], Javier Cotignola[1,2], Geraldine Gueron[1,2]

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Buenos Aires, Argentina. [2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina. [3]Universidad Argentina de la Empresa (UADE), Instituto de Tecnología (INTEC), Buenos Aires C1073AAO, Argentina

Prostate cancer (PCa) is a progressive disease involving multiple molecular alterations. Some PCas that are histopathologically classified as intermediate risk can differ significantly in outcome. The aim of this study was to identify molecular biomarkers that could behave independently from the commonly used PCa histopathological grading, to improve risk prediction of PCa. An in-depth proteomic analysis (LC/ESI-MS/MS) was performed on human prostate adenocarcinoma and benign prostate hyperplasia (BPH) tissues. Results rendered 89 PCa enriched proteins compared with BPH. These proteins were further evaluated in-silico using a custom-made pipeline. For this purpose, differential expression analysis was performed using 12 publicly available transcriptomic PCa datasets (n=2.668). A Shiny-based R tool was then built to allow visualization across multiple datasets. Results depicted 58 candidate proteins highly expressed across PCa datasets. Multivariable survival analyses were then performed using clinicopathological parameters as covariates including histological grading and TMPRSS2-ERG fusion status. Of note, CRIP2 and POSTN displayed significant association with high risk of death, independently from the other co-variates across multiple PCa datasets ($p<0.05$). Further, MTPN, a PCa enriched gene, significantly outperformed the histological grading when predicting progression of disease within intermediate risk patients ($p<0.05$). Overall, our proteomics analysis yielded PCa enriched proteins, and our custom pipeline provided the tools to explore transcriptomic and survival data, while straddling the limits of individual studies. CRIP2, POSTN and MTPN rise as potential prognostic markers that showed significant association with poor prognosis, independent from known risk factors and predictors of PCa prognosis.

SAIC - FUNDACIÓN BIGAND AWARD FOR YOUNG INVESTIGATORS *Saturday, November 19, 16-18 hr*
Juries: Claudia Pérez Leirós, Daniel Gomez, Fernando Dominici

MOTHERS EXPOSED TO CHILDHOOD ADVERSITY EXHIBIT AN INSECURE INFANT-MOTHER ATTACHMENT STYLE AND ALTERATIONS IN DNA METHYLATION

Bruno G. Berardino[1], Ariel A. Berardino[2], Estefanía A. Fesser[1], Christine Mai[3], Julia Redondo[3], Pamela Scorza[3], Eduardo T. Cánepa[1]

[1]Laboratorio de Neuroepigenética y Adversidades Tempranas, Departamento Química Biológica, FCEyN, UBA – IQUIBICEN, CONICET, Buenos Aires, Argentina. [2]Fundación Instituto Leloir, CONICET, Buenos Aires, Argentina. [3]Columbia Population Research Center, Columbia University Medical Center, Columbia University, NY 10032, United States

Adverse childhood experiences (ACEs) can have negative effects on an individual's physical and mental health and further detrimental intergenerational consequences, producing an unfortunate cycle. Although little is known about how ACE exposure influences child development, epigenetic modifications, particularly DNA methylation (DNAm), constitute one of the potential transmission mechanisms. We aimed to find peripheral DNAm signatures related to ACEs in mothers and their offspring from a prospective longitudinal study at the *Hospital Fernández* in Buenos Aires, Argentina. ACEs were assessed using the ACE Study Questionnaire. Mothers with ACE 0 were assigned to control group (CTR) and mothers with ACE 1 or more, to childhood adversity group (ADV). Association between ACE and attachment style (Massie-Campbell Scale) was analyzed using a proportion Chi-square test. DNAm from maternal and fetal blood was evaluated with *Illumina EPIC Array*. The association between ACE and DNAm was examined using the MWW test for two samples, CTR (n=11) and ADV (n=13) for all gene-associated CpGs. We performed an enrichment analysis (Toppgene Suite) for genes neighboring the first 1000 CpGs order by increasing unadjusted p-value. ADV group was significantly associated with insecure attachment style. Altered DNAm between CTR and ADV groups mapped genes associated with biological processes related to neuron morphology and development for maternal and fetal samples. We analyzed the direction of change in methylation level between CTR and ADV for the altered CpG methylation and found that a significant proportion of genes matched the direction when compared to the expected matched frequency by chance. We suggest that DNAm may represent a potential biological transmission pathway for altered developmental consequences derived from childhood trauma. Furthermore, peripheral methylation measurement of candidate CpGs could be a molecular marker for this transmission.

MULTITARGET COMPOUNDS FROM ESSENTIAL OILS AS NOVEL ANTHELMINTIC DRUGS

Guillermina Hernando, Ornella Turani, Noelia Rodríguez Araujo and Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Argentina.

Essential oils (EOs) have a long history in traditional medicine all over the world. In this work we evaluated the anthelmintic potential of different EOs and their main constituents and deciphered the molecular bases of their activity. We use the nematode *Caenorhabditis elegans* as a model for anthelmintic drug discovery. We combined behavioral assays of wild-type and mutant worms with electrophysiological recordings from cultured cells to identify EOs with potential anthelmintic activity and to reveal the active components, their target sites, and mechanisms of action. We found that six different EOs produced rapid paralysis of worms with different potencies, showing EC50 values between 0.02-2%. All EOs also inhibited egg at similar concentrations than for adult stage, indicating that they can mediate both rapid and long-term anthelmintic effects. For the identification of EOs drug targets, we focused on ligand-gated ion channels (LGIC) involved in worm locomotion and behavior. By testing mutant worms, we identified the muscle GABA receptor and two nicotinic receptors, L-AChR and N-AChR, as targets of EOs and of a main component, trans-cinnamaldehyde (TC). Worms lacking glutamate-activated chloride channels (GLuClR) were partially resistant to TC whereas those lacking a serotonin-activated chloride channel (MOD-1) behaved as the wild-type. To confirm that the receptors are targets of TC, we performed whole-cell and/or single-channel recordings from *C. elegans* muscle cells and/or mammalian cells heterologously expressing worm receptors. We found that TC significantly reduced muscle L-AChR channel activity (∼55% at 100 μM), inhibited GluClR currents elicited by glutamate, and did not affect MOD-1 function. The analysis indicated that TC acts as an allosteric inhibitor of several LGICs that are conserved in nematodes. By modulating receptors with key roles in worm motility, EOs emerge as sources of multitarget compounds with high potential for anthelmintic therapies.

MICROGLIAL DYSTROPHY DURING AGE-ASSOCIATED DISEASES: POTENTIAL THERAPEUTIC ROLE OF METFORMIN IN ALZHEIMER'S DISEASE AND TYPE 2 DIABETES MELLITUS.

Carlos Pomilio[1], Nicolás González Pérez[1], Ángeles Vinuesa[1], Melina Bellotto[1], Ismael Calandri[2], Lucía Crivelli[2], Melisa Bentivegna[1], Amal Gregosa[1], Jessica Presa[1], Gustavo Sevlever[2], Juan Beauquis[1], Flavia Saravia[1].
[1]Instituto de Biología y Medicina Experimental (CONICET) y Departamento de Química Biológica (FCEyN-UBA).
[2]Centro de Memoria y Envejecimiento, Fleni, Buenos Aires, Argentina.

Alzheimer's disease (AD) is the leading cause of dementia and there is no effective cure available at day. Brain metabolism is early affected, and type 2 diabetes mellitus (T2D) is a strong risk factor for AD. Both pathologies negatively impact brain function, affecting cellular metabolism and causing chronic neuroinflammation by activating microglial cells, responsible for local immune response. We and others reported in association with aging, AD and T2D, that neuroinflammation is linked to loss of proteostasis and altered cell metabolism in microglial cells, altogether termed as microglial dystrophy. During the last years, it was reported that metformin, the first-line drug used for T2D treatment, exhibits pleiotropic effects on these alterations. So, this line of research was designed to characterize the cellular alterations related to dystrophy in microglial cells during AD and T2D and their potential reversion by metformin. Employing transgenic mice and cultured microglial cells as validated models for AD, we found increased activation, impaired autophagy, lysosomal dysfunction and protein aggregation in microglial cells from AD groups compared to controls. The impairment in autophagy was also evidenced in brain slices from AD patients. Microglial dystrophy was also evidenced in a mouse model of T2D by high-fat diet administration, and employing microglial cells exposed to palmitate, the main saturated fatty acid present in a western diet. In both cases, we found an increased neuroinflammatory response and microglial activation in association with decreased autophagic flux. In T2D mice, this condition also caused impaired spatial memory, and decreased brain insulin signaling compared to control mice. Interestingly, metformin administration in both in vivo and in vitro experimental models restored most of these alterations. Moreover, diabetic patients enrolled in the observational and multicenter study ADNI also showed a better cognitive performance in neuropsychological tests when they were treated with metformin compared to other antidiabetic drugs. Considering diabetic patients diagnosed with AD, metformin-treated patients showed a better cognitive profile, reduced pathological biomarkers for AD in CSF and reduced brain atrophy. Altogether these results suggest that metformin could be proposed as a potential therapeutic approach for brain dysfunction in AD and T2D, and that its mechanism of action could be partially mediated by reversing microglial dystrophy.

RED BLOOD CELLS MAY BE CRITICAL FOR CORONAVIRUS TO ORCHESTRATE A MULTIORGAN CONQUER

Ayelén Toro[1,2], Ana Paula Arevalo[3], Gaston Pascual[1,2], Agustina Sabater[1,2], Marianoel Pereira[4], Jorge Porfido[3], Sofia Lage-Vickers[1,2], Juan Bizzotto[1,2], Pablo Sanchis[1,2], Elba Vázquez[1,2], Javier Cotignola[1,2], Gonzalo Moratorio[4], Martina Crispo[3], and Geraldine Gueron[1,2]

[1]Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina; [2]CONICET-Universidad de Buenos Aires. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina; [3]Unidad de Biotecnología en Animales de Laboratorio, Institut Pasteur de Montevideo, Uruguay; [4]Laboratorio de Evolución Experimental de Virus, Institut Pasteur d e Montevideo, Uruguay.

Increasing evidence exhibits that long COVID is a multisystemic disease with a wide range of persistent symptoms whose manifestations vary broadly among patients and do not resolve over the course of time. Thus, it is imperative to delineate the cell types and anatomical sites in association with replication-competent forms of the virus to increase long COVID-19 treatment effectiveness. In this work, we analyzed single cell RNA-seq data from healthy donors, symptomatic and asymptomatic COVID-19 patients and evidenced multiorgan presence of viral receptors. Next, we assessed viral organotropism in association with clinical manifestations in a validated murine model of coronavirus infection. BALB/cJ mice were randomly distributed in 2 groups: non-infected (n=10) and infected (n=10). Mice were infected with the Mouse Hepatitis Virus (MHV, 6000 PFU), a single stranded RNA coronavirus. Five days post-infection, mice were euthanized and multiorgan dissection was performed for histological and viral load (VL) analyses. Peripheral blood samples were collected pre- and post-infection to determine biochemical and hematological parameters. Liver, lung and brain presented the higher VLs. Hepatic VL was positively associated with liver weight ($p<0.01$) and macroscopic organ alterations. Moreover, hepatic transaminase levels were increased in infected mice while albumin levels were reduced ($p<0.05$ and $p<0.05$, respectively). Further, infection was also associated with lower count of red blood cells (RBC) and hematocrit ($p<0.01$ and $p<0.01$, respectively). Blood fractionation was performed and VL was evaluated in plasma and RBC. Results showed the presence of viral genome in both fractions, identifying the higher VL levels in RBCs. In conclusion, our results evidence the multiorgan extent of coronavirus infection and reveal a viral association with RBC.

REPROGRAMMING THE IMMUNOSUPPRESSIVE MICROENVIRONMENT OF COLORECTAL CANCER BY TARGETING GALECTIN-GLYCAN LATTICES.

Alejandro J. Cagnoni

Laboratorio de Glicómica Funcional y Molecular, Instituto de Biología y Medicina Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas, C1428ADN Buenos Aires, Argentina; y Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas, C1428ADN Buenos Aires, Argentina.

Colorectal cancer (CRC) represents the third most common malignancy and the second leading cause of cancer-related deaths worldwide. Although immune checkpoint blockade therapies have achieved long-term responses in several malignancies, in CRC, patients' clinical benefit is only observed in heavily mutated tumors that are mismatch-repair-deficient or have high microsatellite instability. Thus, identification of novel immune escape mechanisms and design of additional immunotherapeutic modalities is required. Galectin-1 (Gal-1), an endogenous glycan-binding protein, induces tolerogenic programs and contributes to evasion of anti-tumoral responses. We found that Gal-1 confers immune privilege to CRC by increasing the frequency of CD8+ regulatory T cells (Tregs) and accentuating their immunosuppressive activity in the azoxymethane (AOM)-dextran sodium sulfate (DSS) model of colitis-associated CRC (CACRC). Furthermore, analysis of CRC patient datasets revealed a "poor prognosis signature" characterized by high Gal-1 expression and elevated Treg score. Transcriptomics analysis of AOM-DSS treated WT and Gal-1 KO (Lgals1-/-) mice revealed dysregulation of immune and angiogenesis-associated pathways in KO mice. Perturbation-response analysis of cancer-associated processes showed that these animals presented lower VEGF, hypoxia, TNF and NF-kB activation ($p<0.01$). Additionally, immune infiltrate deconvolution revealed a higher immunosuppressive tumor microenvironment in Lgals1-/- mice. To validate Gal-1 as a therapeutic target, we designed and biochemically characterized, a new neutralizing anti-Gal-1 monoclonal antibody (mAb-3). In contrast to anti-PD-1 mAb which showed no beneficial effect in the AOM-DSS model, therapeutic administration of mAb-3 resulted in a significant decrease in number of tumors and tumor volume and an increase in tumor-associated T cell responses. Thus, targeting Gal-1-glycan lattices may represent a promising immunotherapeutic modality for treating CACRC.

SAIC - CONSEJO DE GENÉTICA AWARD *Saturday, November 19, 14-16 hr*
Juries: Liliana Daín, Ezequiel Surace, Carlos De Brasi

IDENTIFICATION OF SINGLE NUCLEOTIDE VARIANTS AND FUSION GENES THROUGH TRANSCRIPTOMICS OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS FROM A MULTICENTER CLINICAL STUDY IN ARGENTINA.

María Sol Ruiz[1,2], Mercedes Abbate[1,2], Daniel Avendaño[1,2], María Cecilia Riccheri[3], Elba Vazquez[1,2], Geraldine Gueron[1,2], Javier Cotignola[1,2]

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina; [2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina. [3]Hospital Nacional Posadas, Servicio de Pediatría, El Palomar, Buenos Aires, Argentina.

Patients diagnosed with Acute Lymphoblastic Leukemia (ALL) are stratified into risk groups based on biochemical, cytogenetic and molecular signatures. While considerable progress has been made on treatment efficacy and survival rates, relapse is the most important cause for treatment failure and occurs in about 15-30% of patients. In approximately one-third of B-ALL patients, major pathogenic molecular abnormalities have yet to be identified. Aims: to characterize molecular profiles associated with disease outcome and improve molecular classification of ALL patients from Argentina. Methods: we studied single nucleotide variants and fusion genes in 39 pediatric ALL patients that are part of a national multicenter clinical protocol (ALL IC GATLA 2010), through transcriptome sequencing of bone marrow samples at diagnosis. RNAmut software was used to evaluate SNVs/InDels in 114 selected genes and 79 selected fusion genes in RNA-seq data. Discovery of fusion genes was complemented with STAR-Fusion software. Variants were analyzed and filtered based on an in-house pipeline. Variants were assessed in silico through Variant Effect Predictor, OncoKB, Varsite, ProteinPaint and COSMIC-3D. Confirmation was performed by RT-PCR and Sanger sequencing. Results: We found a total of 9,594 variants. After annotation and filtering, 20 SNVs/InDels were identified in 11/34 patients (32.3%). Variants that had not been previously described in ALL were found in CREBBP, CSF3R, ETV6, TP53, ATM and DUX4. The group of patients harboring point mutations had significantly lower relapse-free survival ($p<0.05$). Fusion genes previously reported in ALL were found in 8/34 patients (23.5%). Combination of RNAmut and STAR-Fusion increased sensitivity of fusion gene detection. Conclusions: This is the first comprehensive molecular characterization of pediatric ALL patients in Argentina. Transcriptomic sequencing allowed the simultaneous identification of multiple molecular features of clinical relevance.

GENERATION AND CHARACTERIZATION OF IPSCS FROM A PATIENT WITH MUSCULAR DYSTROPHY WITH A MISSENSE MUTATION IN FHL1 (P.C126T) FOR IN VITRO DISEASE MODELING AND PERSONALIZED THERAPY DEVELOPMENT

Castañeda Sheila[1], Zabalegui Federico[1], Amín Guadalupe[1], Belli Carolina Bárbara[1], Waisman Ariel[1], La Greca Alejandro Daniel[1], Sevlever Gustavo Emilio[1], Miriuka Santiago Gabriel[1], Moro Lucía Natalia[1].

[1]Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Instituto de Neurociencias (INEU)-CONICET.
[2]Instituto de Medicina Experimental (IMEX), Academia Nacional de Medicina (ANM)-CONICET.

The *FHL1* gene locates in the Xq26 region, encodes four and a half LIM domain protein 1 and is expressed in skeletal and cardiac muscle. It has been related to cytoskeletal remodeling, myoblasts differentiation, sarcomere assembly and autophagy regulation. Mutations in *FHL1* are related to muscular dystrophy (MD) with a limited life expectancy. The aim of this work was to generate and characterize an induced pluripotent stem cells (iPSCs) line derived from a patient with MD (MD-iPSC) that carries a pathogenic heterozygous missense mutation in *FHL1* (c.377G>A, p.C126T) for *in vitro* disease modeling and personalized therapy development. *In silico* analysis revealed a misfolding of FHL1 p.C126T protein. To generate MD-iPSC, a blood sample was taken from the patient and erythroblasts were reprogrammed by transduction with STEMCCA lentiviral vector. After iPSCs clonal isolation and expansion, we confirmed the c.377G>A mutation, STEMCCA silencing and normal karyotype of MD-iPSCs. For pluripotency validation, alkaline phosphatase activity and pluripotency genes expression were assessed. Moreover, MD-iPSC was capable of differentiating into cells of the three germ layers by embryoid body formation. MD-iPSC derived cardiomyocytes were also obtained and characterized by observing high expression of cardiac markers by RT-qPCR, immunohistochemistry, and western blot by day 23 of differentiation. As gene editing could be a possible *in vivo* therapy, we corrected the patient mutation in MD-iPSC by CRISPR-mediated homologous recombination. In summary, MD patient-derived iPSC line was efficiently generated and differentiated to muscle cells (cardiomyocytes), and the pathogenic mutation was corrected by CRISPR. With these results,

we are able to study the patient's disease *in vitro* and develop a personalized therapy. In this sense, we are generating associated adenovirus (AAV) particles as a vector of the CRISPR system to transduce the patient cardiomyocytes simulating an *in vivo* therapy.

PNPLA6 AS A NOVEL GENE IN THE ETIOLOGY OF COMBINED PITUITARY HORMONE DEFICIENCY

Maria Andrea Camilletti[1,2], Sebastián Alexis Vishnopolska[1,2], Lucía Candela Iglesias García[1], Julian Martinez-Mayer[1], Michelle Brinkmeier[3], Roxana Marino[4], Pablo Ramírez[4], Natalia Pérez Garrido[4], Marta Ciaccio[4], Maria Isabel Di Palma[4], Alicia Belgorosky[4,6], Robert Hufnagel[5], James Liu[5], Marcelo Adrián Marti[2], Jacob Otto Kitzman[3], Sally Ann Camper[3] and María Inés Perez-Millán[1]

[1]Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. [2]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Universidad de Buenos Aires, Argentina. [3]Dept. of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48198-5618 USA. [4]Servicio de Endocrinología, Hospital de Pediatría "Prof. Dr. J. P. Garrahan", Buenos Aires, Argentina. [5]Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, 20892, USA. [6]Consejo Nacional Cientifico Tecnologico, Buenos Aires, Argentina (C.O.N.I.C.E.T)

Patatin-like phospholipase domain-containing protein 6 (PNPLA6) (also known as Neuropathy Target Esterase NTE) is a conserved phospholipase, first described for its role in neuronal degeneration in *Drosophila*. In humans, loss of function of NTE is associated with rare diseases such as Oliver-McFarlane syndrome, characterized by trichomegaly, hair anomalies, retinal degeneration and/or hypopituitarism. The expression of *PNPLA6* in human embryonic pituitaries has been documented, however its specific role during pituitary development remains unknown. Panel sequencing of a large cohort of pediatric patients with hypopituitarism, allowed the identification of a novel, likely pathogenic variant in *PNPLA6*(c.3343C>A) in a child with Combined Pituitary Hormone Deficiency (CPHD), retinitis pigmentosa, long face and bushy eyelashes. The *PNPLA6;*c.3343C>A variant was found in heterozygosity in the child and the healthy mother, and was predicted to result in replacement of a Threonine by a Proline at amino acid position 1115 (p.T1115P) likely disrupting the patatin-like domain, critical for esterase activity. *In vitro* testing of NTE enzymatic activity in HEK293T cells transfected with p.T1115P variant showed a significantly reduced activity compared with cells transfected with WT PNPLA6. However, as reported variants in PNPLA6/NTE were described as homozygous or compound heterozygous, we hypothesized that an additional variant was missing and conducted Whole-Genome Sequencing of the trio. Interestingly, we found a splicing variant in *PNPLA6*(c.3428-44G>A),in the patient and its father, predicted to add 14 amino acids in-frame to the protein. The effect of the variant on splicing was confirmed by RT-PCR on RNA extracted from blood samples. Next, we explored the role of PNPLA6 in pituitary development by analyzing its expression in WT mice embryos at different embryonic stages and after birth. We found PNPLA6 is expressed in Rathke's pouch from embryonic day 10.5 to 15.5 and becomes stronger postnatally (P0). Double IHC assays against pituitary transcription factor SOX2 revealed PNPLA6 is expressed in SOX2+ cells, suggesting a role for PNPLA6 in pituitary stem cells. In addition, the expression of PNPLA6 in hormone expressing cells was evaluated by colocalization with pituitary hormones at P0 and in adult pituitaries, showing that PNPLA6 is mostly expressed in GH-cells. Collectively, our results provide supporting evidence that PNPLA6 is involved in pituitary development, and thus, mutations in PNPLA6 are likely the cause of CPHD in our patient. Our findings expand the spectrum of phenotypes involved in PNPLA6-opathies and are of particular importance for the precise molecular diagnosis of hormonal deficiencies.

SAIC - HORACIO REPETTO AWARD *Saturday, November 19, 15-16 hr*
Juries: Fernando Polack, Mauricio Caballero, Fernando Ferrero, Mar²a Marta Amaral

INHERITED PULMONARY SURFACTANT METABOLISM DISORDERS IN ARGENTINA: DIFFERENCES BETWEEN PATIENTS WITH SFTPC AND ABCA3 VARIANTS

Camila Mallie[1], Juan Balinotti[1, 2], Alberto Maffey[1], Alejandro Colom[1], Ralph Epaud[3], Alix De Becdelievre[4], Pascale Fanen[4], Céline Delestrain[3], Martín Medín[5], Alejandro Teper[1]

[1]Centro Respiratorio, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [3]Centro Hospitalario Intercomunal de Créteil, Servicio de Pediatría General, Centre de Références des maladies respiratoires rares, Creteil, Île-de-France, Francia. [4]Hospital Henri Mondor, Departamento de Genética, Creteil, Île-de-France, Francia. [5]Servicio de Patología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

Background: patients with inherited pulmonary surfactant metabolism disorders have a wide range of clinical outcomes and imaging findings. Response to current anti-inflammatory therapies has been variable and efficacy is unclear. Objective: to describe and compare genetic, clinical, histological, and computed tomography (CT) outcomes in a cohort of patients with variants in the genes encoding surfactant protein C (SFTPC) or adenosine tri-

phosphate-binding cassette transporter A3 (ABCA3) in Argentina. Methods: observational cohort retrospective study. Patients carrying variants in genes encoding SP-C and ABCA3 proteins were included. Results: 14 patients met the inclusion criteria: SFTPC n=6, ABCA3 n=8 (seven were heterozygous and one compound heterozygous). Neonatal respiratory distress was more frequent and severe in neonates with variants in the ABCA3 gene. The onset of the disease occurred in infancy before the age of 20 months in all cases. Patients with ABCA3 pathogenic variants had a severe clinical course, while long term outcomes were more favorable in individuals with SFTPC variants. Initial CT findings were ground glass opacities and intraparenchymal cysts in both groups. Over time, signs of lung fibrosis were present in 57% of patients with ABCA3 variants and in 33% of the SFTPC group. The efficacy of anti-inflammatory interventions appears to be poor, especially for patients with ABCA3 pathogenic variants. Conclusions: clinical, histological, and radiological features are similar in patients with SFTPC and ABCA3 variants, however the latter have more severe clinical course. Current anti-inflammatory regimens do not appear to stop the progression of the disease.

SAIC - EUGENIA SACERDOTE DE LUSTIG AWARD *Saturday, November 19, 14-15 hr*
Juries: Elisa Bal de Kier Joffé, Geraldine Guerón, Ignacio Demarco

IMPORTANCE OF BIOIMAGING IN ASSESSING THE PRECLINICAL SAFETY AND *IN VIVO* BIODISTRIBUTION OF COVIFAB, AN RBD-SPECIFIC F(AB′)2 FRAGMENT DERIVED FROM EQUINE POLYCLONAL ANTIBODIES.

Facundo Salinas [a, 1], Belkis E. Marelli [a, b, 1], Santiago Sanguineti [c], Fernando Goldbaum [c,d,e], Luciana Muñoz [c], Lucas Etchevers [a], Paula Silvestrini [a], Ulises S. Notaro [a], Natalia R. Salvetti [a,b], Vanesa Zylberman [c,f,2], Hugo H. Ortega [a,b,*,2]

[a] Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Esperanza, Santa Fe, Argentina. [b] Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina. [c] INMUNOVA S.A., Av. Patricias Argentinas 435, Ciudad de Buenos Aires C1405BWE, Argentina. [d] CRIP-Centro de Rediseño e Ingeniería de Proteínas UNSAM, Campus Miguelete, San Martín, Provincia de Buenos Aires, Argentina. [e] Laboratorio de Inmunología y Microbiología Molecular, Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina. [f] Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET), Argentina.[1] F Salinas and BE Marelli should be considered sa joint first authors. [2] V Zylberman and Ortega HH should be considered as joint senior authors.

The urgency of COVID-19 treatments precluded traditional drug discovery pathways, given their long times, highlighting the need for suitable tools to accelerate the process. In this study, preclinical biodistribution and safety of an antibody-based treatment were assessed under good laboratory practices with the aim of validating an *in vivo* bioimaging approach as a tool for improve drug studies. CoviFab (INM005), F(ab′)2 fragment derived from equine polyclonal antibodies labeled with IRDye® 800CW, was administered intravenously at a dose of 4 mg/kg in male BALB/cCmedc mice, 6-7 weeks old, 21+/-1.5 g at 0 and 48h. Mice were imaged *in vivo* at different times after injections with the Pearl® Trilogy Imager LICOR imaging system in the near infrared (NIR). At 96 and 144h, mice (n=6) were sacrificed for *ex vivo* imaging and hematological, serum, pathological and histopathological analyses. CoviFab was rapidly localized *in vivo* in all regions analyzed. In liver and ears (metabolization and distribution, respectively), fluorescence was higher than basal throughout the study. In kidney and bladder, it was clearly visualized 24h after each injection. No toxicological or macroscopic changes were observed in the animals. Relative organ weights were similar in treated and control animals. The *ex vivo* study supports the *in vivo* biodistribution data, confirming that CoviFab remains in circulation for more than 144h after the first administration (96h after the second), consistent with that described for others mono and polyclonal antibodies. Therefore, fluorescence in the lungs demonstrates the arrival of the drug at the target organ of the virus. CoviFab was considered safe, with no observable adverse effects and *in vivo* and *ex vivo* results demonstrate its localization and permanence in organs of interest for COVID-19. In agreement with previous studies, these results reaffirm that *in vivo* bioimaging studies could be strong predictors of biodistribution during the drug development.

COVID-T: A FUNCTIONAL PLATFORM TO MONITOR SARS-COV-2-SPECIFIC T CELL RESPONSES IN VACCINATED INDIVIDUALS AND COVID-19 RECOVERED PATIENTS

Montana N. Manselle Cocco[*1], Florencia Veigas[*1], Yamil D. Mahmoud[1], Tomas Dalotto Moreno[1], Alejandro J. Cagnoni[1], Ada G. Blidner[1], Alejandro Benatar[1], Pablo Hockl[1], Juan C. Stupirski[1], Alicia Sirino[2], Valeria Wiersba[3], Nicolas I. Torres[1], Camila A. Bach[1], Marco Scheidegger[1], Marina Pifano[4], Jorge Geffner[5], Gabriel A. Rabinovich[1]

**These two authors contributed equally to this work. [1] Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [2] Hospital General de Agudos Doctor Ignacio Pirovano, Buenos Aires, Argentina. [3] Hospital Universitario Austral, Pilar, Provincia de Buenos Aires, Argentina. [4] Ministerio de Salud de Provincia de Buenos Aires, Argentina. [5] Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires, CONICET, Ciudad de Buenos Aires, Argentina.*

T cell responses emerged as major determinants of the clinical outcome of SARS-CoV-2 infection, underpinning long term protection and vaccine efficacy. Whereas viral variants may escape neutralizing antibodies, T cell responses encompass broad recognition of viral epitopes, providing cross-protection against emerging mutations. To assess the nature, magnitude and persistence of SARS-CoV-2-specific T-cell immunity in vaccinated individuals and recovered patients, we developed 'COVID-T', a multiparametric and versatile T-cell monitoring platform. Briefly, isolated peripheral blood mononuclear cells are cultured with or without SARS-CoV-2 peptide pools. Next, flow cytometry is performed to identify CD4+ and CD8+ T cells reactive to these peptides by analyzing cytokine production and activation markers. During optimization, a machine learning selection algorithm was applied, revealing the importance of CD154+TNF+ and TNF+IL-2+ T cells in delineating SARS-CoV-2-specific T-cell responses. First, we studied T-cell responses in 292 individuals vaccinated with different strategies and schemes at different time periods. Initial findings revealed a significant increase in CD154+TNF+ and TNF+IL-2+ CD4 T cells in individuals receiving a single dose of Sinopharm (inactivated virus) ($p<0.05$ vs non-immunized). Likewise, a significant rise in T cell response was observed in individuals fully immunized with adenoviral-based Sputnik V vaccine ($p<0.05$ vs non-immunized). Moreover, heterologous schemes comprising a broad range of second dose vaccines, revealed no significant differences among groups. More recently, a comprehensive longitudinal analysis of the nature and magnitude of T cell responses elicited by relevant vaccination strategies has been conducted. Thus, during the COVID-19 pandemic, COVID-T emerged as a robust and reliable platform to monitor SARS-CoV-2-specific T cell responses, offering critical information to optimize public health decisions and develop vaccine trials.

SAI - LEONARDO SATZ AWARD *Saturday, November 19, 16-19:30 hr*
Juries: Norberto Zwirner, Diego Croci, Susana Salva

THE GLYCOLYTIC STATUS OF MONOCYTES DURING TUBERCULOSIS LIMITS THE GLYCOLYSIS-POWERED MIGRATORY CAPACITY OF DENDRITIC CELLS IN RESPONSE TO MYCOBACTERIUM TUBERCULOSIS

Mariano Maio[1], Marine Joly[1,2], Zoi Vahlas[2], Joaquina Barros[1], José Luis Marín Franco[1], Melanie Genoula[1], Federico Fuentes[1], Mónica Vermeulen[1], Rafael J Argüello[4], Sandra Inwentarz[3], Rosa Musella[3], Lorena Ciallella[3], Pablo González Montaner[3], Domingo Palmero[3], María del Carmen Sasiain[1], Christel Verollet[2], and Luciana Balboa[1,*]

[1]Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. [2]Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, Toulouse, France. [3]Instituto Prof. Dr. Raúl Vaccarezza, Hospital de Infecciosas Dr. F.J. Muñiz, Buenos Aires, Argentina. [4]Aix Marseille Univ, CNRS, INSERM, CIML, Centre d´Immunologie de Marseille-Luminy, Marseille, France

Mycobacterium tuberculosis (Mtb) is a pathogen that interferes with dendritic cells (DCs) functions impairing the development of adaptive immunity. Herein we wondered how the metabolic pathways influence DCs activation in Tuberculosis (TB). We found that Mtb-stimulated DCs derived from monocytes of healthy donors displayed a predominant glycolytic profile, together with an increase in the expression of HIF1a and glycolytic genes ($p<0.05$). The inhibition of either HIF1a or glycolysis resulted in a low 3D-migration throughout collagen and a reduced chemotaxis towards CCL21 by Mtb-stimulated DCs, without harnessing their ability to activate Mtb-specific T cells ($p<0.05$). We also demonstrated that either dexamethasone-induced tolerogenic DCs or DCs differentiated from monocytes of TB patients are deficient at triggering glycolysis and at initiating migration in response to Mtb ($p<0.05$). Interestingly, by promoting HIF1a activity with DMOG, DCs restored their glycolytic activity and chemotactic activity ($p<0.05$). Based on these results, we focused on dissecting the metabolic profile of monocytes (DCs precursors) and found that CD16+ monocytes from TB patients display a higher glycolytic capacity compared to healthy donors ($p<0.05$). Since we have previously demonstrated that CD16+ monocytes from TB patients generate aberrant DCs, we hypothesize that the premature glycolytic status exhibited by this subset will give rise to DCs with an altered glycolytic activity and a lower migration capacity. For that purpose, we prematurely

activated HIF1a during the first 24hrs of differentiation of healthy monocytes and found that the resulting DCs exhibited a lower migratory capacity as well as low glycolytic induction in response to Mtb, resembling DCs from TB patients (p<0.05). In conclusion, we demonstrated that glycolysis plays a major role in DCs migration, and its modulation appears as a promising tool to develop therapeutic strategies to manipulate immune responses.

FLAGELLIN H7 AS A POTENTIAL VACCINE COMPONENT TO PREVENT H EMOLYTIC UREMIC SYNDROME

Alan Mauro Bernal1, Fernando Nicolás Sosa1, María Florencia Todero1, Mónica Vermeulen[1], Daniela Romina Montagna[1], Romina Jimena Fernández-Brando[1], María Victoria Ramos[1], Agustina Errea[2], Martín Rumbo[2], Marina Sandra Palermo[1]

[1]Instituto de Medicina Experimental – CONICET – Academia Nacional de Medicina, Ciudad Autónoma de Buenos Aires, Argentina. [2]Instituto de Estudios Inmunológicos y Fisiopatológicos – CONICET – CCT LA PLATA, La Plata, Buenos Aires, Argentina.

Argentina has the highest global incidence of hemolytic uremic syndrome (HUS), a systemic complication secondary to Escherichia coli O157:H7 (EHEC) infections. Local humoral response plays a key role in controlling intestinal colonization of EHEC and preventing HUS. Thus, the objective of this work was to analyze the ability of flagellin H7 (H7) to induce a local protective response. For this, BALB/c mice were intranasally immunized with 3 doses of purified H7 (10 μg/mouse) at 10-day intervals. At different times, stools and serum samples were taken to analyze the levels of anti-H7 antibodies by ELISA. 21 days post 3 rd immunization (dp3i) we studied ex vivo the specific proliferation of CFSE-stained lymphocytes from spleen (S) and lung-draining lymph nodes (LN) of immunized mice versus controls. The production of cytokines was measured in the proliferation supernatants to determine Th1/Th2/Th17 profile. To assess protection, immunized and control mice were challenged orally with 10 5 colony-forming units of EHEC 21 dp3i. We observed significant levels of IgG and IgA anti-H7 in plasma and feces, respectively, in immunized mice at all times evaluated from the 7th day after the 2 nd dose (p<0.05). We observed specific proliferation only in lymphocytes from the S and LN of immunized mice compared to controls (LN MFI CFSE: 39.5±5.5 vs 95.3±6.2, p<0.001; S MFI CFSE: 48.6±9.1 vs 95.1±16.8, p<0.05 immunized vs controls). Also, significant levels of IL-4, IL-17 and IFN-γ were detected in these supernatants (p<0.01). When challenged, all immunized mice survived up to 15 days post-infection (dpi) (p<0.01), with basal levels of urea (p<0.0001) and slight neutrophilia (p<0.001) compared to controls that died by 8 dpi with increased urea levels and neutrophilia. These results show that H7 is an immunogen capable of eliciting a specific humoral, Th1/Th2/Th17 cellular and protective response, suggesting its inclusion in a potential human vaccine.

DISCOVERY OF A NOVEL ADJUVANT FOR ORAL VACCINE FORMULATIONS

Celeste Pueblas Castro [1] , Lorena Coria [1] , Laura Bruno [1] , Lucas Saposnik [1] , Philippe Hermand [2] , Stéphane Temmerman [2] , Karina Pasquevich [1] , Juliana Cassataro [1] .

[1]Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, Buenos Aires, Argentina [2]GSK, Rixensart, Belgium

In our laboratory, we have shown that U-Omp19 from Brucella spp. is an oral vaccine adjuvant because it can inhibit gastrointestinal proteases while it induces adaptive immune responses. To our knowledge there are no other reports describing the use of protease inhibitors from bacteria as vaccine adjuvants. Thus, to discover new oral adjuvants we performed an in-silico and in vivo screening of different bacterial protease inhibitors where Ecotin from Salmonella spp. was selected as a candidate. Here, we evaluate if this protease inhibitor could be used as an adjuvant for oral vaccines. For this aim, we orally immunized BALB/c or C57BL/6 mice with two different viral antigens (Ags) simultaneously or both co-administered with Ecotin. When co-administered with the Ags, Ecotin induced a sustainable specific IgA response in feces compared to the administration of Ags alone (p<0.05 t test). This was evaluated by ELISA measuring specific IgA normalized by total IgA in feces. Ecotin also induced higher levels of specific IgG and IgA at serum compared to the groups of Ags alone by ELISA (p<0.05 t test). We evaluated B and T cells by flow cytometry and ELISPOT 30 and/or 53 days after last dose. We found that Ecotin induced higher levels of specific IgA + B cells and plasmablasts at Peyer's patches, specific IgG + cells at bone marrow and specific CD19 + B220 + IgD - CD38 + at spleen (p<0.05 t test). When T cells were evaluated, we found higher percentage of T follicular helper cells at Peyer's patches and specific CD4 + IFN +, CD4 + IL-17 + and CD8 + IL-2 + cells at mesenteric lymph nodes in Ags+Ecotin, compared with Ags alone (p<0.05 t test). Altogether, these results show that Ecotin could be used as adjuvant for oral vaccines, since it is able to induce a specific sustainable adaptative immune response when co-administrated with two different antigens. Further evaluation with other antigens and other routes of immunization are needed to shed light over the efficacy of this adjuvant.

THE TUMOR MICROENVIRONMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): VENETOCLAX RESISTANCE INDUCED BY T CELLS.

Valeria Judith Sarapura Martinez[1], Brenda Buonincontro[1], Chiara Cassarino[1], Juliana Bernatowiez[1], Ana Colado[1], Gregorio Cordini[1,2], Rosario Custidiano[3], Carolina Mahuad[4], Miguel Pavlovsky[5], Paula Soledad Pérez[6], Matias Ostrowski[6], Mónica Vermeulen[1,7], Mercedes Borge[1,7], Mirta Giordano[1,7] and Romina Gamberale[1,7]

[1]Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM), Buenos Aires, Argentina. [2]Hospital de Clínicas, José de San Martín, UBA, Buenos Aires, Argentina. [3]Instituto Alexander Fleming, Buenos Aires, Argentina. [4]Hospital Alemán, Buenos Aires, Argentina. 5FUNDALEU, Buenos Aires Argentina. [6]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), UBA-CONICET, Buenos Aires, Argentina. [7]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, UBA, Buenos Aires, Argentina.

CLL cells survive and proliferate in lymphoid tissues surrounded by activated T cells, myeloid cells and stroma. Venetoclax (VEN) is a BCL-2 inhibitor used for CLL treatment. We have previously reported that autologous activated T cells (aaT cells) generate VEN-resistant (VEN-r) CLL cells and that sphingosine kinases (SphKs) are involved in CLL cell survival. We aimed to characterize the factors involved in the generation of VEN resistance and to evaluate whether SphK inhibitors, SKI-II and opaganib, can reduce it. VEN-r cells were generated with PBMC from CLL patients cultured for 48h with anti-CD3 (aCD3) and then with VEN for 24h. SphK inhibitors were present during all the culture or once VEN-r cells were generated. Purified CLL cells (pCLL) were cultured for 48h with supernatants from aCD3-stimulated PBMC cultures, activated purified CD4 + T cells (pCD4 +) or extracellular vesicles (EVs) produced by pCD4 + cells, and then with VEN. The survival and activation of CLL (CD86, PD1 expression) and T cells (CD40L, CD69 expression) were evaluated by flow cytometry and the expression of SphKs by western blot in viable pCLL. We found that aaT cells generate VEN-r CLL cells through secreted factors and by direct cell contact ($p^{-}0.05$, n= 6). Activated pCD4 + T cells and EVs produced by pCD4+ T cells generate VEN resistance ($p<0,01$, n=7) and EVs increase CD86 ($p<0,001$, n=10) and PD1 expression on leukemic cells ($p<0,05$, n=8). AaT cells enhance SphK2 expression in CLL cells ($p<0,01$, n=9), which is higher in VEN-r cells ($p<0,05$, n=9). SphK inhibitors reduce T cell and CLL activation ($p<0,05$, n=8), impair the generation of VEN resistance ($p<0,001$, n=20) and, in combination with VEN, favor VEN-r CLL cell death ($p<0,05$, n=3). Conclusion: CD4 + T cells participate in the generation of VEN-r CLL cells by cell contact and secreted factors, including EVs. Given that SphK inhibitors reduce VEN resistance, a combined therapy may be a promising treatment option for CLL patients in the future.

SAI - INMUNMOLOGÍA CLÍNICA AWARD *Saturday, November 19, 16-19:30 hr*
Juries: Eliane Piaggio, Oscar Bottasso, Marta Toscano

COVID-19 VACCINATION RESPONSES WITH DIFFERENT VACCINE PLATFORMS IN PATIENTS WITH INBORN ERRORS OF IMMUNITY

Lorenzo Erra[1], Ignacio L. Uriarte[2], Ana Colado[3], María Virginia Paolini[4], Gisela Seminario[5], Julieta Fernández[1], Lorena Tau[6], Juliana Bernatowiez[3], Ileana Moreira, MD,[5] Sebastián Vishnopolska[1], Martín Rumbo[6], Chiara Cassarino[3], Gustavo Vijoditz[7], Ana Laura López[4], Renata Curciarello[6], Diego Rodríguez[2], Gastón Rizzo[6], Malena Ferreyra[6], Leila Romina Ferreyra Mufarregue[7], María Noel Badano[3], María Inés Pérez Millán[1], María Florencia Quiroga[8], Patricia Baré[3], Itatí Ibañez[9], Roberto Pozner[3], Mercedes Borge[3], Guillermo Docena[6], Liliana Bezrodnik[5], María Belén Almejun[1]

[1]Departamento de Fisiología, Biología Molecular y Celular, Instituto de Biociencias, Biotecnología y Biología Traslacional (IB3) e IQUIBICEN, FCEN, UBA, CONICET, CABA, Argentina, [2]Escuela Superior de Medicina, Universidad Nacional Mar del Plata - Hospital Interzonal Especializado Materno Infantil Don Vitorio Tetamanti, Mar del Plata, Buenos Aires, Argentina, [3]Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina, CABA, Argentina, [4]Hospital General de Agudos C. G. Durand, CABA, Argentina, [5]Centro de Inmunología Clínica. CABA, Argentina, [6]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), UNLP, CONICET, asociado a CIC PBA, Facultad de Ciencias Exactas, Departamento de Ciencias Biológicas, La Plata, Argentina y Laboratorio de Salud Pública de la Facultad de Ciencias Exactas, UNLP, La Plata, Argentina, [7]Hospital Nacional Profesor Alejandro Posadas, Buenos Aires, Argentina, 8Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), CONICET, CABA, Argentina, 9Instituto de Química Física de los Materiales, Medio Ambiente y Energía (INQUIMAE), CONICET, FCEN, UBA, CABA, Argentina.

Patients with Inborn Errors of Immunity (IEI) in Argentina were encouraged to receive licensed Sputnik, AstraZeneca, Sinopharm, Moderna, and Pfizer vaccines, even though most of the data on available vaccines comes from trials conducted in healthy individuals. We aimed to evaluate the safety and immunogenicity of the different vaccines in IEI patients in Argentina. The study cohort included adults and pediatric IEI patients (n=118) and age-matched healthy controls (HC) (n=37). Samples were collected before, 28+/-3 days after the first and the second dose of the vaccine. B-cell response was evaluated by measuring IgG anti-Spike(S)/RBD and anti-nucleocapsid(N) antibodies by ELISA. Neutralization antibodies were also assessed with an alpha-S protein-expressing

pseudo-virus assay. The T-cell response was analyzed by IFN-γ secretion on S or N-stimulated PBMC by ELISPOT and the frequency of S-specific circulating T follicular-helper cells (TFH) was evaluated by flow cytometry. No moderate/severe vaccine-associated adverse events were observed. Regarding the antibody response, anti-S/RBD titers showed significant differences in both pediatric and adult IEI patients versus the age-matched HC cohort ($p<0.05$). Neutralizing antibodies were detected in 71/99 patients and were also significantly lower in the patient cohort than age-matched HC ($p<0.01$). Positive S-specific IFN-γ response was observed in 84.5% of IEI patients and 82.1% presented S-specific TFH cells. In conclusion, COVID-19 vaccines showed safety in IEI patients and, although immunogenicity was lower than HC, they showed specific anti-S/RBD IgG, neutralizing antibody titers and T-cell-dependent cellular immunity with IFN-γ secreting T-cells. These findings may guide the recommendation of vaccination in IEI patients to prevent COVID-19 disease.

EVOLUTION OF CRITICAL IMMUNE PARAMETERS IN HOSPITALIZED COVID-19 PATIENTS

Yanina Luciana Mazzocco1,2, Gastón Bergero1,2, Sebastián Del Rosso1,2, Natalia Eberhardt2, Andrés Castellaro1,2, Denise Ochoa[1,2], Alex Saka[1,2], Claudia Sola[1,2], Sofía Villada[3], José Luis Bocco[1,2], Maria Pilar Aoki[1,2].

[1]Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina, [2]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Córdoba, Argentina, [3]Hospital Privado, Córdoba, Argentina.

We have reported that IL-6 released early after a systemic infection exerts a potent anti-oxidative effect that regulates the lethal release of nitric oxide (NO). Recently, we demonstrated that monocyte-derived NO causes protein nitration of CD8 + T-cells limiting CD8-cytotoxic effector functions. The present work aims to determine the evolution of the immune response in hospitalized COVID-19 patients focusing on IL-6 and NO production. To this aim, we comparatively study the immune profile of peripheral blood samples from 27 adult patients (18 male and 9 female) obtained at hospital admission and discharge with moderate pneumonia and positive serology for SARS-CoV2 infection. Patients with No-COVID-19 pneumonia (6 male and 5 female) were used as control. Univariate linear models adjusted for potential confounders showed that higher levels of IL-6, TNF-α, IL- 8, and IL-10 significant predicted longer hospitalization time. At admission, NO production by CD14 + CD16 + monocytes inversely correlated to IL-6 levels while, at discharge, this correlation was reverted for both NO + CD14 + CD16 + and NO + CD14 ++ CD16 - cell populations. In line with this, principal component analysis showed that NO production properly discriminated COVID patients from controls. Compared to discharge, at admission there was a higher frequency of nitrated (NT) CD8 + T-cells ($p=0.008$), and the NT rate positively correlated with NO + monocytes. Interestingly, NO production, and NT label in T-cells were restored at discharge. Our results suggest that in mild COVID-19, the increase in IL-6 levels could be critical to controlling NO production and balancing the oxidative environment. Moreover, the data highlight that NO could have a dual role in acting as a microbicidal metabolite and regulating CD8 + T-cell function. The parameters are restored at the time of discharge and seem related to an effective recovery. These unreported data help to depict the etiopathogenic mechanisms associated with COVID-19 outcome.

RELAPSES IN MULTIPLE SCLEROSIS: ROBUST T CELL RESPONSE BUT NOT SARS-COV-2-PEPTIDE REACTIVE T CELL RESPONSE REDUCES THE PRODUCTION OF A NEUROPROTECTIVE FACTOR

Paula Ayelen Lamas[1], Carla Lucero Remolins[1], Guido Vazquez[2], Franco Appiani[2], Kevin Israel Matamoros Alcivar[1], José Ignacio Valenzuela[1], Alejandra Martinez[3], María Curbelo[3], Daniel Pirola[4], Diego Guerrieri[1], Natalia Basile[5], Adriana Carra[3], Eduardo Chuluyan[1]

[1]Centro de Estudios Farmacológico y Botánicos (CEFyBO), Facultad de Ciencias Médicas de la UBA/ CONICET, CABA, Argentina; [2]Instituto de Neurociencias- Fundación Favaloro/ INECO, CABA, Argentina; [3]Área de Enfermedades Desmielinizantes del Hospital Británico, CABA, Argentina; [4]Hospital Universitario Fundación Favaloro, CABA, Argentina; [5]Dirección Médica y Científica, Gador S.A., CABA, Argentina.

Multiple sclerosis (MS) is a demyelinating disease in which viruses play an important role in its development. Given some reports of MS relapses after the COVID-19 vaccination, there were concerns about its side effects and disease exacerbation. Additionally, it has been demonstrated that secretory leukocyte protease inhibitor (SLPI) is strongly upregulated in experimental models of MS and it is being considered a neuroprotective factor. Therefore, considering SLPI as a biomarker of susceptibility for relapses, we analyzed the production of SLPI in response to SARS-CoV-2-derived peptides or an unspecific T cell stimulator (Tcs) in MS patients (MSp) vaccinated against COVID-19. PMBCs were isolated from heathy donors (HD) and MSp. Cells were cultured with DMSO (control), polyclonal Tcs or immunogenic SARS CoV-2 S-peptide (Sp) for 5 days. Cell culture supernatants (CS) were harvested and SLPI was measured by ELISA. Also, serum SLPI and SARS-CoV2 IgG and IgM were quantified by ELISA. There were no differences between HD and MSp in serum levels of SARS-CoV-2

IgG and IgM. However, serum SLPI level was higher in MSp than in HD (p=0.028) and it is correlated with the levels of SLPI found in the CS of PBMCs treated with Tcs (r=0.638; p<0.0001). The CS-SLPI levels did not change when cells were treated with Tcs or Sp in HD. Remarkably, SLPI levels decreased significantly when patient´s PBMCs were treated with Tcs (p=0.04) but not with Sp. Overall, these results show that SARS-CoV-2 derived peptides do not modify SLPI levels, suggesting that exposure to SARS-CoV-2 peptides present in the vaccine do not increase the risk of relapses in MSp. On the other hand, polyclonal stimulation (such as virus infection) could increase the risk of MS relapses due to a decrease in SLPI levels.

HCV CLEARANCE BY DIRECT ANTIVIRAL AGENTS IS ASSOCIATED WITH DECREASED EXPRESSION OF INTERFERON STIMULATED GENES BUT NOT WITH THE SIZE OF THE INDUCIBLE HIV RESERVOIR

Alejandra Urioste[1], Alejandro Czernikier[1], Ana Paletta[1], Federico Remes Lenicov[1], Ariel Osegueda[1], María Laura Polo[1], Alicia Sisto[2], Florencia Quiroga[1], Gabriela Turk[1], Yanina Ghiglione[1], Natalia Laufer[1].

[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), UBA-CONICET. Ciudad Autónoma de Buenos Aires. Argentina. [2]Hospital General de Agudos Dr. Juan A. Fernández. Ciudad Autónoma de Buenos Aires. Argentina.

Purpose: Previous results from our group indicated that HCV clearance by direct antiviral agents (DAAs) in HCV/HIV coinfected individuals results in higher HIV RNA expression. Here, we extended this finding and evaluated the impact of HCV clearance on the size of the inducible HIV reservoir, the magnitude of the HIV-specific T-cell response and the expression of interferon induced genes (ISGs). Methods: prospective longitudinal study. 22 HIV/HCV coinfected individuals were enrolled (12 with METAVIR fibrosis scale compatible with F4 and 10 with F0/F1). All individuals were on successful antiretroviral therapy and began HCV treatment with DAAs. Peripheral blood samples (PBMC) were obtained before DAAs (BSL), at the end of treatment (EOT) and 12 months post-treatment (12MPT). ISGs: OAS-1, Mx-1 and IRF-7, were analyzed by RT-qPCR, HIV specific-response was evaluated by flow cytometry after stimulation with Nef and Gag peptides. Specific cells were identified by intracellular cytokine staining. Tat/Rev induced limiting dilution assay (TILDA) was used to quantify the inducible HIV reservoir. Results: A no significant increase on the HIV reservoir between BSL and 12MPT was observed for F4 patients, whereas a decrease was observed for F0. No differences were found in the magnitude of the HIV-specific T-cell response. However, a significant decline in the expression of OAS-1 [mean±SD: 0,12±0,23 vs 0,04±0,06(–ΔCt)2, p=0,0313] and Mx-1 [0.65±1.09 vs 0.06±0.05(–ΔCt)2, p=0,0156] was found between BSL and 12 MPT samples. Conclusions: After HCV clearance ISGs showed a lower cell expression. This might be due to the elimination of HCV-antigen chronic stimulation. Nevertheless, we could not find a modification on HIV parameters within the timeframe evaluated, such as the inducible reservoir and the HIV-specific response secondary to this reduction in the ISG pathway. Larger time of follow up and number of individuals could help to further evaluate these parameters.

ADAPTIVE IMMUNITY *Thursday, November 17, 9-10 :30 hr*
Chairs: Luciano D´attilio - Ariana Díaz- Silvana Spinelli- Samanta Funes - Cinthia Araujo

1. (42). **ANALYSIS OF THE ATP/CD39 AXIS IN PEDIATRIC RSV INFECTION**

Russo C[1], Raiden S[2], Algieri S[3], De Carli N[4], Sarli M[3], Bruera MJ[3], Cairoli H[2], Morales I[2], Gonzalez F[2], De Lillo L[2], Seery V[1], Sananez I[1], Simaz N[3], Alfiero G[3], Moya N[3], Ferrero F[2], Geffner J[1], Arruvito L[1].

1. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA. UBA-CONICET, CABA, Argentina.
2. Hospital General de Niños Pedro de Elizalde. CABA, Argentina.
3. Hospital Nacional Prof. Alejandro Posadas. Buenos Aires, Argentina.
4. Clínica del Niño de Quilmes. Buenos Aires, Argentina.

Background: Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis in young children. Extracellular ATP founded in inflammatory environments is regulated by CD39. There is no data regarding the role of purinergic signaling in the lung inflammation during RSV infection. Aims: 1) to quantify ATP levels in serum, nasopharyngeal aspirates (NPA) and the supernatants of stimulated PBMCs; 2) to analyze the frequency and phenotype of CD4+CD39+ cells in peripheral blood; 3) to explore the biologic response of CD4+ T cells upon incubation with an antagonist of CD39.
Methods: Plasma, NPA and PBMCs from children with non-severe (n=46) and severe RSV infection (n=25) and controls (n=17) were used. Results: We found higher levels of ATP in plasma from RSV children, compared with controls ($p<0.0001$). Importantly, severe children had increased levels of ATP in NPA ($p<0.001$) and the stimulation of their PBMCs *in vitro* result in the release of higher levels of ATP ($p<0.005$) compared with non-severe. We observed a higher frequency of CD39+CD4+T cells in RSV children compared with controls ($p<0.01$ and $p<0.001$ for non-severe and severe). Moreover, CD39 expression preferentially occurred on CD4+ T cells displaying activation markers (high CD25, low CD127 and CD45RA-). Additionally, CD4+CD39+ T cells from RSV children produced higher levels of IFN-γ ($p<0.0001$), IL-17A ($p<0.0001$), IL-10 ($p<0.0001$) and IL-8 ($p<0.01$) compared with CD4+CD39- T cells. After confirming that CD4+CD39+ T cells are more susceptible to apoptosis than CD4+CD39- T cells in RSV children ($p<0.0001$), we found that an antagonist of CD39 (ARL67156) delay the rate of apoptosis ($p<0.005$) and modify the cytokine production in these cells ($p<0.01$). Conclusions: Our observations indicate that RSV infection in children induces up-regulation of CD39 in CD4+ T cells modifying their function. Levels of ATP in plasma and NPA could be a useful biomarker of disease severity.

2. (54) **CLUSTERIN PROTECTS MATURE DENDRITIC CELLS FROM REACTIVE OXYGEN SPECIES (ROS) MEDIATED CELL DEATH**

Alvaro López Malizia[1], Melina Sager[1], Antonela Merlotti[2], Agustina Cazala[3], Guadalupe García[3], Sebastián Amigorena[2], Jorge Geffner[1], Juan Sabatté[1]

[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, UBA-CONICET, [2]Institute Curie INSERM U932, Immunity and Cancer, [3]División Anatomía Patológica del Hospital General de Agudos Dr. Juan A. Fernández

Introduction: Clusterin is a multifunctional glycoprotein present in almost all tissues and body fluids. It is involved in a number of physiological and pathological processes including apoptosis, protein homeostasis, Alzheimer´s disease and cancer. Although clusterin expression by myeloid cells has been reported, its influences on dendritic cell (DC) function have not been analysed. Here we show that clusterin expression by mature DCs plays a role on DC survival. Results and Methods: We have previously shown that BDCA2+ blood DCs and Monocyte derived DCs express clusterin after LPS induced maturation. To analyse clusterin function on mature DCs we silenced clusterin expression using siRNA carrying lentivirus. We found that phenotypic maturation markers (DR, CD80, CD86, CD40 and PDL1) and cytokine secretion (IL-1, IL-6, TNF, IL-12 and IL-10) were not modified after 18 hs of LPS stimulation on clusterin silenced DCs. However, after 48hs, silencing of clusterin expression (CLU-KD) resulted in an increased cell death of LPS stimulated DCs (annexin-V/propidium staining: 63.7 ± 11.8, 19.7 ± 4.9, and 25.2 ± 6.5% of death cells, for LPS-stimulated CLU-KD DCs, LPS-stimulated scramble DCs, and unstimulated DCs, respectively) (mean ± ES, n=4, $p < 0.01$). As expected, CLU-KD dendritic cell ability to expand CD4 T cells was found to be strongly diminished (n=6, Paired t test $p<0.01$). We analyzed ROS production by LPS activated DCs. LPS activated DCs produced mitochondrial ROS peaking 4 hs after stimulation. As expected, LPS induced ROS detection was inhibited with the ROS inhibitor N-acetyl-l-cysteine (NAC) ($p<0.001$). The induction of cell death mediated by LPS on CLU KD-DCs was inhibited by treatment with NAC ($p<0.01$), demonstrating that clusterin protects stimulated DC of their own ROS production. Conclusion: we show that clusterin protects mature dendritic cells from ROS mediated cell death

3. (71) **TH1 IN VITRO DIFFERENTIATION IS IMPAIRED BY GLYPHOSATE AND CHLORPYRIFOS BASED PESTICIDES**

María Eugenia Ordóñez[1], Mariana Gantov[1], Belén Camila Lozada Montanari[1], María Natalia Rubinsztain[1], Julieta Erramouspe[1], Jessica Mariel Sierra[1], María Victoria Regge[1], María Cecilia Santilli[1], Aldana Trotta[1], Florencia Secchiari[1], Carolina Inés Domaica[1], Mercedes Beatriz Fuertes[1], Norberto Walter Zwirner[1,3], Adrián David Friedrich[1,2].

[1]Laboratorio de Fisiopatología de la Inmunidad Innata, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [2] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología, Buenos Aires, Argentina. [3] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Chlorpyrifos (CFP) and Glyphosate (GLY) based pesticides (CbP and GbP, respectively) are widely used in agriculture. There is evidence suggesting that GbP is genotoxic and both GbP and CbP are associated with an increased frequency of malignancies observed in highly fumigated rural areas. However, their effect on immunosurveillance has been poorly explored. We have previously demonstrated that CbP and GbP, individually, impair IFN-γ production by NK cells and cytotoxicity against K562 cell line *in vitro*. Here, we focused on the impact of CbP, GbP and the combination of both formulations (CbP+GbP) on the adaptive immune response. First, we developed an equi-toxic and a non-equitoxic model on Jurkat cells to determine how CbP and GbP may interact by measuring cell death. Surprisingly, our results indicate that in both models tested, CbP and GbP display an antagonistic behavior. Furthermore, we analyzed the ability of T cells isolated from healthy donors to differentiate *in vitro* into the Th1 subtype in the presence of sub-apoptotic doses of CbP, GbP and CbP+GbP. Our results show that CbP+GbP but

not CbP or GbP alone decreased both T-bet and IFN-γ expression in $CD4^+$ T cells evaluated by flow cytometry ($p< 0,05$). Moreover, CbP+GbP impact strongly on T cell proliferation capacity. Altogether, our results indicate that CbP+GbP but not CbP or GbP alone alter T cell function, suggesting that both formulations may have a cooperative effect at low doses that weaken the immune system´s ability to mount Th1 responses. Since the concentrations of GLY and CPF used here were far below the No-Observed Adverse Effect Level (NOAEL) for each active principle, this work reinforces the need of reevaluating pesticide safety using commercial formulations (and active principles) interactions.

4. (88) **HYPOGAMMAGLOBULINEMIA IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): ROLE OF LEUKEMIC CELLS**

Juliana Bernatowiez[1], Chiara Cassarino[1], Ana Colado[1], Valeria Sarapura Martinez[1], Brenda Buonincontro[1], Martín Bertini[2], Fernando Bezares[2], Monica Vermeulen[1], Romina Gamberale[1], Mercedes Borge[1], Mirta Giordano[1].

1.Laboratorio de Inmunología Oncológica, Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM), Buenos Aires, Argentina, 2.Hospital General de Agudos Dr. Teodoro Álvarez, Buenos Aires, Argentina.

Hypogammaglobulinemia (HGG) is a common immune deficiency in CLL and can cause a high morbidity due to respiratory infections. In our cohort of patients from a single center we found that 14 of 29 had IgG HGG and 10 of 29 had IgM HGG at diagnosis (IgG<700 mg/dl; IgM<40 mg/dl). The precise mechanism responsible for HGG is unknown. The fact that HGG is present even at initial stages of the disease suggests that it cannot be attributed to infiltrative accumulation of clonal cells in the bone marrow. To gain insight into the mechanisms involved in HGG, we took advantage of the CLL mouse model Eu-TCL-1 that develops leukemia in aged mice. The leukemic cells from TCL-1 donors are usually transplanted into syngeneic wt (C57) mice to accelerate the disease course. We found that leukemic mice have significantly lower levels of IgG but not IgM in plasma compared to control animals (IgG 1180 ± 0.08 versus 410 ± 0.05 mg/dl; n=12; $p<0.05$, Mann Whitney test). To determine if leukemic cells impair the secretion IgG, we activated purified B cells from C57 spleen ($2x10^5$/ml) with LPS (2.5 ug/ml) in the presence or not of leukemic cells ($1x10^5$/ml) for 5 days. IgG levels in supernatants were evaluated by ELISA. Our results show that co-culture of activated B cells and leukemic cells significantly decreased IgG (B cells alone: 12.31 ± 0.06 ng/ml; B cells in the presence of leukemic cells: 7.40 ± 0.91 ng/ml, n= 5, $p<0.05$, Friedman test, followed by Dunn's multiple comparisons test). In addition we found that leukemic cells impaired the proliferative capacity of B cells in response to LPS as measured by the CFSE dilution assay and flow cytometry (% of proliferation of B cells alone 72.53 ± 0.97 vs % of proliferation of B cells in the presence of leukemic cells 55.45 ± 3.02; $p<0.05$, Wilcoxon matched-pairs signed rank test). We conclude that malignant cells exert an inhibitory effect on B lymphocytes that might explain, at least in part, HGG in CLL.

5. (97) **STAPHYLOCOCCAL ENTEROTOXIN N PROMOTES PBMCs PROLIFERATION AND CYTOKINE RELEASE**

Daniela Maria Redolfi, Sofia Noli Truant, Maria Belen Sarratea, Laura Valeria Iannantuono Lopez, María Belén Antonoglou, Emilio Luis Malchiodi, Marisa Mariel Fernández.

Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina.

Bacterial Superantigens (SAgs) are enterotoxins that interact simultaneously and in a non-classical way with the TCR Vβ chain and the MHC-II. As this interaction occurs, it induces an exacerbated T cell proliferation and a massive cytokine release producing Toxic Shock Syndrome, among other affections. The present study aimed to analyze the capacity of Staphylococcal enterotoxin N (SEN), which belongs to the *egc* operon, to activate the immune system by evaluating its interaction with PBMCs from healthy donors. For this purpose, SEN was cloned in a prokaryotic plasmid, produced as a recombinant protein in *E. coli* and purified by Ni^{++}/NTA column followed by Size Exclusion Chromatography. Further, SEN was identified by Mass Spectrometry. PBMCs from donors non-exposed to SAgs were incubated with different concentrations of SEN to evaluate proliferation in presence of [methyl-3H] thymidine. Supernatants were collected for quantitative analysis of IL-6, IL-12, IL-10, TNFα and IFNγ using ELISA. PBMCs were stimulated with 0.001-10 µg/ml of SEN and proliferated even when treated with as low as 0.001 µg/ml ($p<0.01$). In addition, 0.1, 1 and 10 µg/ml of SEN induced a higher production of IL-6, IL-12, IL-10, TNFα and IFNγ on PBMCs compared to untreated cells ($p<0.001$). In conclusion, these results show that the SAg SEN induced naive PBMCs proliferation with an associated release of pro-inflammatory cytokines, IFNγ, TNFα and IL-12, promoting a Th1 differentiation profile. However, it also increased IL-6 production, which is associated with Th17 activation, and IL-10, an anti-inflammatory cytokine related to immunomodulation. These effects, reported previously for other SAgs of the *egc* operon, highlight the complexity of the immune response profile elicited by these toxins.

6. (129) **G PROTEIN-COUPLED RECEPTOR KINASE 2 (GRK-2) MODULATES THE ACTIVATION AND MIGRATION OF LEUKEMIC AND T CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS**

Chiara Cassarino[1], Juliana Bernatowiez[1], Valeria Sarapura Martinez[1], Brenda Buonincontro[1], Martín Bertini[2], Fernando Bezares[2], Monica Vermeulen[1], Mirta Giordano[1], Romina Gamberale[1], Ana Colado[1], Mercedes Borge[1].

1.Laboratorio de Imunologia Oncológica, Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM), Buenos Aires, Argentina, 2.Hospital General de Agudos Dr. Teodoro Álvarez, Buenos Aires, Argentina.

Leukemic B cell proliferation mainly occurs in the lymph nodes in response to signals provided by the tumor microenvironment, such as CD40L and cytokines secreted by activated T cells. GRK2 regulates B cell homing to lymph nodes by inducing S1PR1 (Sphingosine-1 phosphate receptor-1) downregulation, which allows the lymphocyte to overcome the S1P-mediated retention in the blood, and to follow the chemokine gradient into the tissue. GRK2 also modulates other cellular functions such as activation and survival in different cancer cells. We have previously found that a GRK2 inhibitor, CMPD101 (CMP) increases leukemic-cell migration to S1P without affecting spontaneous or drug-induced apoptosis. Here we aim to expand the study on the effect of GRK2 inhibition in the activation and migration of leukemic and T cells from CLL patients. Leukemic and T cells were obtained from CLL patient´s peripheral blood, and from the CLL mouse model Eμ-TCL-1. Transwell migration assay was used to evaluate chemotaxis. T cells were activated with plate bound-anti-CD3 mAb. Activation markers were assessed by flow cytometry and cytokines by ELISA. Statistical significance was analyzed with the GraphPad Prism software. We found that CMP (3-30 μM) increased the migration towards S1P of leukemic cells from CLL patients and also from the CLL mouse model ($p<0.05$, n=10), while it did not modify leukemic cell migration in response to CXCL13, CXCL12 and CCL21. Also, CMP increased T cell migration in response to S1P, while it decreased migration towards CCL21 ($p<0.05$, n=8). Moreover, CMP decreased CD40L expression and IL-10 and IFNɣ secretion by activated T cells and reduced the expression of the activation marker CD86 on leukemic cells induced by activated T cells ($p<0.05$, n=8). Our results suggest that GRK2 inhibition may be useful for: 1) to induce CLL cell mobilization from lymphoid tissues by increasing cell migration towards S1P and 2) to impair CLL cell activation by reducing T cell signaling.

7. (133) **NANOENCAPSULATED ESSENTIAL OIL MODULATES IMMUNOLOGICAL AND OXIDATIVE PARAMETERS IN WEANED PIGS**

Ivana Dalila Montironi[(1)], Dardo Andrés Roma[(2)], Sofía Arsaute[(3)], María Eugenia Cecchini[(3)], María Luján Pedraza [(4)], Agustina Pinotti [(4)], Fernando Mañas[(2)], Fernando Bessone[(4)], Fabrisio Eduardo Alustiza[(4)], Romina Valeria Bellingeri[(5)], Laura Noelia Cariddi [(3)]

(1) Cátedra de Farmacología, Facultad de Agronomía y Veterinaria-UNRC, Río Cuarto, Córdoba, Argentina. (2) INCIVET CONICET-UNRC, Río Cuarto, Córdoba, Argentina. (3) INBIAS CONICET-UNRC, Río Cuarto, Córdoba, Argentina. (4) Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Marcos Juárez, Marcos Juárez, Córdoba, Argentina. (5) IITEMA CONICET-UNRC, Río Cuarto, Córdoba, Argentina.

In a previous study, we demonstrated that *Minthostachys verticillata* essential oil (EO) modulated mucosal and systemic immune response in a mouse model. The aim of this work was to evaluate immunomodulatory effect of nanoemulsion based on *M. verticillata* EO (NEO) in weaned pigs. NEO was developed with EO (20%v/v), Tween 80 (0.75%v/v), Span 60 (0.25%w/v) and water (79%v/v) and was synthesized by the high-energy method. Biocompatibility studies on Caco-2 cells showed that NEO was not toxic up to 500 µg/ml. NEO immunomodulatory effect was evaluated in male weaned pigs (n=36) divided into six groups (n=6) that received orally for 30 consecutive days the followed: Group 1: Control (ClNa 0.9%), Group 2: Vehicle control (Tween 80, Span 60 and water), Group 3: EO (10 mg/Kg/day), Groups 4-6: NEO (2.5, 5 or 10 mg/Kg/day). An increase in body weight gain was observed in EO (10 mg/Kg/day) and NEO (10 mg/Kg/day) groups compared to control ($p<0.05$). Feces consistency was normal in all groups. At day 30, blood samples were obtained and animals were sacrificed to extirpate digestive organs. Triglycerides levels was lower in EO (10 mg/kg/day) ($p<0.001$) and NEO (2.5, 5 or 10 mg/kg/day) ($p <0.01$, $p<0.001$, $p <0.001$) groups. No differences in total cholesterol, serum albumin, glucose, ALT and AST levels were observed. $CD4^+$ T cells number was higher in NEO (10 mg/Kg/day) group compared to other groups ($p<0.001$, $p<0.05$, $p<0.001$). No differences in $CD8^+$ T cells number was observed. In addition, lipid peroxidation was evaluated and a decrease in malondialdehyde (MDA) in liver was observed in all NEO groups compared to control ($p<0.05$). An increase in MDA in liver ($p<0.001$) and stomach ($p<0.01$) was observed in EO (10 mg/Kg/day) group. MDA in kidney was not altered. Results showed that nanoencapsulation enhanced the biological activity of EO. NEO oral administration was safe, modulated systemic adaptive immunity and showed a greater antioxidant effect than non-encapsulated EO.

8. (186) **KINETICS OF TONSILLAR LYMPHOID PROLIFERATIVE RESPONSES EX VIVO**

Rocío A. Pastor[1], Juliana Puyssegur[1], Lindybeth Sarmiento Varón[1], Gonzalo Husain[1], M. Elena Arabolaza[2], Bibiana Paoli[2], and Eloísa I. Arana[1,3]

[1]Institute of Immunology, Genetics and Metabolism (INIGEM), Clinical Hospital 'Jose de San Martín', University of Buenos Aires (UBA), National Council for Scientific and Technological Research (CONICET), Buenos Aires, Argentina, [2]Pediatric Otorhinolaryngology Service, Otorhinolaryngology Division, Clinical Hospital 'Jose de San Martín', UBA, Buenos Aires, Argentina, [3]Department of Immunology, School of Medicine, UBA, Buenos Aires, Argentina

The mucosa of the oronasopharynx is the gateway for the majority of pathogens. Efforts in vaccine development aimed at local mucosal immune responses would yield benefits particularly relevant in the current pandemic context. Although is known to be induced partly via the tonsils and adenoids, the responses at this site are poorly understood. Our aim was to characterize the proliferative response for the different tonsillar lymphocyte population ex vivo. Cell proliferation was monitored after tonsillar mononuclear cells (TMC) were stimulated with either IL2/IL4 or IL2/IL4/CpG/CD40L in the culture, by scoring $Ki\text{-}67^+$ cells using FACS. We analyze 3 lymphoid populations (CD20+, CD3+CD4+ and CD3+CD8+) at 3 different time points (24, 48 and 72 hours) for 5 patients. We found that B and T cells steadily expanded from 24 hs up to 72 hs in IL2/IL4/CpG/CD40L cultures. Completed 72hs of culture, around one third of tonsillar B cells expressed Ki-67 (30% ± 13,7%). Maximum levels reached by CD4+ and CD8+ T cells were 11,4%± 4,1% and 11,7%± 4,2% Ki-67+ cells, respectively. On the other hand, T cells showed a better response than B cells in IL2/IL4 cultures. At the last time point, while the CD3+CD4+Ki-67+ cells represented 8,9%± 7,2% of the CD4+ T cells and CD3+CD8+Ki-67+ were 8,6%± 1,8% of the CD8+ T cells, proliferating B cells accounted for only 3,8% ± 1,2% of total CD20+ cells. Finally, we investigated the expression of CD73, known to promote stemness and exhaustion in peripheral T cell populations, on stimulated tonsillar B and T cells. CD73 appeared downregulated in all stimulated lymphocytes. The ability for vigorous proliferation upon re-infection is a trait of adaptive immunity and the basis of vaccination. CpG is a Toll like receptor ligand, experimentally used as a vaccine adjuvant. We propose our system of culture to rapidly test mucosal vaccine and adjuvants candidates. Of note, mucosal vaccine responses in mice are often poorly predictive of results in humans.

9. (661) **MATERNAL STRESS DURING PREGNANCY ENHANCES ALLERGIC AIRWAY INFLAMMATION RISK IN THE OFFSPRING IN AN AIRWAY SENSITIZATION ASTHMA MODEL**

Estefania Nicole Morales, Guido Rattay, Alejandra Goldman, Ignacio Martin Fenoy

Rationale: Allergic asthma is increasing worldwide. The presence of atopic diseases in the mother propagates the onset of allergic diseases in the offspring considerably stronger than atopic diseases of the father. Such observation challenges genetic predispositions as the sole cause for allergic diseases. Epidemiological studies suggest that caregiver stress in the perinatal periodmay predispose offspring to asthma. Using an OVA/alum intraperitoneal asthma model we have previously shown that maternal stress during pregnancy results in an increase of litter susceptibility to develop allergic lung inflammation. In this work we aim to study whether this phenomenon is still observed using observed using an asthma model that involves airway aero-allergen sensitization in the absence of adjuvant. Methods: Pregnant BALB/c mice (day 15 and 17) were subjected to 2 restraint stress exposure. On week 6 after birth, offspring were treated intranasally with papain (30 µg) during 5 consecutive days. After 3 days allergic airway inflammation was evaluated (S group). Negative controls included offspring of non-stress dams subjected to the same protocol (C group) or mock-sensitized with PBS (N group). Results: Offspring of stress, but not control dams, showed increased eosinophils infiltrate in bronchoalveolar lavage ($p<0.05$). Perinatal stress resulted in pathological changes of pulmonary allergic inflammation. These changes included eosinophils and mononuclear cell infiltration around airway and vessels with no goblet cell hyperplasia. This increase was accompanied by high levels of IL-5 in bronchoalveolar lavage fluid ($p<0.05$). Conclusions: Maternal stress during pregnancy resulted in an increase of litter susceptibility to develop allergic lung inflammation in a natural exposure asthma model.

10. (745) **IgE-MEDIATED UPPER AIRWAY INFLAMMATION IN A RUSSIAN THISTLE POLLEN-INDUCED MURINE MODEL**

Marcelo Javier Galvez, Gisela Giorgi, Ileana Lencinas, María Carla Crescitelli, Agustina Kenny, Adriana Martínez, María Gabriela Murray, María Inés Prat

INBIOSUR (Instituto de Ciencias Biológicas y Biomédicas del Sur), Departamento de Biología, Bioquímica y Farmacia, CONICET-Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina

Allergic rhinitis (AR) has worldwide distribution with an increasing prevalence every year. Also, allergic rhinitis and chronic rhinosinusitis are frequently associated with asthma. In our region, Russian thistle (*Salsola kali*) is a common weed causing pollinosis. The aim of this study was to establish a mouse model of AR based on *Salsola kali* pollen proteins, and to define the extent that this could have in the lower airways. BALB/c mice (n = 5/group) were administrated weekly with PBS or *S. kali* pollen extract through i.p. route and later challenged by nasal instillation of PBS or *S. kali* pollen respectively on days 21, 22 and 23. Bronchoalveolar lavage (BAL) were obtained. Serum total IgE and *S. kali*-specific IgE were measured by ELISA. After sacrifice, the noses and lungs were fixed and paraffin embedded for histological analysis (H&E, toluidine blue and

periodic acid-Schiff). After nasal challenge, frequency of sneezing in sensitized group were higher than the control (p<0,001). Goblet cell hyperplasia and eosinophil infiltration was observed in nasal lateral mucosa (257±40 in sensitized mice vs. 16±2 in control group, p<0,05). IgE values were increased seven times in sensitized mice compared to control group (p<0,01). Moreover, mice in the sensitized group had significantly higher levels of *S. kali*-specific IgE than the control (p<0,05). Sensitization induced moderate to severe inflammatory infiltration in lungs. Significant increased number of total inflammatory cells were observed in BAL from sensitized mice compared to control group (p<0,05) including eosinophils. Our results confirm an upper airway inflammation associated to an IgE-mediated hypersensitivity reaction induced by the aeroallergen exposure. In addition, associated effects were demonstrated in the lungs, although more research needs to be done to clarify this upper-lower airway interaction. The later could be helpful since AR and asthma respond to similar treatment strategies.

11. (827) **ABSENCE OF LEUKOCYTE SPECIFIC PROTEIN 1 (LSP1) ALTER ACTIVATION AND PROLIFERATION OF CD4⁺ AND CD8⁺ T LYMPHOCYTES**

GA Lopez Menichetti[1], MM Pascual[1], F Ruiz Moreno[1], MI Crespo[1], BA Maletto[1], G Morón[1], ND Dho[1].
1. CIBICI–CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Leukocyte-specific protein 1 (LSP1) is a 52 kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes and endothelial cells. We have previously shown that *Lsp1*$^{-/-}$ mice have an impaired CTL response after antigen exposure, with *Lsp1*$^{-/-}$ dendritic cells (DCs) failing to induce a strong CTL response in vivo and a lower CD4+ T cell-mediated response. In order to study the role of LSP1 in T cell functionality, we evaluated the capacity of *Lsp1*$^{-/-}$ CD4⁺ and CD8+ T lymphocytes to activate and proliferate in vitro. T cells were purified from spleen of *Lsp1*$^{-/-}$ and WT mice by cell sorter after labeling splenocytes with anti-CD8**β** (to purify T CD8+ cells), anti-CD11b and anti-CD4 antibodies to purify T CD4+ lymphocytes. Sorted T cells were then stained with eF670 proliferation dye and finally cocultured with Ab anti-CD28 in a well plate sensitized with anti-CD3. Three days later, a significantly lower percentage of activated cells expressing CD69 (p<0.001) or CD44 (p<0.0001) was observed in *Lsp1*$^{-/-}$ CD4+ T Lymphocytes and inside the activated cells gate, the proliferating percentages was lower too in *Lsp1*$^{-/-}$ CD4+ T Lymphocytes (p<0.01 and p<0.0001 respectively). Something similar was seen in *Lsp1*$^{-/-}$ CD8+ T Lymphocytes, with a significantly lower percentage of proliferating cells (p<0.01) and CD44 expression (p<0.001) than their WT counterpart cells. To assess the lymphocytes activity in the previous assay, we measure IL-2, IL-17, TNF and IFN_γ in supernatant by sandwich ELISA. We found that in *Lsp1*$^{-/-}$ CD4+ T Lymphocytes produced less IL-2 and IFN_γ (p<0.05 and not quantifiable for WT respectively) and significantly more IL-17 (p<0.05) than WT CD4⁺ T cells. *Lsp1*$^{-/-}$ CD8+ T Lymphocytes produced significant less TNF (p<0.001) and, in contrast to WT CD8+ T cells, not measurable IL-2. These results suggest that LSP1 deficiency also affects T cell activation and proliferation, which could impair induction of effector responses in mice.

12. (848) **ALTERED FOLLICULAR HELPER T CELL (TFH) DIFFERENTIATION IN TONSILS OF CHILDREN WITH TRISOMY 21**

Jeremias Dutto[1]; Paula Araya[2]; Lucía Boffelli[1]; Nicolás Nuñez[1], Joaquin M. Espinosa[2]; Mariana Maccioni[1].
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina. [2]Linda Crnic Institute for Down Syndrome, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA.

Down Syndrome, which is caused by trisomy 21 (T21), is characterized by immune dysregulation, anatomical differences in the upper respiratory tract and higher rate of comorbidities. Since tonsils are the first barrier against airborne pathogens, we studied if the immune response generated in these secondary lymphoid organs could be related to some of the differences observed.
We characterized the T cell compartment obtained from hypertrophied tonsils in children with T21 versus age-matched controls (n=4/group). We studied the frequency of activated non-Tfh cells (CD3+ CD4+ CD45RA- CXCR5-PD1+), Tregs (CD3+ CD4+ CD45RA- Foxp3+), pre-Tfh (CD3+ CD4+ CD45RA- CXCR5low PD1int) and Tfh (CD3+ CD4+ CD45RA- CXCR5hi PD1hi) by multiparametric flow cytometry and multiplex immunofluorescence. The expression of CXCR3 (related to a Th1 profile) and the cytokines IL21, IFNγ and IL17 were analyzed. Lymphoid follicles exhibited smaller size in T21 tonsils compared to controls, which was not accompanied of altered frequencies of CD3+, CD4+, CD8+, Tregs and CD19+ cells. However, we found a 40% increase in the fraction of activated non-Tfh cells (p<0.05) and a 30% decrease of Tfh (p<0.3). When cytokines were studied, an increase in the fraction of Tfh IFNγ+, IL21+ and IFNγ+IL21+ was observed. The pre-Tfh population showed an increased frequency (p=0.08). Interestingly, a higher percentage of CXCR3+ pre-Tfh population (p<0.01) was observed. To explore if dendritic cells could be involved in promoting a Th1 bias, we phenotypically analyzed the different populations, but we could not detect significant changes in their frequencies. We also performed exploratory studies using multiplexed immunofluorescence to analyze the expression of BCL6, CD21, CD3, CD4, CXCR3, Ki67, PD1, Tbet through whole slide scanning of the T21 tonsils.
Our results suggest that T21 children have an altered tonsil T cell compartment, with a skewed Tfh differentiation and a Th1 profile among the pre-Tfh population.

13. (870) **Gain and loss of function novel variants in CARD11 in patients with Inborn Errors of Immunity**

Julieta Belén Fernandez[2,3] ; Luciano Urdinez[*1*]; Lorenzo Erra[2,3*]; Alejandro M. Palma[1]; María F. Mercogliano[2,3];, Emma Prieto[1]; Verónica Goris[1]; Mariana Villa[1]; Carolina Bouso[1]; Lucia Caputi[1]; Belen Quesada[1]; Daniel Solis[1], Anabel Aguirre[1] ; Matías Oleastro[1]; Silvia Danielian[1] ; María B. Almejun[2,3]
[1]Servicio de Inmunología y Reumatología, Hospital Nacional de Pediatría Juan P. Garrahan, Buenos Aires, Argentina
[2] Laboratorio de Biofisicoquímica de Proteínas, Departamento de Química Biológica, IQUIBICEN, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[3] Laboratorio de Genética en Endocrinología, IB3, Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[4] Servicio de Dermatología, Hospital Nacional de Pediatría Juan P. Garrahan, Buenos Aires, Argentina
**These authors share first authorship*

CARD11 encodes a lymphocyte-specific scaffold protein necessary inter alia for proper NF-κB activation in B- and T- cells. Germline pathogenic variants in CARD11 have been linked to Inborn Errors of Immunity (IEI) with diverse clinical phenotypes: BENTA (heterozygous GOF), CADNIS (heterozygous LOF/DN) and SCID (biallelic null). Functional impact of novel CARD11 variants identified by NGS cannot be predicted with absolute certainty. Therefore, functional assessment must be used for a proper interpretation. Our aim is to evaluate two novel mutations in CARD11 identify in IEI patients NGS in the Hospital Garrahan: p.T43R and p.Q249P. Both CARD11 variants were absent from gnomAD and ExAC, presented a high degree of amino acid conservation by Clustal Omega and predicted to be damaging by PolyPhen2. We assessed the ability for the novel variants to alter TCR signaling by transfection with WT and mutant CARD11 expression constructs into JPM50.6 and Jurkat cell lines. We use the previously reported LOF (p.R30W) and GOF (p.G123S) mutations as controls. Compared to WT, the novel variant p.T43R barely induced NF-κB activation upon anti-CD3/CD28 stimulation and disrupted WT CARD11 ability to activate NF-κB when is co-transfected in JPM50.6, in similar levels as with the previously described p.R30W. On the other hand, p.Q249P induced a constitutive GFP expression in the absence stimulation like to the p.G123S GOF mutation. The immunofluorescence assays performed in

transfected HEK293T revealed that p.Q249P variant and the GOF, p.G123S, exhibited cytoplasmic aggregates in absence of stimuli which were previously described as indicative of active signaling. Overall, novel variant p.Q249P was considered GOF while the new variant p.T43R behaved as LOF/DN. The identification of novel CARD11 mutations in IEI patients together with the functional studies contribute to better understanding the mechanisms underlying CARD11 pathways in the development of primary immunodeficiencies.

14. (874) **KINETICS OF CROSS-PRESENTATION ASSESSED EXPERIMENTALLY AND BY A MODEL OF THE COMPLETE ENDOMEMBRANE SYSTEM**

Facundo Garrido[1,a] , Franco Nieto [1,a] , Sofía Dinamarca [1] , Ignacio Cebrian [1], Luis S. Mayorga [1,2]

1 Instituto de Histología y Embriología de Mendoza (IHEM) - Universidad Nacional de Cuyo - CONICET, Mendoza 5500, Argentina.

2 Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza 5500, Argentina.

a. These authors contributed equally.

Dendritic cells (DCs) have a specialized endomembrane system capable of presenting exogenous antigens in the context of MHC class I (MHC-I) molecules. This process, named cross presentation, is crucial to activate CD8+ T lymphocytes and initiate cytotoxic immune responses. In this report, we present an Agent Based Model in combination with Ordinary Differential Equations with enough complexity to reproduce cross-presentation. The model embraces the secretory and endocytic pathways, in connection with the plasma membrane, the endoplasmic reticulum and the cytosol. Key molecules required for cross-presentation were included as cargoes. In the simulations, the kinetics of MHC-I uptake and recycling, and cross-presentation accurately reproduced experimental values. The model proved to be a suitable tool to elaborate hypothesis and design experiments. In particular, the model predictions and the experimental results obtained indicate that the rate-limiting step in cross-presentation is the loading of complexes after the proteasomal processing of the antigenic proteins.

AUTOIMMUNITY *Thursday, November 17, 9-10:30 hr*
Chairs: Ruben Motrich - Silvina Villar

15. (104) **LOW EXPRESSION OF COMPLEMENT REGULATOR PROTEINS IN MYASTHENIA GRAVIS PATIENTS**

Mariano E Justo[1,2], Florencia Aguirre[3], Juliana Leoni[2], Andrés Villa[3], Mariela L Paz[1,2]

1 Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología. CABA, Argentina.

2 CONICET-Universidad de Buenos Aires. Instituto de Estudios de la Inmunidad Humoral (IDEHU), Facultad de Farmacia y Bioquímica. CABA, Argentina

3 División Neurología. Hospital Gral. de Agudos Dr. José María Ramos Mejía, Bs. As, Argentina. Centro Argentino de Neuroinmunología - CADENI

Myasthenia gravis (MG) is an autoantibody-mediated autoimmune disease that affects the neuromuscular junction. These autoantibodies, mainly directed against the acetylcholine receptor, can activate the classical pathway of the complement system, leading to the destruction of the muscle fiber. Complement is a cascade system which comprises over 50 proteins, many of them involved in the inhibitory regulation of the activation sequence. Different complement regulator proteins (CRPs) have been evaluated in animal models, but little has been examined in MG patients. We measured CD59, CD46 and CD55 expression, three membrane-bound CRPs, on white blood cells (WBCs) from 6 MG patients and 6 healthy controls (HCs). Blood samples were collected in heparinized tubes and processed 24h later. Isolated WBCs were incubated with anti CD59-FITC, CD55-APC and CD46-PE monoclonal antibodies during 30min at RT. Expression of the three markers was analysed by flow cytometry on whole WBCs and on the granulocyte's subpopulation (the most abundant population and the one with the higher expression of CRPs). Additionally, the severity of the disease was determined and registered through ADL and MGC clinical scores. We found a lower mean fluorescence intensity (MFI) on granulocytes for the three CRPs in MG patients compared to HCs. Only CD46 expression (MFI) showed a statistically significant (ss) difference with HCs (MG: 5796 ± 1507 vs. HCs: 7822 ± 1302, p=0.03). No differences were observed when analysing the whole WBCs' population. Preliminary, with this initial small and heterogeneous group of patients, we couldn't find a correlation between CRPs expression and MG severity. In conclusion, this study suggests that a diminished expression of these three CRPs, especially CD46, might be involved in a higher susceptibility to complement damage in MG patients, although further studies involving a higher number of participants are needed.

16. (818) **THE ABSENCE OF TNF RECEPTOR 1 (TNFR1) INCREASES SERUM SEROTONIN LEVELS AND FAVORS THE SUBSEQUENT DEVELOPMENT OF REACTIVE ARTHRITIS IN MICE**

Samanta Celeste Funes[1], Juan Eduardo Silva[1,2], Claudia Aguilera Merlo[2], Roberto Carlos Davicino[1,2], Matías Distel[1], María Elena Arce[1,2], Javier Elicabe[1,2], María Silvia Di Genaro[1,2]

[1]Instituto Multidisciplinario de Investigaciones Biológicas-San Luis (IMIBIO-SL, CONICET-UNSL), Argentina. [2]Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina.

Serotonin (5-HT) is synthesized mainly in the gut and can regulate innate and adaptive immune functions. Multiple inflammatory factors, such as infections, modulate 5-HT levels in the body. In this sense, antidepressants (as fluoxetine) that modify 5-HT availability also affect inflammatory responses. However, its ability to reduce the severity of autoinflammatory diseases remains to be elucidated. This work aims to study the impact of 5-HT changes in the development of reactive arthritis (ReA). In our study model, mice deficient in TNF receptor 1 (TNFR1 KO) develop ReA as a sequela after infection with *Yersinia enterocolitica* (Ye). Thus, non-infected TNFR1KO and WT mice were euthanized, and the distal portion of the small intestine was removed and evaluated by immunofluorescence with an anti-serotonin receptor (SERT) antibody. Serum samples were additionally assayed for 5-HT by HPLC. In addition, WT and TNFR1 KO mice were orally infected with Ye O:3 (1-5x10^8 bacteria/mouse). From infection day, fluoxetine (20 mg/kg/day) or water (control) was administrated in the drinking water. The ReA was detected on day 14, and the score was recorded. On day 21, mice were euthanized, and sera samples were taken for 5-HT levels evaluation. Besides, the joints were obtained for histopathological evaluation and cytokines determination by ELISA. Our results indicate that non-infected TNFR1 KO mice have a higher basal serum 5-HT level, although no significant differences are observed in the ileum SERT expression. On day 21, fluoxetine-treated mice showed lower serum 5-HT levels and less clinical symptoms severity than controls. Although a significant reduction in proinflammatory cytokines was not detected, the reduction in the clinical score was consistent with less histological damage in the joints. To conclude, increased availability of 5-HT contributes to the development of ReA; however, blockade of SERT with fluoxetine and their consequent reduction in serum 5-HT can attenuate joint damage.

17. (833) **INTERFERON ALPHA SIGNALING IS NEEDED FOR THE INDUCTION OF SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE IN A TLR-7 INDUCED MODEL**

Fernando Nicolas Ferreyra[1], María Sol Martínez[1], Carolina Olivera[1], Daniela Andrea Paira[1], Juan Pablo Mackern-Oberti[2], Rubén Darío Motrich[1], Virginia Elena Rivero[1]

[1]CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. [2]Instituto de Medicina y Biología Experimen-

tal de Cuyo, IMBECU CCT Mendoza-CONICET, Mendoza, Argentina

Several studies have demonstrated that altered Toll-like receptor (TLR) signaling contributes to the initiation and/or exacerbation of Systemic Lupus Erythematosus (SLE). In recent years, it has become apparent that TLR-7, which sense single-stranded RNA, contributes to the development of SLE. Taking into account that TLR-7 signaling strongly induces the production of IFN-α, C57BL/6 mice and IFNAR knockout mice were treated topically into the skin with Imiquimod 5%, 3 times weekly for 8 weeks. Serum anti-DNA, anti-histone antibodies and spleen lymphocytes populations were analyzed by ELISA and immunofluorescence cytometry respectively. Kidney infiltration was investigated looking for the presence of $CD45^+$ leukocytes using immunofluorescence cytometry. B6 mice treated with Imiquimod (IMQ) developed higher spleen weight and cellularity when compared to untreated mice($p<0,05$). Increased amounts of $CD69^+$, $CD44^{hi}$ T cells, Th1 and Th17 were observed in spleen of IMQ treated mice when compared to untreated B6 mice($p<0,04$). In addition, B6 treated mice showed increased $CD19^+$ counts with elevated levels of $CD19^+CD11c^+$ and $CD19^+Tbet^+$ aged-associated B cells. A significant increase in anti-dsDNA and anti-histone IgG antibodies was detected in serum samples at 4 weeks and continued to increase at 8 weeks of treatment($p<0.02$).
IMQ treatment in IFNAR knockout mice did not induced splenomegaly. Although increased amounts $CD69^+$ T cells and high levels of Th1 cells were observed, no significant differences were detected in $CD44^{hi}$, Th17, $CD19^+$ and $CD19^+CD11c^+$ or $CD19^+Tbet^+$ subpopulations when compared to untreated IFNAR KO mice. Serum samples were negative for the presence of auto-antibodies. Finally, when we looked for the presence of $CD45^+$ cells in kidney, B6 but not IFNAR KO treated mice showed significant leukocyte infiltration ($p<0,05$).
Our data showed that disrupting IFN-α signaling protects mice from immune dysregulation and organ infiltration in a model of TLR-7 induced lupus.

BIOINFORMATICS AND THERAPEUTIC TARGETS I

(17/11 9-10:30 hs)
Chairs: Ezequiel Lacunza - Diego Mengual Gómez - C. David Bruque

18. (87) **BIOMARKERS OF THE BACTERIAL, VIRAL AND HUMAN GUT TRANSCRIPTOME IN METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD) IN ARGENTINA**

María Florencia Mascardi[1,2], Flavia Mazzini[1], Bárbara Suárez[1,2], Vera Ruda[3,4], Sebastián Marciano[5], Paola Casciato[2,5], Adrián Narvaez[5], Leila Haddad[5], Margarita Anders [6], Federico Orozco[6], Ana Jesica Tamaroff[7], Frank Cook[8], John Gounarides[8], Susana Gutt[7], Adrián Gadano[5], Celia Méndez García[4,9], Martín Marro [10,11], Alberto Penas Steinhard[2,12], Julieta Trinks[1,2]

[1]Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) – CONICET - Instituto Universitario del Hospital Italiano (IUHI) - Hospital Italiano de Buenos Aires (HIBA), Ciudad Autónoma de Buenos Aires, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [3]Biotherapeutic and Analytical Technologies, Novartis Institutes for Biomedical Research, Cambridge (NIBR), MA, United States of America; [4]Chemical Biology & Therapeutics, NIBR, Cambridge, MA, United States of America; [5]Liver Unit of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [6]Liver Unit of Hospital Alemán, Ciudad Autónoma de Buenos Aires, Argentina; [7]Nutrition Department of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [8]Analytical Sciences & Imaging Department, NIBR, Cambridge, MA, United States of America; [9]Chemical Biology & Therapeutics, NIBR, Basel, Switzerland; [10]Cardiovascular and Metabolic Disease Area, NIBR, Cambridge, MA, United States of America; [11]Tectonic Therapeutic, Inc., Watertown, MA, United States of America; [12]Universidad Nacional de Luján, Departamento de Ciencias Básicas, Laboratorio de Genómica Computacional, Luján, Buenos Aires, Argentina.

The functional dynamics of the gut microbiome and its interactions with the human transcriptome represents a niche for non-invasive biomarkers to risk-stratify MAFLD. In order to identify gut transcriptomic signatures associated with MAFLD in Argentina, we obtained stool samples, diet, demographic and clinical data from 33 biopsy-proven MAFLD patients (12 simple steatosis -SS- and 21 steatohepatitis -SH-) and 19 healthy volunteers (HV). NovaSeq6000® was used for RNA-seq. Data were analyzed with Maaslin2-v1.2.0, bioBakery-v1.8 and DESeq2-v4.1. In MAFLD patients, particularly in those with SH, BMI was significantly higher ($q=4.49x10^{-6}$). After adjusting for BMI, functional profiling of bacterial and viral differentially expressed genes (DEGs) revealed augmented bacterial sulfur and uric acid metabolisms, viral life cycle and viral regulation of host immune system in MAFLD and SH patients when compared to HV and SS, respectively. Inflammatory regulation, lipid, iron and carbohydrate metabolism, and response to oxidative stress were enhanced among human DEGs. The most active bacterial families in all groups were *Bactereoidaceae*, *Rikenellaceae*, *Oscillospiraceae* and *Prevotellaceae*. *Bifidobacteriaceae* expression arose mostly in HV, while *Prevotellaceae* prevailed in MAFLD patients. The Firmicutes/Bacteroidetes ratio was higher in MAFLD and SH when compared to HV and SS, respectively. *Myoviridae*, *Podoviridae*, *Siphoviridae* and *Microviridae* were the most transcriptionally active viral families in all groups. *Myoviridae* and *Microviridae* showed enhanced activity in MAFLD (FDR=0.006 for *Microviridae*) and SH (FDR=0.01 and $4.2x10^{-6}$, respectively), whereas *Podoviridae* and *Siphoviridae* were less active in these groups. In conclusion, we identified specific signatures of the microbial and human gut transcriptomes that could be useful as non-invasive biomarkers of MAFLD diagnosis and progression.

19. (90) **THE GUT MICROBIOME ENVIRONMENT IN METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD): A CORRELATION NETWORK APPROACH FOR THE ANALYSIS OF METATRANSCRIPTOMICS AND METABOLOMICS DATA**

María Florencia Mascardi[1,2], Flavia Mazzini[1], Bárbara Suárez[1,2], Vera Ruda[3,4], Sebastián Marciano[5], Paola Casciato[2,5], Adrián Narvaez[5], Leila Haddad[5], Margarita Anders [6], Federico Orozco[6], Ana Jesica Tamaroff[7], Frank Cook[8], John Gounarides[8], Susana Gutt[7], Adrián Gadano[5], Celia Méndez García[4,9], Martín Marro [10,11], Alberto Penas Steinhard[2,12], Julieta Trinks[1,2]

[1]Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) – CONICET - Instituto Universitario del Hospital Italiano (IUHI) - Hospital Italiano de Buenos Aires (HIBA), Ciudad Autónoma de Buenos Aires, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [3]Biotherapeutic and Analytical Technologies, Novartis Institutes for Biomedical Research, Cambridge (NIBR), MA, United States of America; [4]Chemical Biology & Therapeutics, NIBR, Cambridge, MA, United States of America; [5]Liver Unit of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [6]Liver Unit of Hospital Alemán, Ciudad Autónoma de Buenos Aires, Argentina; [7]Nutrition Department of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [8]Analytical Sciences & Imaging Department, NIBR, Cambridge, MA, United States of America; [9]Chemical Biology & Therapeutics, NIBR, Basel, Switzerland; [10]Cardiovascular and Metabolic Disease Area, NIBR, Cambridge, MA, United States of America; [11]Tectonic Therapeutic, Inc., Watertown, MA, United States of America; [12]Universidad Nacional de Luján, Departamento de Ciencias Básicas, Laboratorio de Genómica Computacional, Luján, Buenos Aires, Argentina.

Functional interactions of the gut microbiome could determine key regulators of MAFLD useful as non-invasive biomarkers of disease progression. Stool samples were obtained from 33 patients with MAFLD (12 simple steatosis -SS- and 21 -SH-) and 19 healthy vol-

unteers (HV) to identify gut microbiome signatures of MAFLD using an integrated analysis of transcriptomics and metabolomics data. HPLC and flow injection analysis with MS/MS in tandem were used for metabolomics. RNA-seq was performed in NovaSeq6000®. Metabolites and human, bacterial, fungal, viral, archaeal and protozoan genes were differentially identified between groups (MAFLD vs HV/SH vs SS), and used to build weighted correlation networks with Spearman's coefficients. Networks were analyzed with Hmisc-v4.7-0, igraph-v1.2.7 and Cytoscape-v.3.9.1. Degree (DS), betweenness (BS) and eigenvector (ES) scores were used to infer the regulatory relevance of each node. The MAFLD *vs* HV network consisted of 51 nodes and 109 edges or correlations, being the strongest one between *Desulfobacteraceae bacterium* and *Faecalibacterium prausnitzii Mushu phage*. Nodes with the highest DS were encoded by *Shigella phage SfIV*, *Mushu phage* and *D. bacterium*, whereas the latter node also showed the highest BS. Top 5 nodes with the highest ES belonged to transcripts of *Mushu phage*. The SH *vs* SS network consisted of 62 nodes and 187 correlations, being the strongest ones between *Fusobacterium proliferatum*, *Methanolacinia paynteri* and *phage phiX174*. The highest DS were found among genes encoded by *Alistipes putredinis*, *F. prausnitzii*, *Shigella phage SfIV*, *D. bacterium*, *Methanoculleus chikugoensis*, *Mushu phage* and *Fonticula alba*. Uppermost BS corresponded to transcripts of *Marssonina brunnea* and *F. alba*. The highest ES were found among transcripts of *Mushu phage* and *F. prausnitzii*. In conclusion, the activity of *F. alba, F. prausnitzii* and its *Mushu phage* in the gut microbiota could act as key regulators of the progression of MAFLD to SH.

20. (92) **ANALYSIS OF THE FUNGAL AND PROTOZOAN GUT TRANSCRIPTOME IN METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD) IN ARGENTINA**

Barbara Suarez[1,2], Flavia Mazzini[1], María Florencia Mascardi[1,2], Vera Ruda[3,4], Sebastián Marciano[5], Paola Casciato[2,5], Adrián Narvaez[5], Leila Haddad[5], Margarita Anders [6], Federico Orozco[6], Ana Jesica Tamaroff[7], Frank Cook[8], John Gounarides[8], Susana Gutt[7], Adrián Gadano[5], Celia Méndez García[4,9], Martín Marro [10,11], Alberto Penas Steinhard[2,12], Julieta Trinks[1,2]
[1]Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) – CONICET - Instituto Universitario del Hospital Italiano (IUHI) - Hospital Italiano de Buenos Aires (HIBA), Ciudad Autónoma de Buenos Aires, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [3]Biotherapeutic and Analytical Technologies, Novartis Institutes for Biomedical Research, Cambridge (NIBR), MA, United States of America; [4]Chemical Biology & Therapeutics, NIBR, Cambridge, MA, United States of America; [5]Liver Unit of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [6]Liver Unit of Hospital Alemán, Ciudad Autónoma de Buenos Aires, Argentina; [7]Nutrition Department of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [8]Analytical Sciences & Imaging Department, NIBR, Cambridge, MA, United States of America; [9]Chemical Biology & Therapeutics, NIBR, Basel, Switzerland; [10]Cardiovascular and Metabolic Disease Area, NIBR, Cambridge, MA, United States of America; [11]Tectonic Therapeutic, Inc., Watertown, MA, United States of America; [12]Universidad Nacional de Luján, Departamento de Ciencias Básicas, Laboratorio de Genómica Computacional, Luján, Buenos Aires, Argentina.

Fungi and protozoa are the least known members of the gut microbiome, but their presence represents a niche for biomarkers discovery to risk-stratify MAFLD patients. In order to identify fungal and protozoan gut transcriptomic signatures of MAFLD, we obtained stool samples from 33 biopsy-proven MAFLD patients (12 simple steatosis -SS- and 21 steatohepatitis -SH-) and 19 healthy volunteers (HV) from Argentina. NovaSeq6000® was used for RNA-seq. Data were analyzed with Maaslin2-v1.2.0, bioBakery-v1.8 and DESeq2-v4.1. Differentially expressed genes (DEGs) of opportunistic pathogenic fungi *Fusarium proliferatum* and *Candida sorbophila* were up-regulated in MAFLD and SH patients when compared to HV and SS, respectively. Biological processes related to acetylenic compound synthesis, reactive oxygen species synthesis, viral procapsid maturation and viral entry into host cell were overrepresented in MAFLD patients, being the latter two also enriched in SH. Among the 151 active fungal families detected in these samples, *Saccharomycetaceae* prevails among MAFLD patients, whereas *Aspergillaceae* family predominates in HV. In the protozoan transcriptome, DEGs of *Blastocystis spp.* and *Fonticula alba*, a bacteria-feeding amoeba, were upregulated in MAFLD and SH patients. Enriched biological processes in the MAFLD group were related to ubiquitination, non-autophagic vacuolization and L-alanine catabolic process, whereas copper metabolism, peptidoglycan turnover and non-autophagic vacuolization were overrepresented in SH. Among the 42 protozoan families active in these samples, *Blastocystidae* was more common in MAFLD and SH when compared to HV (FDR=0.2) and SS groups (FDR=7.1e-06), respectively. Although the activity of the *Fonticulidae* family was scarce, its abundance showed significant differences between MAFLD and HV (FDR=1.26e-07), as well as between SH and SS (FDR=2.9e-18). In conclusion, the activity of several members of the gut microbiome influence MAFLD progression.

21. (103) **ANALYSIS OF THE ARCHEAL TRANSCRIPTOME IN THE METABOLIC ASSOCIATED FATTY LIVER DISEASE (MAFLD) GUT MICROBIOME IN ARGENTINA**

Barbara Suarez[1,2], Flavia Mazzini[1], María Florencia Mascardi[1,2], Vera Ruda[3,4], Sebastián Marciano[5], Paola Casciato[2,5], Adrián Narvaez[5], Leila Haddad[5], Margarita Anders [6], Federico Orozco[6], Ana Jesica Tamaroff[7], Frank Cook[8], John Gounarides[8], Susana Gutt[7], Adrián Gadano[5], Celia Méndez García[4,9], Martín Marro [10,11], Alberto Penas Steinhard[2,12], Julieta Trinks[1,2]
[1]Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) – CONICET - Instituto Universitario del Hospital Italiano (IUHI) - Hospital Italiano de Buenos Aires (HIBA), Ciudad Autónoma de Buenos Aires, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [3]Biotherapeutic and Analytical Technologies, Novartis Institutes for Biomedical Research, Cambridge (NIBR), MA, United States of America; [4]Chemical Biology & Therapeutics, NIBR, Cambridge, MA, United States of America; [5]Liver Unit of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [6]Liver Unit of Hospital Alemán, Ciudad Autónoma de Buenos Aires, Argentina; [7]Nutrition Department of Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [8]Analytical Sciences & Imaging Department, NIBR, Cambridge, MA, United States of America; [9]Chemical Biology & Therapeutics, NIBR, Basel, Switzerland; [10]Cardiovascular and Metabolic Disease Area, NIBR, Cambridge, MA, United States of America; [11]Tectonic Therapeutic, Inc., Watertown, MA, United States of America; [12]Universidad Nacional de Luján, Departamento de Ciencias Básicas, Laboratorio de Genómica Computacional, Luján, Buenos Aires, Argentina.

Non-invasive biomarkers are urgently needed to risk-stratify MAFLD patients. The role of the members, beneficial or deleterious, of the still poorly known domain *Archaea* in the human gut remains to be determined as they could represent a niche for biomarkers discovery. In order to identify archaeal gut transcriptomic signatures related to MAFLD, we obtained stool samples, diet, demographic and clinical data from 33 biopsy-proven MAFLD patients (12 simple steatosis -SS- and 21 steatohepatitis -SH-) and 19 healthy volunteers (HV) from Argentina. NovaSeq6000® was used for RNA-seq. Data were analyzed with Maaslin2-v1.2.0, bioBakery-v1.8 and DESeq2-v4.1.
In the archaeal transcriptome, differentially expressed genes (DEGs) of several methanogenic archaeas were highly expressed in MAFLD and SH patients when compared to HV and SS, respectively. These DEGs were subjected to a GO functional enrichment analysis which revealed over-representation of viral capsid assembly and phage shock processes in MAFLD and SH patients, whereas translation ribosomal small subunit biogenesis and ribosome assembly were enriched in MAFLD, but depleted in SH patients. Among the 37 functional archaeal families identified in the gut microbiome, active members of *Methanosarcinaceae* family were more commonly observed among the HV when compared to MAFLD, but showed a

similar abundance between SH and SS. On the contrary, the active members of the *Nanopusillaceae* family were more prevalent among the MAFLD and the SH patients when compared to the HV and SS groups, respectively. Although the activity of the beneficial *Methanomassiliicoccaceae* family was scarce in the global functional composition, it was significantly lower among patients with a more severe stage of liver disease (SH) when compared to those with SS (FDR=8.1e-10). In conclusion, archaeal expression signatures and their functional taxonomic composition could be useful as non-invasive biomarkers of MAFLD progression in Argentina.

22. (499) **INTEGRATED BIOINFORMATICS ANALYSIS IDENTIFIED lncRNA-miRNA-mRNA COMPETING ENDOGENOUS RNA NETWORKS (ceRNA) THAT MIGHT BE INVOLVED IN PROSTATE CANCER PROGRESSION TO CASTRATION RESISTANCE**

Presenting Author: Sabrina Ledesma-Bazan
Authors: Sabrina Ledesma-Bazan[1,2], Florencia Cascardo[1,2], María Sol Ruiz[1,2], Elba Vazquez[1,2], Geraldine Gueron[1,2], Javier Cotignola[1,2].
[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina.
[2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

Long non-coding RNAs (lncRNAs) regulate several biochemical pathways and contribute to the pathophysiology of cancer. We have previously identified 12 lncRNAs whose expression respond to hormone therapy in prostate cancer patients. Furthermore, in castration-resistant prostate cancer (CRPC), their levels were restored to those observed in therapy-naïve primary tumors. However, how these lncRNAs contribute to tumorigenesis is yet to be understood. Considering that lncRNAs might be sponges of miRNAs, we aimed at identifying lncRNA-miRNA-mRNA networks altered during prostate cancer progression. We performed a bioinformatics-based analysis using public datasets comprising 40 primary prostate tumors (11 paired pre- and post-androgen deprivation therapy (ADT) and 29 pre-ADT) and 8 CRPC. We identified 105 mRNAs differentially expressed in both post- vs pre-ADT primary tumors and CRPC vs post-ADT. Next, to create possible lncRNA-miRNA-mRNA networks, we used miRNet (a miRNA-centric network visual analytics platform) to seek miRNAs that target both lncRNAs and mRNAs. Of note, we found that *PCGEM1* (lncRNA) expression correlates with *ASPM* (mRNA) expression (r=0.51, p=8.2E-13), and these two RNAs have target sequences for the binding of hsa-miR-506-3p and hsa-miR-124-3p. These results identified a possible *PCGEM1-hsa-miR-506-3p/hsa-miR-124-3p-ASPM* ceRNA involved in prostate cancer progression to castration resistance. In addition, Gene Set Variation Analysis (GSVA) revealed that this ceRNA network might regulate Wnt signaling pathway in prostate tumors. Other ceRNAs identified included *RAB3B* mRNA (RAS oncogene family) and are: *PCGEM1-hsa-miR-124-3p-RAB3B, LINC01087-hsa-miR-130a-3p-RAB3B, and LINC01671-hsa-miR-145-5p-RAB3B.* These results give new insights in ceRNA dysregulation in prostate cancer; and ultimately, they might help to identify new prognostic biomarkers for prostate cancer and new druggable targets for avoiding/delaying progression to CRPC.

23. (545) **CHARACTERIZATION OF BREAST TUMORS WITH UNBALANCED LEVELS OF PROGESTERONE RECEPTOR ISOFORMS: A PROTEOMIC APPROACH**

Andrés Elía[1], Jana Sánchez[4], Rui Vitorino[2], Leo Saldain[1], Paula Martínez Vazquez[3], Javier Burruchaga[3], Eunice Spengler[3], Javier Muñoz[4], Paola Rojas[1], Luisa Helguero[2], Claudia Lanari[1].
1Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina; 2Institute of Biomedicine (IBIMED), University of Aveiro, Portugal; 3Hospital de Agudos "Magdalena V de Martínez", General Pacheco, Argentina; 4Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

It is well established that progesterone receptors (PR) play a relevant role in breast carcinogenesis and that a misbalanced expression of PR isoforms differentially affects breast cancer progression and antiprogestin treatment responsiveness. Moreover, we have shown, in preclinical and clinical studies, that mifepristone exerts antiproliferative effects in tumors expressing higher levels of PR isoform A (PRA) than B (PRB), named PRA-H, suggesting the necessity to develop methods to identify these patients using non-invasive technologies. Our aim was to compare the proteome profile of PRA-H breast carcinomas with those with the opposite ratio (PRB-H). Nuclear and cytosolic protein fractions were obtained from 18 breast cancer samples classified as PRA-H or PRB-H and were studied by LC-MS/MS (UltiMate 3000 LC system - Q Exactive HF-X mass spectrometer - Thermo). We observed 289 differentially regulated proteins in cytosol (164 down and 125 up) and 301 in nuclear extracts (131 down and 170 up; logFC > 1, pval < 0.05) from both groups. Enrichment analysis showed biological processes related to intracellular transport (FDR = 9.58e-08), metabolic (FDR = 0.0028) and actin filament-based processes (FDR = 1.79e-05) in PRB-H tumors; while in PRA-H tumors, pathways related to oxoacid metabolic (FDR = 0.0015), immune effector (FDR = 1.59e-08) and electron transport chain (FDR = 3.35e-06) processes were enriched. Finally, using the differentially expressed proteins, we studied those that might be secreted. Among them, we found CPB1, whose differential mRNA expression has already been reported in a similar setting in previous assays. This study underscores the biological differences between PRA-H and PRB-H breast carcinomas and provides candidates that might be used as surrogate markers in plasma to identify patients that may benefit from an antiprogestin treatment.

24. (609) **DISSECTING TUMOR *VS.* STROMA TRANSCRIPTOME IN PDAC XENOGRAFTS: MRP4 AS AN INDUCTOR OF STROMAL ACTIVATION**

Sahores A[1], Gancedo S[1], De Sousa-Serro MG[1], González JF[1], Gómez N[1], May M[1], Shayo C[2], Davio CA[1], and González B[1].
1. Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, C1113AAD, Buenos Aires, Argentina.
2. Instituto de Biología y Medicina (IBYME-CONICET), Buenos Aires, Argentina.

The xenobiotic transporter MRP4/ABCC4 is involved in the regulation of cAMP signaling by extrusion to the extracellular compartment. MRP4 is highly expressed in pancreatic ductal adenocarcinoma (PDAC), and is linked to increased proliferation, a mesenchymal phenotype and poor prognosis. PDACs are characterized by a dense fibrotic (desmoplastic) stroma, mainly composed by cancer activated fibroblasts (CAFs) and extracellular matrix, that determines the interaction between cancer cells and their microenvironment, and also promotes tumor progression and chemoresistance. The aim of our study was to evaluate the effect of tumor MRP4 overexpression on the stromal compartment. We established PDAC xenografts by sc. inoculation of BxPC3 cells in NSG mice, with stable transfection of either ABCC4 (MRP4+) or empty vector (mock). These xenografts consist of human parenchyma and murine stroma, mostly fibroblasts. Tumortranscriptome was evaluated by RNAseq, and the obtained FASTQ files were aligned to human and mouse reference genomes. The obtained BAM files were processed with Xenofilter, an algorithm specifically developed to discriminate human and mouse reads. The mouse count matrix was analyzed with DESeq2, and the differentially expressed genes (DEGs) were evaluated for functional enrichment (gene ontology and pathway analysis). We detected 49 DEGs in murine stromal cells from MRP4+ *vs.* mock xenografts. Particularly, stromal upregulated genes showed significative enrichment (FDR<0.05) in terms related with purinergic and adenosine receptor signaling, autophagy and collagen biosynthetic processes, while downregulated genes associated with TGFβ signaling. Analysis of the TCGA-PAAD database (178 samples) showed a positive correlation of ABCC4 with 17 marker genes of myofibroblastic and inflammatory CAFs subtypes. Our results suggest that MRP4 overexpression in PDAC augments stromal adenos-

ine levels and primes fibroblasts to increase collagen production and promote desmoplasia.

25. (635) **THE ABUNDANCE OF CYTOTOXIC CELLS IN THE BONE MARROW TUMOR MICROENVIRONMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS IS ASSOCIATED WITH SPECIFIC CLINICAL AND TRANSCRIPTOMIC FEATURES**

Daniel Avendaño [1,2], Mercedes Abbate [1,2], María Cecilia Ricicheri [3], Elba Vazquez [1,2], Geraldine Gueron [1,2], Javier Cotignola [1,2], María Sol Ruiz [1,2]

1Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina; 2CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina. 3Hospital Nacional Posadas, Servicio de Pediatría, El Palomar, Buenos Aires, Argentina.

Acute lymphoblastic leukemia (ALL) is characterized by bone marrow immature lymphoid blasts overproduction and is the most incident pediatric cancer worldwide. Although overall survival and treatment efficacy has increased in recent decades, some patients develop treatment-related acute toxicity and about 15-30% of patients relapse. Whether the components of the tumor microenvironment (TME) can contribute to the clinical heterogeneity observed in patients is yet to be deciphered. In this work, we aimed at characterizing the immune components of the TME in ALL by gene expression analysis and assess its prognostic value. RNA-seq was performed in bone marrow aspirates from Argentinian pediatric ALL patients at diagnosis (N=32). *MIXTURE* deconvolution method was used to estimate the proportion of immune cells. $CD4^+$ T cells proportions were increased in patients that died or relapsed (p-val=5.6E-4). Next, we calculated a "cytolytic score" (CS) based on the expression of 5 specific genes for cytotoxic T lymphocytes and Natural Killer cells by RNA-seq and performed validation by RT-qPCR. Results showed that B cell proportions correlated with blasts percentage at diagnosis by blood smear (Spearman rho=0.628, p-val<0.0005). We developed a custom-made method to measure the CS by RT-qPCR; results were expressed relative to healthy donor-derived activated peripheral blood mononuclear cells. *NRF1* was selected as the reference gene (*NormFinder*). The CS values by RT-qPCR and RNA-seq were highly correlated (Spearman rho=0.817, p-val=1.56E-6) and showed >95% agreement (Bland-Altman analysis). *CX3CR1* (adj.p-val<0.05) and *HAVCR2* (*TIM-3*, adj.p-val=0.067) RNA levels were increased in patients with high vs low CS. Overall, the TME immune component of ALL patients showcased high heterogeneity. Further, transcriptional features suggest an inflammatory and exhausted phenotype in the TME with high CS patients.

26. (700) **MITOCHONDRIAL AEROBIC METABOLISM PLAYS A KEY ROLE IN HER2+ TRASTUZUMAB-RESISTANT BREAST CANCER TUMOR SPHEROIDS**

Ivana Tapia[1], Davide Perico[2], Antonella De Palma[2], Virginia Wolos[1], Marcela Villaverde[1,3], Fernanda Rocca[1], Marianela Abrigo[1], Dario Di Silvestre[2], PierLuigi Mauri[2], Gabriel Fiszman[1,3].

[1]Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigación, Departamento de Inmunobiología, Buenos Aires, Buenos Aires, Argentina. [2]Proteomics and Metabolomics Unit, Institute for Biomedical Technologies (ITB-CNR), Segrate (MI), Italy. [3]CONICET, Argentina.

Breast cancer (BC) is the leading cause of death in women worldwide. Around 15-20% of BC tumors overexpress HER2, which is associated with poor disease-free survival. Targeted anti-HER2 therapies such as Trastuzumab (Tz), provide an option for these patients. However, ~60% of them acquire a resistant phenotype during the first year of treatment. In order to identify protein profiles associated with Tz resistance, a comparative proteomic study was performed. Two differential Tz response levels in a 3D culture model were mimicked using a HER2+ human mammary adenocarcinoma cell line (BT-474) and its derived resistant cell line developed in our laboratory (partial Responder Spheroids RS and non-Responder Spheroids n-RS, respectively). During 15 days both spheroids were treated with Tz (50 μg/ml) (%inhibition, RS: 51.2±11.1 and n-RS: 23.1±6.3; ANOVA, p<0.01) and pre- and post-treatment samples of each condition were collected. Afterwards, HPLC-coupled mass spectrometry analysis was performed (3 biological replicates with 2 technical replicates; FDR 1%). A total of 3,881 proteins were identified and label-free compared. Next, those statistically significant proteins that allowed to discriminate between conditions (Linear Discriminant Analysis p<0.05) were determined and the subsequent functional enrichment analysis (Cytoscape, STRING) revealed changes in several pathways. Among them, while RS exhibited a predominance of anaerobic energy metabolism, n-RS were characterized by increased mitochondrial aerobic metabolism. *In vitro* treatment with 2.5M metformin (Met, mitochondrial electron chain inhibitor) significantly prevented growth n-RS ($\%inhibition_{Tz}$= 15.0±3.6 vs $\%inhibition_{Tz+Met}$= 41.1±2.7, t-test p<0.05) and accentuated Tz response in RS. Interestingly, Met affected 3D architecture of n-RS. To conclude, these results highlight a key role of mitochondrial aerobic metabolism in Tz resistance and propose Met as a potential therapeutic alternative for this phenotype.

27. (765) **CHARACTERIZING SPECIFIC ROLES OF VAV FAMILY MEMBERS IN MELANOMA**

Aylén Avila[1]; Luciano Anselmino[2,3]; Nahuel Cesatti Laluce[2,3]: Macarena Mamberto[2,3], Xosé R. Bustelo[4], Mauricio Menacho-Márquez[1,2,3]

1. Centro de Investigación y Producción de Reactivos Biológicos (CIPReB, Facultad de Ciencias Médicas-UNR); 2. Instituto de Inmunología Clínica y Experimental de Rosario. Universidad Nacional de Rosario (IDICER, UNR-CONICET); 3. CONICET; 4. Centro de Investigación del Cáncer de Salamanca, España.

aylen.avila@gmail.com

Vav proteins are Rho guanine nucleotide exchange factors with roles in physiological and pathological processes. The three members of this family normally show functional redundancy and are associated with proactive functions in cancer, but their role in melanoma was poorly explored. We aimed to characterize by bioinformatic approaches processes differentially regulated by each member of this family in melanoma. All 'packages' were used in R. Gene expression data of skin cutaneous melanomas was obtained by 'TCGABiolinks' from the Cancer Genome Atlas (TCGA). We divided patients according to Vav1, Vav2 and Vav3 expressions. Survival plots were constructed using the Kaplan-Meier estimator. High Vav2 expression was associated with a worst prognosis (p=0.037) while Vav1 and Vav3 correlated with patient survival (p=0.0056 and 0.00011). Differentially expressed genes between the groups were considered for |FC|>1.5 and FDR<0.01 using 'edgeR'. Enrichment analyses were performed with ShinyGO 0.76 and 'ReactomePA'. Immune cell infiltration signatures were evaluated by eight different algorithms, finding a strong positive correlation between Vav1 and immune cell signatures (p<2e-16). No correlation was observed for Vav2 or Vav3 expression. We modulated Vav2 and Vav3 expression in B16F0 mouse melanoma cells using shRNAs and plasmid expression techniques. Total RNA was extracted and samples processed at the Genomic Unit (Centro de Investigación del Cáncer, Salamanca, Spain) using the "GeneChip WT PLUS Reagent kit". After 16-18h of hybridization at 45ºC, staining and washing, the ClariomS mouse Arrays were scanned, The CEL files obtained were analyzed using 'limma' and 'oligo'. The analysis of functional notations associated with Vav2 and Vav3 partially overlapped the ones obtained from TCGA data. Altogether, our data indicate that Vav1 expression is associated with immune cell infiltration, while Vav2 and Vav3 modulate different processes in melanoma cells, including tumor immunity.

28. (772) **BIOINFORMATIC COMPLEMENTATION OF THE ROLE OF GENETIC VARIANTS IN THE MANIFESTATION OF PORPHYRIAS**

Priscila Ayelén Pagnotta[1,2], María Laura Buscalia[3], Johanna Zuccoli[3], Viviana Melito[2,3], Victoria Parera[3] y Ana María Bu-

zaleh[2,3]
1 Instituto de Biología y Medicina Experimental (IBYME); 2 Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales (FCEN, UBA); 3 Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP), UBA-CONICET

Porphyrias are a group of metabolic diseases caused by failures in heme biosynthesis. Porphyria Cutanea Tardia (PCT), caused by a deficiency in the Uroporphyrinogen decarboxylase enzyme, is highly associated with HIV infection. The mutation in Porphobilinogen deaminase is not enough for the appearance of symptoms in Acute Intermittent Porphyria (AIP). Experimentally, we observed that symptomatic AIP and PCT-HIV individuals present a higher frequency of non-wild type variants of *ABCB1* and Glutathion S-transferases (*GST*s). The aim was to use databases to complement this study. Pubmed and Scielo (meta-analysis following the guidelines of PRISMA), 1000Genomes, PharmGKB, Gene Expression Omnibus (GEO, GSE44228), UniProt, Ensembl, and GenBank were used. The experimental results of the control group matched with the meta-analysis and with that reported in 1000Genomes. Gene frequencies varied between different regions of the planet. Some drugs for the treatment of HIV increase the probability of toxicity in individuals with experimentally tested variants: Efavirenz and Nelfinavir (rs1045642, *ABCB1*), Atazanavir (rs2032582, *ABCB1*) and Nevirapine (rs1045642, *ABCB1*; Presence, *GSTM1*). Using an array (GSE44228), individuals treated with protease inhibitors had lower expression of *ABCB1* (FC=0.83, p adj<0.05) than non-nucleoside reverse transcriptase inhibitors and differential expression of 17 *ABC*s (65% overexpressed), 21 *CYP*s (71% overexpressed) and 11 *GST*s (64% under expressed). Cases of probability of systemic toxicity were found in relation to triggering drugs of AIP and genetics variants: rs1045642 (12), rs1128503 (2) and rs1128503 (2), *null GSTT1* (3), *null GSTM1* (8) and rs1695 (7). The evidence on the role of variants in xenobiotic transporter genes and drug metabolizing system in PCT-HIV association and the manifestation of AIP became greater with this further study, reinforcing the relevance of advancing towards personalized medicine and pharmacogenetics.

29. (802) **TRANSCRIPTIONAL EFECTS OF INGAP-PP PEPTIDE IN THE CONTROL OF THE PANCREATIC β-CELL MASS AND FUNCTION: ADVANTAGES OF COMBINE EPIGENETIC AND TRANSCRIPTOMIC ANALYSIS**

Agustín Romero[1,2], Ana C. Heidenreich*[1,2], Carolina L. Roman*[3], Macarena Algañarás[3], Juan J. Gagliardino[3], Bárbara Maiztegui[3], Luis E. Flores[3], Santiago A. Rodríguez[1,2].
1 Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET), Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina.
2 Departamento de Fisiología, Biología Molecular y Celular, Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Buenos Aires, Argentina.
3 CENEXA-Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET-CeAs CICPBA), Facultad de Ciencias Médicas UNLP, La Plata, Argentina.
** Equal contribution*

Diabetes mellitus is characterized by chronic hyperglycemia and can lead to pancreatic β-cell loss. An attractive therapeutic approach is to harness the innate regenerative potential of the pancreas itself. The Islet Neogenesis-Associated Protein pentadecapeptide (INGAP-PP) has been shown to improve β-cell mass and function. The aim of this work is to yield light into the mechanism of action underlying such effects. We performed RNA-seq analysis on rat pancreatic islets treated in vitro with INGAP-PP and integrated epigenetics data to further characterize transcriptomic effects of this treatment. We identify 1,669 differentially expressed genes by INGAP-PP treatment (Linear Mixed Effects model p<0.05), including dozens of previously unannotated rat transcripts. Among these we found genes that are selectively expressed in different cell types within pancreatic islets, proposing that INGAP-PP effects may potentially be reached by interaction of different cell populations. In-depth analysis reveals previously reported and novel signaling pathways potentially modulated by the peptide treatment, like Vegf, Pi3k, and Tgf-β pathway, as well as biological processes including angiogenesis and extracellular matrix organization. We report and validated by qRT-PCR (ANOVA p<0.05) the upregulation of: Mlxipl (coding for ChREBP, involved in activation of genes in response to carbohydrates), Tcf3 (angiogenesis), Col6a3 (extracellular matrix organization) and Nfatc3 (β-cell activation). Taking advantage of the epigenomic analyses, we also identify 14 novel long non-coding RNAs that are express in β-cells. Moreover, our results suggest that INGAP-PP might coordinate the activation of Hif1a-, Nfat- and Vitamin D receptor-regulated programs, ultimately leading to enhanced glucose-stimulated insulin release. Taken together, these results reveal novel potential mechanisms that could underlie the positive physiological effects of INGAP on β-cell function and mass.

30. (852) **HIF1A/HIF3A RELATION IN ACUTE MYOCARDIAL INFARCTION PROGNOSIS: IN SILICO ANALYSIS**

Nuñez Pedrozo CN[1]; Peralta TM[1]; Castillo MG[1]; Locatelli P[1]; Olea FD[1]; Crottogini A[1]; Cuniberti L[1].
[1]Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET).

Objectives: The hypoxia-inducible factor-1 (Hif1) is a major regulator of the hypoxic response after acute myocardial infarction (AMI). It is known that mammalian hearts have limited regeneration capacity. Over the last years, molecular therapies have sought to enhance Hif1 expression to induce angiogenesis and cell survival. In the present research we evaluated the Hif1a/Hif3a relation in the transcriptomes of infarcted mice belonging to the regenerative (R) and non-regenerative (NR) evolutionary stages. Methods: The bulk RNA-Seq files (n=36) were retrieved from GEO (GSE142366). The heart samples were taken at 1.5, 3 and 7 days post-AMI for both neonatal (R) and infant mice (NR) and non-infarcted mice at equal times. The files were processed on UseGalaxy employing the FastQC/HISAT/featurecount pipeline. Differential gene expression (FDR < 0.05) and gene ontology analysis was performed with edgeR and PathFindR. Results: Bulk analysis showed that at 1.5 days post AMI, R mice had significant lower expression of Hif1a (logFC: -1.24) and higher expression of Hif3a (logFC: 2.15) when compared to NR mice (p < 0.001). Gene ontology analysis showed a significant alteration of the biological process named positive regulation of angiogenesis at 1.5 days (FE: 1.54; p <0.05) and 7 days (FE: 1.87; p <0.05) post-AMI in genes with differential expression between R and NR stages. Conclusion: This study shows that within the first 36 hours post-AMI Hif1a expression is down-regulated in R mice compared to the NR mice. Furthermore (and surprisingly), Hif1a post-IAM in the R condition is also down-regulated compared to the non-infarcted R condition. This temporary silencing is associated with a higher expression of Hif3a in the R infarcted mice compared to the NR infarcted mice. These results support the existence of a Hif1a/HIif3a relation that may fine-tune the positive regulation of angiogenesis post-AMI.

31. (873) **CHARACTERIZATION OF A TRASTUZUMAB-RESISTANT HER2-OVEREXPRESSING BREAST CANCER CELL MODEL USING SHOTGUN PROTEOMICS**

Marianela Abrigo[1], Virginia J. Wolos[1], Ivana J. Tapia[1], Miriam J. Diament[1], Elisa D. Bal de Kier Joffé[1,3], Ezequiel Lacunza[2,3], Gabriel L. Fiszman[1,3].
[1] Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigación, Buenos Aires, Argentina.
[2] CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Buenos Aires, Argentina.
[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

HER2 is overexpressed in 15-20% human breast tumors and is associated with poor disease-free survival. Trastuzumab (Tz), a HER2-targeted monoclonal antibody, is the therapy of choice for HER2+ breast cancer patients. However, more than half of them develop resistance during treatment. In our laboratory, we established a Tz-resistant breast cancer cell line, BT-474R, obtained by continuous treatment of HER2+ BT-474 cell line with 10 µg/ml Tz for

6 months. The aim of this work was to identify protein profiles associated with the resistant phenotype. To this end, we compared BT-474 and BT-474R through a LFQ proteomic approach. A *label-free mass spectrometric* protein *quantification followed by* Proteome Discoverer analyses was performed (FDR 1%). For differential protein expression analysis, we employed DEqMS in R/Bioconductor. Results were visualized in a Volcano plot (fold change≥2). We identified 176 differentially abundant proteins from a total of 1,381, among which 62 were upregulated and 114 downregulated in BT-474R cells ($p<0.05$). Then, to identify the cellular processes involved in this modulation, we performed a GeneSet Enrichment Analysis (GSEA) using clusterProfiler in R/Bioconductor. The most highlighted processes that could be associated with Tz resistance were oxidative phosphorylation, fatty acid metabolism, mTORC1 signaling ($p<0.001$) and G2-M checkpoint ($p<0.01$). Cell cycle proteins were analyzed, finding Cdk1 upregulated in BT-474R cells ($p<0.001$). Furthermore, pirin and DCLK1 proteins, known as positive regulators of cell proliferation and tumor progression, were also upregulated in the resistant cell line. Accordingly, we found that BT-474R cells have a significantly shorter doubling time than BT-474 cell line (30.23 h vs 92.37 h, $p<0.05$). Our findings suggest a more proliferative phenotype associated with Tz-resistant cells. Further studies are needed to understand the proteomic changes involved in resistance acquisition.

BIOINFORMATICS AND THERAPEUTIC TARGETS II

Friday, November 18, 14-15:30 hr

Chairs: María Sol Ruiz - Ayelén Toro - Juan Bizzotto - Fernanda Rubio

32. (6) **THE TRADEOFFS BETWEEN CLINICAL AND LABORATORY BIOMEDICAL RESEARCH**

Oscar Bottasso[1]

[1]*Instituto de Inmunología Clínica y Experimental de Rosario (IDICER, UNR-CONICET)*

The need to achieve a greater understanding of the diverse array of medical problems imposes a kind of conducting force capable of pushing this continuously shifting exploration border towards areas of greater confidence. The science and the medical practice digging their backgrounds through hypotheses, experimental verifications, and subsequent interpretations are presently getting the input of Translational Medicine viewed as a goal of biomedical research aimed at offering better control tools. Something that is closely linked to the origins of the very medicine. The transition between basic and clinical research is complex requiring appropriate crosstalk helpful enough for the input/output relationship to become fruitful. Partly because the *in vitro* experiments and preclinical studies do not necessarily mirror the clinical situation to sensibly translate the "truth" emerging from laboratory data. This is particularly relevant when trying to develop new biomarkers, potentially predictive scoring systems, biostatistical procedures for combined endpoints, and mostly, the efforts towards the availability of novel therapeutic approaches. Regardless of the precise situation, intermingled communication between players from different fields is necessary, if we are about to establish new conceptual and methodological frameworks likely to facilitate a better theoretical/practical approach. To some extent, it constitutes a sort of burst of the biomedical sciences into the clinical scenario aimed at validating the knowledge arising in the laboratory to become part of some clinical practice or guidelines for health policies, with all the implications therein. Conversely, many clinical observations raise questions that can be elucidated from the experimental ground, as a valuable way of affording some feedback on this interactive process. The way ahead appears as long as challenging, given that the bulk of the investigative work carried out in our setting has been mostly performed in self-confined compartments (biomedical, clinical, and public health). Purportedly, we have lastly realized that field-specific speeches concentrated on themselves end up hindering the portrayal of problems in their different facets, as well as the way of formulating investigative designs addressed to provide improved alternatives.

33. (44) **INFLUENCE OF MEMBRANE LIPID COMPOSITION ON WATER DIFFUSION THROUGH HUMAN AQUAPORIN-1**

Casal J.J., Dorr R.A., Toriano R.

Universidad de Buenos Aires. CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay). Facultad de Medicina, Paraguay 2155 7º (1121) Buenos Aires, Argentina

Introduction. Lipids play important modulatory and structural roles for membrane proteins. Molecular dynamics simulations (MDS) are frequently used to provide insights into the nature of these protein-lipid interactions. We study the influence of the lipid environment on human Aquaporin-1 (hAQP1, PDB code: 4CSK). Two heterogeneous lipid bilayers, representative of mammals (M) and cancer cells (C), and other only with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), were generated around 4CSK. Methods. CHARMM-GUI was used for the generation of the three systems. 4CSK was embedded into a 70 × 70 Å lipid bilayer, with TIP3P water molecule model, neutralizing ions, and a temperature set at 37ºC. The CHARMM36m force field was used at production dynamics of 500 ns. GROMACS, VMD and MDAnalysis were used to evaluate the convergence of the simulation, pore radius profile and water diffusion through the protein channels, distances between selectivity residues (ar/R) and the number of water molecules near them and pore length in the narrow zone. Comparative mean, SD and ANOVA tests were used in statistics. Results. Constriction in the ar/R site: varied from a narrow to a wide conformation. A closed state coincides with the absence of water molecules at the site. Water diffusion through protein in DPPC: 8.3 (0.04), C: 8.62 (0.08) > M: 4.44 (0.01), units: 10^{-14} cm^3s^{-1}. Pore length in C: 20.40 (0.48) > DPPC: 18.94 (0.42) > M: 18.49 (0.37), units: Å. Conclusion. We present quantitative evidence that membrane composition affects AQP1 water dynamics. Our findings confirm the need for further progress in the study of the regulation of aquaporins by their lipid environment. With the emergence of more powerful hardware and advanced simulation techniques and algorithms, we can expect an even larger impact of simulations on our understanding of biological membranes and the role of lipids.

34. (170) **DRUG REPURPOSING STRATEGY TARGETING SEROTONIN-GATED ION CHANNELS**

Noelia Rodriguez Araujo, Guillermina Hernando and Cecilia Bouzat

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Argentina.

Drug repurposing is an effective strategy for identifying new therapeutic use(s) for currently available drugs. We focused on serotonin (5-HT)-gated ion channels of nematodes and vertebrates and tested a series of clinically used drugs by electrophysiological techniques. We studied a nematode serotonin-activated chloride channel, MOD-1, as a novel target for antiparasitic drugs, and the vertebrate 5-HT_3A receptor as a drug target for the treatment of nausea, emesis, and irritable bowel syndrome. Receptors were expressed in mammalian cells and their function was measured by whole-cell recordings. Drug screening assays revealed that piperazine (PZE), an anthelmintic drug acting at nematode GABA receptors, decreased macroscopic currents elicited by 5-HT of MOD-1 (IC_{50} 113±29 μM). The analysis indicated that PZE acts as a negative allosteric modulator of MOD-1. Moreover, motility assays using the nematode model *Caenorhabditis elegans* showed that the negative modulation impacts on worm behavior, thus confirming the inhibition of MOD-1 as a novel anthelmintic mechanism. We tested PZE derivatives acting as H1-antihistamine drugs and found that hydroxyzine inhibited MOD-1 responses whereas cetirizine did not have any effect. We also showed that tryptamine, which has significantly higher agonist efficacy for MOD-1 (α=80%) than for 5-HT_3A (α=27%), affected worm motility, indicating that it can be a novel anthelmintic lead compound. Moreover, we found that sumatriptan, a tryptamine-derivative currently used for migraine, also inhibited MOD-1 currents elicited by 5-HT. In 5-HT_3A studies, we found that PZE also decreased macroscopic responses elicited by 5-HT (IC_{50} 238±89 μM), thus revealing a novel

allosteric inhibitor of this receptor. Our drug repurposing approach contributes to identify new targets and novel pharmacological uses of clinical drugs on a rational basis.

35. (279) **IMPACT OF FECAL MICROBIOTA TRANSPLANTATION IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS**

María Florencia Fernández[1], Martín Ruhle[1], Daniela Borgnia[1], Claudia Hernandez[2], Ana Juliá[3], Adriana Bottero[4], Laura Busquet[4], Carlos Waldbaum[5], Fabiana López Mingorance[5], Carlos Figueroa Turienzo[3], Andrea Mangano[1]

[1]Unidad de Virología y Epidemiología Molecular-CONICET, Hospital de Pediatría "J.P. Garrahan", Argentina, [2]Servicio de Microbiología, Hospital de Pediatría "J.P. Garrahan", Argentina, [3]Servicio de Trasplante de médula ósea, Hospital de pediatría "J.P. Garrahan", Argentina, [4]Servicio de Gastroenterología, Hospital de Pediatría "J. P. Garrahan", Argentina, [5]Departamento de Gastroenterología, Hospital de Clínicas José de San Martín, Argentina.

Background and Aims: The Graft-Versus-Host-Disease (GVHD) is a major cause of mortality related to Hematopoietic Stem Cell Transplant (HSCT). The conditioning regimen for HSCT leads to disruption of microbiota homeostasis, called dysbiosis. Lower alpha-diversity of the Fecal Microbiota (FM) at the time of neutrophil engraftment was associated with higher transplantation-related mortality. For this reason, numerous therapeutic strategies have been proposed to restore FM integrity, including use of Fecal Microbiota Transplantation (FMT). Our goal is to evaluate the changes FMT produces in FM of pediatric HSCT recipients and describe their clinical evolution. Methods: We monitored 15 pediatric HSCT recipients 7 days before and after FMT, including the donors. Library preparation was based on 16S Metagenomic protocol and Illumina MiSeq sequencing. Metataxonomic and statistical analysis was performed by SHAMAN software. Results: Microbiome alpha diversity, related to detect Operational Taxonomic Units (OTUs), was compared between the 3 groups (donors, pre-FMT and post-FMT). We found α-diversity increased significantly post-FMT (p=0.00045). Donors conferred 48 new OTUs after FMT. Moreover, Bacteroides acquired bacterial dominance (>37.8%) and Ruminococcus increased significantly (1.8% to 17.1%) after FMT. In our cohort, 8 patients developed grade 2 GVHD, 1 patient grade 3 GVHD and 6 did not present GVHD complications. Furthermore, no transplant-related mortality was observed. Conclusions: To our knowledge, this is the first study using metagenomic sequencing in a pediatric cohort undergoing FMT after-HSCT. These findings suggest that increase in diversity results from transferring new OTUs from the donor during the FMT. Despite the fact that there were no HSCT-related deaths or severe GVHD, the small patient population makes it infeasible to correlate to any specific genera. Further FM characterization may provide insights into other factors influencing clinical outcomes.

36. (289) **TRANSCRIPTIONAL ANALYSIS SHEDS LIGHT ON RUSSIAN STURGEON MECHANISMS TO COPE WITH BACTERIAL INFECTION AND CHRONIC HEAT STRESS**

Valeria Silva-Álvarez[1,2], Alicia Costábile[3], Mauricio Castellano[3], Marcio Aversa-Marnai[1,2], Ignacio Quartiani[4], Daniel Conijeski[5], Alejandro Perretta[4], Andrea Villarino[3], Ana María Ferreira[1,2]

[1] Unidad de Inmunología, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Uruguay.
[2] Área Inmunología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Uruguay.
[3] Sección Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de la República, Uruguay.
[4] Unidad de Patología, Biología y Cultivo de Organismos Acuáticos, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de la República, Uruguay.
[5] Esturiones del Río Negro S.A., Uruguay.

Sturgeons are chondrostean fish of high economic value and critically endangered due to anthropogenic activities, which has led to sturgeon aquaculture development. Russian sturgeon (*Acipenser gueldenstaedtii*), the second most important species reared for caviar, is successfully farmed in subtropical countries, including Uruguay. However, during the Uruguayan summer, sturgeons face intolerable warmer temperatures that weaken their defences and favour infections by opportunistic pathogens, increasing fish mortality and farm economic losses. Since innate immunity is paramount in fish, for which the liver plays a key role, we used deep RNA sequencing to analyse differentially expressed genes in the liver of Russian sturgeons exposed to chronic heat stress and challenged with *Aeromonas hydrophila*. We assembled 149.615 unigenes in the Russian sturgeon liver transcriptome and found that metabolism and immune defence pathways are among the top five biological processes taking place in the liver. Chronic heat stress provoked profound effects on liver biological functions, up-regulating genes related to protein folding, heat shock response and lipid and protein metabolism to meet energy demands for coping with heat stress. Besides, long term exposure to heat stress led to cell damage triggering liver inflammation and diminishing liver ability to mount an innate response to *A. hydrophila* challenge. Accordingly, the reprogramming of liver metabolism over an extended period had detrimental effects on fish health, resulting in weight loss and mortality, with the latter increasing after *A. hydrophila* challenge. This transcriptomic study describes how chronic heat stressed sturgeons respond to a bacterial challenge, suggesting that liver metabolism alterations have a negative impact on the innate anti-bacterial response of this ancient fish.

37. (328) **INTEGRATION OF COMPUTATIONAL AND EXPERIMENTAL APPROACHES TO DISCOVER NEW POTENTIAL *TRYPANOSOMA CRUZI* OLIGOPEPTIDASE B INHIBITORS**

Melina Mottin[1,2]; Matheus Silva[1]; Milene Andrade[1]; Carolina Horta Andrade[2]; Jaime M Santana[1], Izabela M. D. Bastos[1]; Flávia S. N. Motta[1,3].

1. Laboratório de Interação Patógeno-Hospedeiro (LIPH), University of Brasília, Brasília, DF, Brazil.
2. Laboratory of Molecular Modeling and Drug Design (LabMol), Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil.
3. Faculdade de Ceilândia, University of Brasília, Brasília, DF, Brazil
Corresponding author e-mail: fnmotta@unb.br

Trypanosoma cruzi oligopeptidase B (OPBTc) is proven to participate in the parasite infection process and has been increasingly cited as an important virulence factor in trypanosomatids, since it has no orthologs in mammals. Therefore, OPBTc is a promising target for the rational design of effective and safe inhibitors, with a greater possibility of therapeutic success. In this study, the ChemBridge commercial database was virtual screened (VS) against OPBTc, aiming to prioritize new potential inhibitors. The VS was performed based on the following computational filters: (*i*) structural similarity to known OPBTc inhibitors; (*ii*) molecular docking at the OPBTc catalytic binding site; and (*iii*) lipophilicity (logP) and solubility (logS) properties. Firstly, a similarity search of ChemBridge dataset was performed based on the structure of 13 OPBTc inhibitors, previously identified in our laboratory. A total of 136 compounds were selected for the molecular docking VS. The 3D structure of OPBTc is not experimentally available. Thus, we predicted OPBTc 3D models using different methods: homology modelling, protein folding and *ab initio* (deep learning). Four models were obtained using the Swiss-Model, Itasser, trRosetta and Alphafold servers. All models were evaluated regarding statistical quality parameters, using MolProbity server. The best model was obtained through the Alphafold server. The OPBTc catalytic binding site is composed by the triad His682, Ser562 and Asp647. Then, docking calculations were performed against the best OPBTc 3D model, at the catalytic site, using the virtual screening workflow protocol of the Glide program. The top 10 ranked virtual hits presented docking scores ranging from -8.0 to -2.0 Kcal·mol^{-1}. Lipophilicity and solubility properties of the hits were evaluated and a final hit list with 22 promising OPBTc inhibitors was obtained. Future enzymatic and parasite assays will be performed to evaluate the activity of the prioritized hits against OPBTc.

38. (429) **TRANSIENT INFLAMMATORY GENE EXPRESSION TRIGGERS REORGANIZATION OF CHROMATIN TOPOLOGY ASSOCIATED TO DECIDUALIZATION**

Luciana Ant1, Francois Le Dily2, Julieta Erramouspe1, Carlos David Burque3, Miguel Beato2, Patricia Saragüeta1.
1 CONICET-Intituto de Biología y Medicina Experimental, Capital Federal, Argentina, 2 Centro de Regulación Genómica (CRG), Barcelona, España, 3 Unidad de Conocimiento Traslacional Hospitalaria Patagónica, Hospital de Alta Complejidad SAMIC - El Calafate, Provincia de Santa Cruz, Argentina.

Background: Chromatin topology is known to participate in gene expression changes associated with cell differentiation. To approach this issue in a hormone inducible system, we studied the conversion of stromal endometrial cells (t-HESC) into secretory cells, known as decidualization, which is controlled mainly by progesterone.
Description: We performed Hi-C and RNAseq analysis 1h, 3 and 6 days after hormone treatment. The transcriptional profiles determined by mRNA-seq were already modified at 1h in 96 genes (log2(FC) > l1l, padj<0,05), were TNFα/NFKB pathway was significantly enriched (FDR<0,05). At 3 days, 42 (43,75%) of these early regulated genes showed reorganization of chromatin topology in their regions. Furthermore, 6 of these 42 genes were significantly down-regulated at 3 days (log2(FC) > l1l, padj<0,05), and 36 continued to be expressed. After 3 days, the cells exhibited a secretory profile and down-regulation of cell cycle genes (FDR<0,05). We also analyzed the shift in chromatin state (A/B) at 100kb resolution and found that the majority of the compartments shifted from B to A state (70,39 % of the compartments that changed state). After 6 days, the cells continued expressing a secretory profile along with an increase in a subset of cell cycle genes. Between day 3 and 6, we detected compartment state shifts mainly from A to B (94.24 % of the compartments that changed state). Conclusion: The changes in compartment state at day 3 could be due to the transient cell cycle inhibition, since other gene expression pathways were not modified between day 3 and 6. However, other processes cannot be excluded. The genes upregulated at 1h might play a role setting off the differentiation process. Further studies at a higher resolution or single cell studies of chromatin accessibility could contribute to a better understanding of the relationship between genome topology and gene expression.

39. (485) **STUDY OF HUMAN AQUAPORIN-4 EXTENDED ISOFORM BY HETEROLOGOUS EXPRESSION IN XENOPUS LAEVIS OOCYTES AND MOLECULAR DYNAMICS**

Tomás Molina Ponce[1], Victoria Vitali[2], Juan José Casal[1], Karina Alleva[2], Claudia Capurro[1], Vanina Netti[1]
[1] Laboratorio de Biomembranas, Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
[2] Cátedra de Física. Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Aquaporin-4 (AQP4) is expressed at the plasma membrane as 2 isoforms, M1-AQP4 of 323 amino acids (aa) and M23-AQP4 of 301 aa. Recently, a new AQP4 isoform with a 29 aa C-terminal (Ct) extension (AQP4ex, 352 aa) generated by translational readthrough (TRT) was described, which has not been fully characterized yet. Thus, the aim of our work was to study the properties of the Ct extension by heterologous expression in *Xenopus laevis* oocytes and molecular dynamics (MD) simulations. Human M1-AQP4 and M1-AQP4ex (Biomatik) were subcloned into T7T-derived vector and expressed in oocytes to measure osmotic permeability coefficient (P*f*) in response to a hypotonic shock. For MD simulations, the Ct end was modeled and linked to human AQP4 crystalized structure (3GD8) to build M1-AQP4 and M1-AQP4ex homotetramers by Chimera. Then, a 100 ns MD simulation was run in GROMACS for both isoforms embedded in a bilayer of lipid POPC molecules and solvated with TIP3P as a solvent model. M1ex was built with S324 in the stop codon skipped by TRT in both systems. Our results indicate that M1 and M1ex were expressed at the plasma membrane and functional for water transport, presenting P*f* values different from non-inyected oocytes. Both isoforms presented an increase in P*f* at both acidic (5.8) and alkaline (7.4) intracellular pH, being this increase higher in alkaline conditions. Homology modeling of the extended Ct showed that it is a random coil. MD simulations evidenced that M1ex has a larger root mean square deviation compared to M1, indicating that M1ex would be less compact and stable. The distance from H201 to R216, representative of the selectivity filter (M1: 7.7 ± 4.2Å vs. M1ex: 8.3 ± 2.1 Å, ns), is similar in both isoforms. Our preliminary results indicate that the Ct extension may not be involved in regulation of water permeability of M1ex. However, this is the first report evaluating P*f* values of human M1-AQP4ex with S324 and demonstrating its modulation by acidic/alkaline pH.

40. (566) **COMPARISON OF THE INTERACTION OF MKP3 (DUSP6) AND ITS SPLICE VARIANT WITH ERK BY DOCKING METHODOLOGY**

María Mercedes Bigi, M. Mercedes Mori Sequeiros Garcia, Silvana Nudler, Juan Manuel Cohen Sabban, Fabiana Cornejo Maciel, Paula Maloberti, Cristina Paz.
INBIOMED, UBA-CONICET, Departamento de Bioquímica Humana, Facultad de Medicina, UBA.

MKP-3 is a dual activity enzyme member of the MAP Kinase Phosphatases family. It is induced by proliferative stimuli and specific for phospho-ERK. The human MKP-3 gene generates the full-length transcript or variant L, and an alternative spliced product or variant S, encoding MKP-3S or S protein. MKP-3L is a cytosolic protein regulated by ERK-dependent phosphorylation. S protein lacks the nuclear export signal and a target residue for ERK phosphorylation involved in L protein stability. Accordingly, we have demonstrated differences between variants regarding stability, subcellular localization and enzymatic activity measured by ERK dephosphorylation. The aim of this study was to gain insight on MKP-3 spatial structure by a bioinformatic approach to explain these differences and to predict additional functional differences between L and S proteins. From sequence alignments we found that an "acid loop" region, which includes the D262 residue crucial for the catalysis, is absent in S protein. In contrast, S protein retains its binding domain to ERK2 through a kinase interaction motif (KIM). We simulated possible interactions between MKP-3 variants and ERK through docking methodology. From crystals structures 1HZM and 2FYS (PDB IDs) we simulated the ERK interaction with the KIM domain and MKP-3S. Then, we made the prediction of the interaction of the catalytic domain with phosphatase activity and ERK, through aligning, refining and docking structures 1MKP and 3ZUV. In all cases, the best models obtained achieved a high quality score (CAPRI RANK 3). The results suggest that although S protein could interact with ERK, its catalytic activity would be altered. S protein could act as a negative dominant by interacting through the KIM domain and blocking L interaction with ERK. Based on the analysis and the predictive models obtained, our current efforts focus on performing different mutations and activity measurements on different substrates to validate these results.

41. (623) **COMPARATIVE GENE EXPRESSION PROFILING BETWEEN EX VIVO AND IN VIVO IRRADIATION AT 4 HOURS**

Jerónimo Leberle[1], Vanesa Biolatti[2], Lara Negrin[2], Adriana Cascón[2], Rocio Brezan[2], Julieta Irazoqui[2], Alejandro Alvarez[3], Romina Ventimiglia[3], Marina Perona[4], Irene Ibañez[5], Nicolás Bellora[1].
[1] Comisión Nacional de Energía Atómica (CNEA)- INTECNUS - CONICET, [2] CNEA - INTECNUS, [3] Fundación INTECNUS, [4] Gerencia de Área de Aplicaciones de la Tecnología Nuclear - CNEA - CONICET, [5] Instituto de Nanociencia y Nanotecnología - CNEA - CONICET

Introduction and objectives: The molecular study of the radioinduced response by the massive analysis of the radiomodulated transcriptome has gained great relevance in recent years, both in the field of radioprotection and radiotherapy (RT). The aim of this work was to evaluate the transcriptome of irradiated human leukocytes at 4 hours post-exposure *ex vivo* and *in vivo* in order to identify a trend in the gene expression profile between conditions. Materials and methods: Leukocytes from healthy individuals were X-ray irradiated *ex vivo* at 25, 100 and 200 cGy. Leukocytes were also collected from patients with different tumors which received an *in vivo* irradiation corresponding to a single RT fraction, equivalent to a 1.6 cGy blood dose. Control samples were non-irradiated *ex vivo* or collected prior to the RT session. Then, leukocytes were cultured for 4 hours at 37°C and 5% CO_2 for RNA extraction. The transcriptome was sequenced by RNA-seq (Illumina Platform, 150 bp paired-end). Sequencing reads were mapped to the human genome hg38 (STAR software). A comparative analysis of the transcriptomes was performed between irradiated vs. control samples by applying different bioinformatic pipelines, DESeq2 and limma:voom tools. Results: First, we identified 15737 differentially expressed genes (DEGs) associated with biological variability which were then removed to highlight the DEGs modulated by ionizing radiation in every dose vs. controls (0 Gy). The most significant radioinduced DEGs *ex vivo* were SEMA7A, CD70, PCNA, CDKN1A, DDB2, AEN and FDXR and a long non-coding RNA, LINC02846. Finally, transcripts per million (TPMs) were analyzed, showing consistent gene expression levels between conditions. Conclusion: A similar trend was observed in the expression profile of the most significant radioinduced DEGs between *ex vivo* and *in vivo*, showing that these DEGs have a similar behavior after irradiation.

42. (649) **GLUTATHIONE AND FORMALDEHYDE METABOLISM AS THERAPEUTIC INTERVENTIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA**

Agustín Morellato, Lucas Pontel
Instituto de Investigación en Biomedicina de Buenos Aires (IBIOBA), CONICET - Partner Institute of the Max Planck Society, Buenos Aires, Argentina.

Formaldehyde (FA) is produced inside cells as a byproduct of essential biological processes. We have recently shown that glutathione (GSH) reacts with FA and forms S-hydroxymethyl-GSH (HSMGSH), which is then metabolized by the enzyme alcohol dehydrogenase 5 (ADH5). In this work, we set up to identify those tumors that might be less tolerant to inactivation of GSH and FA metabolism. Using DepMap portal data, we analyzed essentiality and found that cancer cells from hematopoietic origin present negative CHRONOS scores for genes involved in GSH metabolism. Then, we evaluated the sensitivity of a panel of cancer cell lines to the γ-glutamatecysteine ligase (GCL) inhibitor L-buthionine sulfoximine (L-BSO) alone, and in combination with FA in models lacking ADH5. We identified two groups of cell lines: those resistant to L-BSO alone, in which GSH synthesis was epistatic to ADH5 for FA tolerance; and those sensitive to L-BSO, in which GSH can protect cells against FA independently of ADH5. Normalized viabilities against 200 μM L-BSO in resazurin-based viability assays were A549 (lung cancer)= 109.3±1.6 μM; H1299 (lung cancer)= 103.8±1.7 μM (P=0.92); HCT116 (colorectal cancer)= 96.7±4.1 μM (P=0.36); Nalm6 (B-acute leukemia)= 17.0±7.1 μM (P<0.0001); Jurkat (T-acute leukemia)= 2.1±0.3 μM (P<0.0001), (mean ± SEM, Tukey's comparison vs A549). Finally, as GSH can also act as co-factor of peroxidases involved in the detoxification of cellular peroxides, we suggest that a GSH-dependent peroxidase might protect against FA independently of ADH5 in hematopoietic-derived cell lines. Using DepMap portal data we evaluated the expression of glutathione peroxidases and found that only GPX1, GPX4 and GPX7 are expressed in these cells. Also, preliminary data using RSL3 inhibitor in viability assays indicates that GPX4 counteracts FA toxicity in acute leukemia-derived cell lines, overall revealing a selective feature that might shed light into more selective therapeutic interventions.

43. (663) **B LINEAL EPITOPES PREDICTION OF *BLOMIA TROPICALIS* MAJOR ALLERGENS**

Constanza Guerrieri Magrini[1,2], Federico Paez Córdoba[1,2], Guido Scarpati Soto[2], Alejandra Goldman[2], Ignacio Fenoy[2]
1 Both authors contributed equally
2 TECA_ECyT-UNSAM - CONICET

RATIOLE: House dust mite (HDM) allergy is the leading cause of IgE-mediated hypersensitivity. *Blomia tropicalis* has been reported to be a clinically important HDM allergen. Current diagnostic and therapy relay in whole mite extracts. There is an impossibility to obtain homogeneous allergen extract preparations from natural sources. To develop better diagnoses and immunotherapies against allergies there is a need to better understand allergen's immunogenicity. We aim to predict linear B epitopes in *B. tropicalis* allergens. METHODS: *B. tropicalis a*llergens were retrieved from allergen.org. Protein sequences were analyzed for post-translational modifications. Reported epitopes of *B. tropicalis* were retrieved from IEDB.org. B cell linear epitopes (lep) of each major allergen of *B. tropicalis* were predicted using 8 software according to the following pipeline: i) ABCpred with a 16-mer and a 0.75 threshold; ii), found leps must also be identified by Bepipred Linear Epitope Prediction 2.0 or by Bepipred Linear Epitope Prediction. The remaining lep must have more than 4 adjacent residues in at least one of these algorithms using default thresholds; and iii) selected epitopes must also be predicted by at least 3 of the following software: Chou & Fasman, Emini, Karplus & Schulz, Kolaskar & Tongaonkar and Parker (IEDB.org). RESULTS: For *B. tropicalis* 14 major allergens were found (Blo t 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 19, 21). Only Blo t 5, 21, 13 have reported lep in IEDB. After applying the described pipeline we found the following lep: Blo t 21 (1), Blo t 1 (15), Blo t 11 (29), Blo t 3 (12), Blo t 12 (7), Blo t 7 (5), Blo t 5 (1), Blo t 8 (6), Blo t 4 (19), Blo t 10 (9), Blo t 13 (7), Blo t 19 (2), Blot 6 (14) and Blo t 2 (7). These epitopes could be useful for allergy immunotherapy and diagnosis development.

44. (797) **GENOME SEQUENCE AND CHARACTERIZATION OF A HYPERVIRULENT BI/NAP1/027 *CLOSTRIDIOIDES DIFFICILE* (CDC20121308).**

Laureano A. Español[1], Miranda C. Palumbo[2], Federico Serral[2], Angela M. Barbero[1,3], Sabina Palma[1,3], Diego Ruggeri[4], Darío Fernández Do Porto[2], Rodrigo E. Hernández Del Pino[1,3], Virginia Pasquinelli[1,3].
1. CIBA, UNNOBA, Buenos Aires, Argentina, 2. IQUIBICEN, FCEN-UBA, Buenos Aires, Argentina, 3. CIT NOBA (UNNOBA, UNSAdA, CONICET), Buenos Aires, Argentina, 4. Servicio de Bacteriología Sanitaria, INEI-ANLIS "Dr. Carlos G. Malbrán".

Clostridioides difficile (*C. difficile*) is a Gram-positive, obligate anaerobic and spore-forming bacteria that is widespread in the environment. *C. difficile* infection is an important cause of antimicrobial-associated diarrhea, life-threatening pseudomembranous colitis and toxic megacolon. Since 2000, the rapid emergence of the hypervirulent PCR ribotype (RT) 027 complex has been associated with increases in the incidence and severity of disease and mortality. We characterized a BI/NAP1/027 *C. difficile* strain CDC20121308. The genome sequence was obtained using a whole-genome shotgun strategy. The draft genome was 4,188,514 bases in length and the G+C content of the genomic DNA was 29.3 mol%. A total of 3,971 coding sequences (CDS) and 55 tRNAs were predicted. We detected the presence of RT 027 lineage markers (*thyA*, *cdtA*, *cdtB and tcdC* 18bp-deletion), previously characterized by MALDI-TOF. The annotation identified 25% of CDS into RAST subsystems. The genome of CDC20121308 had 11 genes devoted to resistance to toxic compounds, antibiotics (Tetracycline (Tet) and Vancomycin (Van)) and disinfecting agents as predicted using CARD. Indeed, by broth microdilution assay we found that *C. difficile* CDC20121308 is resistant to Van and Tet with minimum inhibitory concentrations of 4 mg/ml and 8 mg/ml, respectively. Crystal violet staining demonstrated biofilm formation, which could be associated with antibiotic resistance and pathogenicity. Moreover, we observed a spreading diffuse growth away from the inoculum stab at 12, 24 and 48 h, suggesting a motile phenotype. In conclusion, our results allowed the characterization of the BI/NAP1/027 *C. difficile* CDC20121308 strain. We de-

monstrated the presence of several genes associated with pathogenesis that were validated by functional assays. This study provides additional data for the use of this highly virulence commercial strain of epidemiological relevance worldwide in research work involving *in vitro* and *in vivo* assays.

45. (862) **GENETIC REGULATION OF SUCCINATE METABOLISM IN THE HYPOXIC HEART OF NEONATAL MICE REVEALED BY BULK AND SINGLE-NUCLEUS RNA SEQUENCING ANALYSIS**

Peralta TM[1], Nuñez Pedrozo CN[1], Ulla S[1], Locatelli P[1], Olea FD[1], Crottogini A[1], Cuniberti L[1].
[1]Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET).

Objective**:** The aim of this research was to compare the expression profile of genes related to succinate metabolism in the infarcted hearts of neonatal and infant mice by bulk and single-nucleus RNA-Seq analysis in order to clarify the molecular basis of the metabolic switch related to myocardial regeneration. Materials and methods: The bulk and single nucleus RNA-Seq files were retrieved from GEO (GSE142366). The heart samples were taken at 1.5, 3 and 7 days post-acute myocardial infarction (AMI) for neonatal (P1) and infant mice (P8). The bulk files were processed on UseGalaxy employing the FastQC/HISAT/featurecount pipeline. Differential gene expression analysis of bulk and single nucleus data was performed with edgeR and Seurat respectively. Results: Bulk analysis showed that at 1.5 days post AMI, neonatal mice had significant downregulation of the Krebs cycle genes Sucla2 (logFC -1.47), Sdha (logFC -1.20), Sdhb (logFC -0.81), Sdhd (logFC -0.78) and Succinate exporters genes Vdac1 (logFC -0.81), Slc16a1 (logFC -0.96) when compared to the infant mice ($p < 0.001$). At 7 days post AMI, neonatal mice showed significant downregulation of Sucla2 (logFC -0.88), Sdha (logFC -0.48), Vdac1 (logFC -0.54) and Slc16a1 (logFC -0.90) compared to infant mice ($p < 0.05$). No significant differences were found at day 3 post AMI. Single-nucleus analysis showed that at 1.5 days post AMI, neonatal murine cardiomyocytes showed significant downregulation of Sucla2 (logFC -0.53), Sdha (logFC -0.56), Sdhb (logFC -1.20), Sdhc (logFC -0.42), Sdhd (logFC -0.78) and Vdac1 (logFC -1.08) compared to infant mice ($p < 0.001$). Conclusion: Mammalian hearts are known to accumulate succinate during myocardial infarction which leads to generation of reactive oxygen species and cell death upon reperfusion. The ability of neonatal mice to reduce the expression of genes involved in succinate metabolism may partially explain the myocardial tolerance to hypoxia required for regeneration.

46. (902) **BIOINFORMATIC EVALUATION OF DIFFERENTIALLY EXPRESSED GENES IN MYELODYSPLASTIC SYNDROMES WITH *SF3B1* VARIANTS**

Lincango, Marco[1]; Larripa, Irene[1]; Belli, Carolina[1].
1. Laboratorio de Genética Hematológica, Instituto de Medicina Experimental (IMEX-CONICET)/ Academia Nacional de Medicina, Buenos Aires

Bioinformatic analysis of cloud databases is an emerging research method to dig the physiopathology of diseases. Pathogenic variants in the splicing factor (SF) *SF3B1* (SF3B1mut) in low risk Myelodysplastic Syndromes (MDS) were recognized within a distinct subtype in 2022, associated with ring sideroblasts, good outcome and response to Luspatercept. Our aim was to individualize differential expressed genes (DEG) related with *SF3B1*mut and a differential outcome in MDS. RNAseq datasets of CD34+ of MDS patients (24 SF3B1mut, 32 SF-wild type-WT) and healthy controls (7 HC) were downloaded from the GEO database GSE114922. Analysis, specified below, were performed in R v4.2.1. A total of 1342 DEG ($p<0.05$) were identified (DESeq2 package) between SF3B1mut, SF-WT and HC. Functional enrichment analysis (gseGo and enrichGO) showed compromised pathways related to ribosomes, oxidative phosphorylation, mitochondrial gene expression and translation. A set of 40 genes were identified as hub genes (STRING, protein-protein interaction networks; CytoHubba, *Stress* algorithm). ROC curve analysis (pROC package, AUC>0.85) to determine DEG related with the presence SF3B1mut highlighted *MRPL2* (0.87), *MRPL52* (0.86), *NDUFA8* (0.89), *MRRF* (0.90), *ALAD* (0.85) and *GAR1* (0.91). SF3B1mut was independently associated (Logistic regression) with higher expression levels of *MRRF* (OR36.3, CI95% 1.6-1827.4, p=0.04) and a tendency for *NDUFA8* (OR25.7, CI95% 0.8-1545.9, p=0.08) and *GAR1* (OR 23.9, CI95% 0.8-3656.9, p=0.14). Regarding survival (Cox's regression), higher expression levels of *NDUFA8* (HR0.2, CI95% 0.04-0.8, p=0.024) and of GAR1 (HR 0.1, CI95% 0.02-0.7, p=0.022) were independently associated with a better outcome, while of *MRPL52* (HR 7.9, CI95% 0.8-79.1, p=0.08) and *MRRF* (HR 21.5, CI95% 0.6-753.9, p=0.091) with a tendency of a worse one. Our in silico approach, which validation is ongoing, underlined four DEG associated with differential survival outcome and the presence of *SF3B1* variants in MDS.

CARDIOVASCULAR AND RESPIRATORY I

Thursday, November 17, 9-10:30 hr

Chairs: Rosana Elesgaray - Martín Donato - Silvia García

47. (12) **EFFECTS OF HYPERBARIC OXYGEN THERAPY (HBOT) ON SARS-COV-2+ HOSPITALIZED PATIENTS WITH HYPOXEMIC RESPIRATORY FAILURE**

Guillermo A. Keller[a,b], Leila Eizaguirre [b], Javier Coria[c], Guillermo Di Girolamo[c] and Silvia Miranda[c]
a-Universidad de Buenos Aires, Facultad de Medicina, Centro de Vigilancia y Seguridad de Medicamentos.
*b-Hospital General de Agudos Donación Francisco J. Santo*janni, Departamento de Urgencias.
c-Universidad de Buenos Aires- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional (IATIMET).

HBOT has been proposed to address COVID-19-associated hypoxia. However, its actions at the molecular level are poorly known.
Method: 50 patients with hypoxemic COVID-19 pneumonia from Hospital Santojanni were recruited between May-August 2021 in a randomized controlled study comparing standard care (C group) versus standard care plus HBOT (H group) in 1:1 proportion. HBOT was performed with Biobarica® chambers at 1.45 atm, 90 min/day for 5 days. Oxygen saturation (SatO2) was followed up. Blood was obtained at t=0 and t=5d. White blood cell (WC) count, lymphocytes (L) and platelets (P) and serum analysis (glucose, urea, creatinine, sodium, potassium, ferritin, D dimer, LDH and CRP) were carried out. Plasma levels of sVCAM, sICAM, sPselectin, SAA and MPO, and of a cytokine panel (IL-1β, IL-1RA, IL-6, TNFα, IFNα, IFNγ, IL-15, VEGF, MIP1α, IL-12p70, IL-2 and IP-10) were measured by multiplex magnetic bead assays (Merck Millipore). Angiotensin Converting Enzyme 2 (ACE-2) levels were determined employing a comercial kit (R&D Systems)
Results: The average basal SatO2 was 85±3%. The days needed to reach SatO2>90% were: H: 3±1 and C: 5±1 ($P<0,01$). At term, H increased WC, L and P counts (all, $P<0,01$). Also, H diminished D dimer levels (H=233±81, C=402± 66ng/ml; $P<0,001$) and LDH concentration (H= 237±92, C: 329±44 UI/L, $P<0.01$]. At term, H showed lower levels of sVCAM, sPselectin and SAA than C with respect to basal values (H vs C: ΔsVCAM: $P<0,01$; ΔsPselectin: $P<0,05$; ΔSAA: $P<0,01$. Similarly, H diminished levels of TNFα (ΔTNFα: $P<0,05$), while increased levels of IL-1RA and VEGF (ΔIL-1RA and ΔVEGF: $P<0,05$). The rest of parameters did not show differences between groups.
Conclusion: Patients underwent HBOT improved SatO2 with lower levels of severity markers (WC and platelets count, D dimer, LDH, SAA). Moreover, this work demonstrated that HBOT reduced proinflammatory agents (sVCAM, sPselectin, TNFα) and increased anti-inflammatory and pro-angiogenic ones (IL-1RA and VEGF).

48. (43) **IMPACT OF MEDIUM PRESSURE HYPERBARIC OXYGENATION THERAPY ON THE CARDIOVASCULAR SYSTEM**

Magdalena Peirone, Christopher Gutierrez, Javier Coria, Silvia Miranda, Guillermo Di Girolamo, Rocío Castilla and Pa-

tricia Bonazzola
Instituto Dr. Alberto Taquini de Investigaciones en Medicina Traslacional (IATIMET) UBA-CONICET

Medium Pressure Hyperbaric Oxygenation therapy (mHBOT) at 1.4 atm and 100% oxygen has been proposed instead of the traditional HBOT at 2.4 atm. It is used as an adjunct therapy in burn wounds, diabetic foot ulcers and treatment of COVID-19 patients with respiratory distress. Up to date no studies were carried out to support the cardiovascular risk of both treatments. Thus, we studied the impact of mHBOT on the cardiovascular system in Sprague Dawley rats subjected to 30 sessions of 60 min at 1.4 atm and 100% O_2. Isolated hearts were perfused and exposed to 30 min ischemia (I) and 45 min reperfusion (R) at 37ºC. Simultaneous mechanical and heat measurements and the infarct area were evaluated. In aorta, histology, nitrite levels and the contractile response to noradrenaline (NA), acetylcholine (Ach) and nitroprusside (SNP) were studied. In plasma, sICAM and sEselectin were analyzed. Hearts from mHBOT-treated rats showed: 1) an increase in resting pressure during I ($p<0.05$), 2) an improvement ($p<0.05$) in post-I contractile recovery (52.4±12.3 vs 23.1±5.9%) at 45 min R, 3) an increase in pre-I +P/P ratio, 4) no changes in total heat rate, 5) increased ($p<0.05$) contractile economy during R (99.2±20.3 vs 37.5±8.9% at 45 min). 6) a reduction in the infarct area. Arteries from mHBOT-treated rats showed: 1) no changes in morphology, response to NA or SNP, 2) a decrease ($p<0.05$) in EC50 (1.8×10^{-8} vs 2.3×10^{-7} M) and in the maximal relaxation (79.8±2.1 vs 60.7±2.5%) by Ach, 3) an increased nitrite levels (60.4±5.4 vs 10±2.2 μM). Plasma from mHBOT-treated rats showed a sICAM increase ($p<0.05$) (10.46±0.72 vs 8.01±0.57 ng/ml) but sEselectin remained unchanged. Conclusion: the mHBOT seems to sensitize the contractile machinery, cardioprotects hearts from I/R injury acting as a preconditioning agent, and increases the endotellium-dependent relaxation probably related to NO production. mHBOT would not activate endothelium and sICAM may be involved in the mHBOT-induced NO production.

49. (81) **POTENTIAL PROTECTIVE EFFECTS OF THE BISPHOSPHONATE ALENDRONATE ON VASCULAR SYSTEM**

Cutini PH, Massheimer VL
Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), CONICET-UNS, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.

Bisphosphonates are commonly drugs used for the treatment of postmenopausal osteoporosis. Since the risk of cardiovascular disease is increased in postmenopausal women, we study the extraosseous actions of the bisphosphonate alendronate (ALN). To that end, cellular and molecular events involved in vascular remodeling and survival were studied, such as cell proliferation and migration, nitric oxide (NO) production, and angiogenesis. Primary cultures of EC and VSMC isolated from murine aorta were used. We showed that ALN significantly stimulated endothelial NO synthesis (115±8 vs 175±15 nmol NO/mg protein, control vs 10 µM ALN, $p<0.02$; Griess method). In turn, 10 µM ALN markedly enhanced EC viability (71% over control, $p<0.001$; 96 h treatment; MTT assay). Since these cellular events are crucial for neovascularization, the effect of ALN on angiogenesis was investigated using EC cultures and the tube formation assay. ALN stimulated tube formation (5.4±0.5 vs 7.2±0.6 mm, control vs ALN 10 µM, $p<0.05$; 96 h of treatment) through a mechanism of action that involves MEK1/2, PKC and PI3K signal transduction pathways participation. Oxidative and inflammatory stress alters VSMC behavior. We demonstrated that 10 µM ALN blocked the LPS-induced reactive oxygen species hydrogen peroxide (H_2O_2) synthesis. Under basal conditions, ALN did not induce changes in H_2O_2 synthesis with respect to the control. The action of ALN on VSMC migration was evaluated using an EC-VSMC co-culture model (transwell system). The evidence obtained shows that ALN completely suppressed LPS-induced VSMC migration. This action was only detected in the co-culture, since in the absence of EC, ALN did not exhibit this effect. In conclusion, the results presented suggest a potential protective effect of ALN on events that compromise vascular architecture through a direct action on vascular cells.

50. (115) **"PATHOPHYSIOLOGICAL ROLE OF INOSITOL 1, 4, 5-TRIPHOSPHATE RECEPTOR (IP3R) BINDING PROTEIN RELEASED WITH IP3 (IRBIT) IN THE MYOCARDIUM"**

Di Mattia Romina[1], Ciarrocchi Sofía[1], Gallo Delfina[1], Blanco Paula[2], Portiansky Enrique[3], Valverde Carlos[1], Bleckwedel Federico[4], Zelarayan Laura[4], Aiello Alejandro[1], Orlowski Alejandro[1]
[1]Centro de Investigaciones Cardiovasculares "Dr. Horacio Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.
[2]Servicio de Cardiología, Facultad de Veterinaria, Universidad Nacional de La Plata. La Plata, Argentina.
[3]Laboratorio de Análisis de Imágenes, Facultad de Veterinaria, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.
[4]Instituto de Farmacología y Toxicología, Centro Médico Universitario Goettingen. Goettingen, Alemania.

Introduction: IP3R binding protein released with IP3 (IRBIT) was originally identified as a competitive inhibitor of the mentioned receptor. When IP3 concentration raises in response to GPCR activation, IRBIT is released from IP3Rs and activates several ion transporters, including Na^+/HCO_3^- cotransporters NBC, Na^+/H^+ exchanger NHE3, Cl^- channel CFTR and Cl^-/HCO_3^- exchanger Slc26a6. Aims: Although IRBIT heart expression has been reported, its function in cardiac tissue is unknown. Thus, we aimed to study the cardiac effects of overexpressing IRBIT to establish its pathophysiological role. Experimental design: 3 months-old male mice were transduced (1×10^{12} vp/kg) with a cardiotropic adenoassociated virus to achieve IRBIT overexpression (AAV9-IRBIT-mCherry), using AAV9-mCherry as control. Echocardiography and electrocardiography analysis were performed and mice were sacrificed after a month. IRBIT expression was assessed in two cardiac hypertrophy models: spontaneously hypertensive rats (SHR) and mice with transaortic constriction (TAC). Data is expressed as mean±SEM. We used Shapiro-Wilk normality test and Student's t-test or two-way ANOVA test as was needed. For non-normal populations, we used Mann-Whitney test. Results: IRBIT overexpressed mice showed an increase in left ventricular mass index (LVMI) and wall thickness measured by echocardiography (LVMI, 28 days: AAV9-mCherry: 2.81±0.22, n=7; AAV9-IRBIT-mCherry*: 3.77±0.32 n=10; $p<0.05$ vs. AAV9-mCherry). Moreover, an augmented heart weight/body weight was found in IRBIT overexpressed group compared to control (AAV9-mCherry: 4.25±0.13, n=7; AAV9-IRBIT-mCherry*: 4.78±0.18, n=10; $p<0.05$ vs. AAV9-mCherry). To further study the connection between IRBIT and cardiac hypertrophy, IRBIT protein expression was measured in SHR rats and IRBIT mRNA levels were measured using single cell RNA sequencing approach in TAC mice. In both models, IRBIT was found significantly overexpressed (Wistar: 2.65±0.48, n=4; SHR*: 5.53±0.63, n=3; Sham: 0.27±0.019, n=413; TAC*: 0.29±0.012, n=852; $p<0.05$ vs. Wistar and Sham respectively). Conclusion: Data presented here support IRBIT as a novel cardiac protein involved in cardiac hypertrophy development.

51. (134) **MELATONIN PROTECTS AGAINST CARDIAC MITOCHONDRIAL DYSFUNCTION IN EARLY TYPE 1 DIABETES**

Ivana A. Rukavina-Mikusic[1,2], Juan S. Adán Areán[1,2], Virginia Vanasco[1,2], Silvia Alvarez[1,2] y Laura B. Valdez[1,2]
[1] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Fisicoquímica; [2]Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Bioquímica y Medicina Molecular (IBIMOL; UBA-CONICET), Fisicoquímica;
irukavina@docente.ffyb.uba.ar

Background: Melatonin has been shown to protect against cardiac dysfunction, with its action being associated with changes in mitochondrial function and dynamics. Aim: To study the effect of melatonin administration against cardiac mitochondrial dysfunction associated to short periods of hyperglycemia, in a type 1 diabetes model.

Methods: Diabetes was induced by Streptozotocin (60 mg/kg, ip) in male rats. On the 3rd day and until the day before sacrifice, rats received a daily injection of melatonin (10 mg/kg day, ip). Four experimental groups were formed: control, control rats injected with melatonin, diabetic rats, and diabetic rats injected with melatonin. Animals were sacrificed at day 14, and heart mitochondrial function was studied. Results: Melatonin did not reverse the hyperglycemia developed in STZ-injected animals, nor the lower weight gain of diabetic rats. However, melatonin prevented the cardiac mitochondrial dysfunction that occurs as direct or indirect consequence of hyperglycemia, avoiding or attenuating: a) the active mitochondrial respiration decline (25%); b) the respiratory complexes dysfunction (~20%); c) the oxidative phosphorylation efficiency decrease (18%); d) the increase in mitochondrial production of H_2O_2, NO and $ONOO^-$ (90%, 50%, 240%); e) the Mn-SOD activity reduction (14%); f) the increase in mitochondrial $[O_2^{\cdot-}]_{ss}$, $[H_2O_2]_{ss}$ and $[NO]_{ss}$ (126%, 43%, 50%); and g) the elevation of lipid oxidation (48%) and protein nitration (35%). The Se-GPx (38%) and catalase (380%) activities as well as the UCP-3 protein levels (180%) remained increased in mitochondria of diabetic animals despite melatonin treatment, contributing to the $[H_2O_2]_{ss}$ maintenance. Conclusion: Although rats remain exposed to hyperglycemia, melatonin administration prevented the heart mitochondrial dysfunction. The cardiac mitochondrial function improvement in early diabetes could delay or prevent the onset of heart failure that occurs after much longer periods of hyperglycemia.

52. (173) **RESTORATION OF CARDIAC MITOCHONDRIAL FUNCTION IS RELATED TO MITOCHONDRIAL DYNAMICS EFFICIENCY AND DEPENDS ON THE SEVERITY OF ENDOTOXEMIA**

Adan Arean Juan Santiago, Pappalettera Bruno, Vico Tamara, Evelson Pablo, Vanasco Virginia, Alvarez Silvia.
Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica. Junín 956, C1113AAD CABA, Argentina.

There is a close relationship between mitochondrial ultrastructure and function. Changes in mitochondrial dynamic processes (fusion, fission, mitochondrial biogenesis, and mitophagy) could act as mechanisms leading to mitochondrial function restoration, which is affected during endotoxemia. Our aim was to analyze if the changes in mitochondrial dynamics are related to cardiac mitochondrial function restoration during mild and severe endotoxemia. Female Sprague Dawley rats (45 days) were ip injected with 0.5 mg/kg (low-grade endotoxemia, LE), 8 mg/kg (severe endotoxemia, SE), or vehicle (control, C), and after 6 or 24 h assays were performed in cardiac tissue. PGC1α y mTFA expression (mitochondrial biogenesis) were increased only at 6h in LE and recovers control expression at 24h, while in SE values remained increased up to 24h. In LE, Parkin expression increased at 6 h while Pink1 only increased at 24 h (mitophagy). In SE, both Pink1 and Parkin increased their expression since 6 h. TEM analysis showed mitochondrial swelling at 6 h in SE and LE. At 24h LE, mitochondrial population showed size variety and structures compatible with mitochondrial remodeling processes. Interestingly, in SE at 24h also evidence of the loss of the myofibrillar structure and mitochondria with altered morphology is observed. To assess mitochondrial function recovery, ATP production and mitochondrial membrane potential were measured. In LE, ATP production was found significantly decreased (38%) at 6h, recovering control values at 24h (control: 225 nmoles ATP/min mg protein, $p<0.05$). However, in SE both ATP production and mitochondrial membrane potential were observed decreased (44% and 22% respectively) up to 24 h. Our results show that the efficiency of mitochondrial dynamics in restoring mitochondrial function in the heart depends on the degree of inflammation and experimental endotoxemia.

53. (192) **"HYPOOSMOTIC STRESS AS A MODULATOR OF THE NLRP3 INFLAMMASOME IN ADULT CARDIOMYOCYTES"**

Racioppi MF, Gonano LA, Pereyra E, Rando M and Vila Petroff M. Centro de investigaciones cardiovasculares Horacio *Cingolani. UNLP-CONICET.*

In most cells, hypoosmotic-induced swelling is followed by a process called "regulatory volume decrease" (RVD) that depends on chloride (Cl^-) efflux (ICl swell). Although it is not completely clear if adult cardiomyocytes can regulate their volume by RVD, this response does appear when Cl^- efflux is increased experimentally with low Cl^- hypotonic solutions. The NLRP3 inflammasome is responsible for inflammatory responses by releasing IL-1ss and IL-18 that can damage the heart. Osmotic stress and Cl^- efflux are known NLRP3 activators in non-cardiac cells. However, if swelling activates NLRP3 in cardiac myocytes has not been studied. We aimed to determine if hypoosmotic stress can activate NLRP3 in adult cardiomyocytes and if this activation is associated with the RVD process, in particular with the activation of ICl swell. Ventricular cardiomyocytes were obtained from 3 months old male Wistar rats by enzymatic digestion. To assess swelling and RVD, cells were perfused with isotonic (300 mOsm) or either normal or low Cl^- hypotonic solutions (HS; 217 mOsm). Cell width was determined by video edge-detection. RVD was absent in normal Cl^- HS but detected in myocytes perfused with a low Cl^- HS solution. Cardiomyocytes were incubated for 2 hours with 1μg/ml bacterial LPS to induce NLRP3 priming and then exposed to either isotonic, normal or low Cl^- HS for 2 hours. As a marker of NLRP3 activation, IL-1ss concentrations in supernatants were quantified by ELISA. No significant difference was detected in IL-1ss levels when cells were exposed to isosmotic or normal Cl^- HS, but a significant increase was found when cells were exposed to low Cl^- HS ($p=0,028$, $n=5$ per group). This increase was significantly attenuated by treating myocytes with the anion channel inhibitor SITS (100 μM), which inhibits Cl^- efflux. Statistical analysis was performed by ANOVA or T Test followed by post hoc-test. Data are expressed as means ± SEM. Differences were considered significant at $p \leq 0.05$. Our results suggest that a Cl- mediated RVD response is necessary for NLRP3 inflammasome activation in adult cardiomyocytes under hypoosmotic stress.

54. (288) **FUNCTIONAL RELEVANCE OF RYR2 PHOSPHORYLATION AT S2814 IN VENTRICULAR MYOCYTES UNDER HIGH PACING FREQUENCY AND BETA-ADRENERGIC STIMULATION**

Agüero R[1], Cely-Ortiz A[1], Racioppi F[1], Rizzardi F[1], Valverde C[1], Wehrens X[2], Lascano E[3], Vila Petroff M[1], Mattiazzi A[1] and Gonano LA[1].
[1]Centro de investigaciones cardiovasculares, CONICET-UNLP Argentina. [2]Departments of Molecular Physiology and Biophysics, Medicine (in Cardiology), Neuroscience, Pediatrics, Center for Space Medicine, Baylor College of Medicine, Cardiovascular Research Institute, Houston, TX. [3] Fundación Favaloro, CONICET Argentina.

While β-adrenergic stimulation can prevent Ca^{2+} alternans, pharmacological inhibition of CaMKII precludes it. However, whether this is due to RyR2 or phospholamban phosphorylation is not known. We aim to determine the relevance of RyR2 phosphorylation at S2814 for the propensity to Ca^{2+} alternans with and without β-adrenergic stimulation. Ventricular cardiomyocytes were obtained from 2-3-month-old gender mixed wild-type and S2814A mice after collagenase-based heart digestion in Langendorff system. Cytosolic Ca^{2+} was monitored loading the cells with Fluo-4 and these were paced at 1,3,4,5,6 and 8 Hertz. The alternans ratio (AR) was calculated by measuring the difference in amplitude between consecutive Ca^{2+} transients normalized by the amplitude of the largest. The alternans threshold was determined as the pacing frequency at which the AR was ≥ 0.1; if not reached, the threshold was considered as 9 Hz. Unpaired T test was applied, and data expressed as mean±SEM. The alternans threshold in WT and S2814A cells was not significantly different (5.82±0.38 and 5.92±0.39) and neither was AR at any frequency (n=28 cells per group). A short incubation (10 min) with isoproterenol 100 nM increased the alternans threshold and reduced the AR at 5 Hz in WT cardiomyocytes (threshold WT=5.82±0.38 and WT+ISO= 7.15±0.31. n= 28 and 13 cells respectively), but there were not significant changes in S2814A treated cells. Interestingly, a longer incubation (60 min), which has been shown to increase RyR2 phosphorylation at a same degree than short treatment, promoted further increase in alternans threshold in the WT group.

These results will be contrasted with additional data of Ca^{2+} release restitution in both groups in absence and presence of isoproterenol. Conclusion: While S2814A cardiomyocytes do not have a different propensity to develop Ca^{2+} alternans compared with WT, the phosphorylation at S2814 is necessary for the protection of adrenergic stimulation against Ca^{2+} alternans.

55. (366) **EXPOSURE TO AIR POLLUTION FINE PARTICULATE MATTER ($PM_{2.5}$) PROMOTES ADIPOSE TISSUE INFLAMMATION AND OBESITY BY IMPAIRING THERMOGENESIS**

Timoteo Marchini[1,2], Sheu-Tijani Olawale Abogunloko[2], Lourdes Caceres[1], Mariana Garces[1], Virginia Vanasco[1], Natalia Magnani[1], Silvia Alvarez[1], Pablo Evelson[1], Dennis Wolf[2]
[1] Universidad de Buenos Aires, CONICET, Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina.
[2] Cardiology and Angiology, Medical Center, University of Freiburg, Freiburg im Breisgau, Germany.

Clinical evidence suggests that the exposure to air pollution fine particulate matter ($PM_{2.5}$) is associated with the development of cardiometabolic disorders. To study the impact of $PM_{2.5}$ on inflammation, metabolism, and obesity, male 8-week-old C57BL/6 mice received 1 mg/kg body weight of a $PM_{2.5}$ surrogate (ROFA, Residual Oil Fly Ash) or saline (control) by intranasal instillation. ROFA-exposed mice showed a biphasic lung inflammatory cell recruitment, with neutrophils peaking at 6 h and macrophages peaking at 72 h, together with significantly increased levels of proinflammatory TNF-α, IL-6, and CCL2. Bulk mRNA sequencing of sorted alveolar macrophages from ROFA-exposed mice revealed a proinflammatory gene expression signature and upregulated pathways for redox and lipid metabolism. Differentially expressed genes, such as CCL3 and other inflammatory mediators, were validated by a customized cytokine bead assay in BAL and plasma, and showed a sustained increase for up to 72 h in ROFA-exposed mice. In parallel, decreased metabolic gene expression (*Ucp1, Elovl3, Adrb3*) in brown adipose tissue suggests reduced lipolysis and thermogenesis, despite ongoing white adipose tissue inflammation. To further explore this observation, another set of mice were exposed to ROFA or saline and monitored in metabolic cages for 48 h. Despite enhanced physical activity, ROFA-exposed mice showed significantly reduced heat production. Lastly, consequences of $PM_{2.5}$ inhalation were evaluated in a real-life mice model of exposure to polluted urban air for 16 weeks. Increased weight gain, impaired glucose homeostasis, and adipose tissue inflammation were observed in mice breathing urban air (27±8 μg $PM_{2.5}/m^3$) versus filtered air (2±1 μg $PM_{2.5}/m^3$), together with altered metabolic gene expression in adipose tissue. Our findings indicate that air pollution $PM_{2.5}$ exposure induce a pulmonary and systemic proinflammatory state that blunts metabolic pathways in adipose tissue and promotes obesity.

56. (400) **CLINICAL VARIABILITY IN CHILDREN WITH CYSTIC FIBROSIS: INFLUENCE OF *CFTR* VARIANTS AND GENETIC MODIFIERS ON THE PRESENTATION AND COURSE OF THE DISEASE**

Carolina Crespo[1], Hilda Giugno[2], Alejandra Visich[1], María Eugenia Foncuberta[1], Abel Gómez[1], Gustavo Dratler[3], Claudio Castaños[2], Lilien Chertkoff[1], Luis Pablo Gravina[1]
1 Laboratorio de Biología Molecular-Genética - Hospital de Pediatría Garrahan, 2 Servicio de Neumonología - Hospital de Pediatría Garrahan, 3 Laboratorio de Pesquisa Neonatal - Hospital de Pediatría Garrahan

Cystic Fibrosis (CF) is a severe autosomal recessive disorder caused by variants in the *CFTR* gene. More than 2000 variants have been identified, being F508del the most frequent one worldwide, but their frequency depend on ethnic origin. The broad clinical spectrum is influenced by *CFTR* variant type, but also by genetic modifiers, such as *MBL2* gene, among other factors. The aim of this study was to characterize the molecular background of Argentinean CF patients, to evaluate the impact of *CFTR* variants on newborn screening (NBS) result, and to assess the influence of *MBL2* genotype on disease severity. A total of 4700 CF genetic analysis carried out at the Garrahan Hospital between 1994 and 2021, including 310 CF patients, were retrospectively revised. Data from 207 CF patients with a NBS result was analyzed. *MBL2* genotype was studied in 106 pediatric CF patients carrying two severe *CFTR* mutations, and classified as MBL-sufficient and MBL-insufficient. Clinical phenotype was defined according to Shwachman score and lung function tests. Among 1593 affected alleles, 85 different variants were identified, five of them had been only reported in Latin American patients, and five were novel. F508del, G542X, N1303K, W1282X, 1717-1G>A and I507del were the most frequent variants. From 203 CF patients who underwent NBS, 21 had a negative result (10%). Patients with at least one residual function variant have 5-fold risk of having a negative NBS result (CI95%: 1.9-14.7, p=0.002). MBL insufficiency-related *MBL2* genotypes were associated with a 3.5-fold risk of having a severe phenotype (CI95%: 1.2–10.3, p=0.03). It was also associated with an earlier onset of infection with *P. aeruginosa* (p=0.035). Molecular analysis of *CFTR* gene contributes to CF diagnosis, and is necessary for indication of modulator therapies. *CFTR* genotype could play a role in the false negative rate of CF NBS. Our study highlights *MBL2* gene as a modulating factor in CF, influencing the course of the disease.

57. (425) **CALCIUM ALTERNANS IN MYOCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS: PROPERTIES AND MODULATION**

Di Marzio G.[1], Mariangelo J.I.E.[1], Zoroza M.P.[1], Gonano L.[1], Mundiña-Weilenmann C.[1], Said M.[1]
[1]Centro de Investigaciones Cardiovasculares CTT-Conicet La Plata-Facultad de Ciencias Médicas UNLP.

Introduction: Cardiac alternans is a dynamical phenomenon which is linked to the genesis of lethal arrhythmias. It has been proposed that alternans is ultimately dependent on alterations in calcium (Ca) cycling dynamics. We demonstrated that the hypertrophied myocardium of the spontaneously hypertensive rats (SHR) shows an increased susceptibility to Ca alternans at early stages of the hypertensive disease. This alternans occurred associated to a lengthened refractoriness of sarcoplasmic reticulum (SR) Ca release and an adverse remodeling of T-tubule network, without alterations in the capacity of SR Ca removal. Objective: To study the subcellular properties of Ca alternans in SHR myocytes and to investigate how extracellular Ca concentration affects Ca alternans. Material and Methods: Frequency-induced Ca alternans was measured in isolated myocytes from 6 and 12 mo-old SHR and compared with age-matched normotensive rats (W). Line scan confocal microscopy was used to examine subcellular Ca dynamics in Fluo-4 loaded myocytes. Global intracellular Ca transient was measured in Fura-2 loaded myocytes by epifluorescence microscopy. Extracellular Ca was increased from 1 to 2.5 mM. Results: When stimulated at 4 Hz, SHR myocytes exhibited inhomogeneities in the Ca signal with different cell zones alternating out-of-phase. This subcellular alternans, known as discordant alternans, was quantified by the discordance index (DI), standard deviation of local alternans ratio. DI was increased in SHR when compared to aged-matched W myocytes (0.48±0.07 and 0.21±0.02 for SHR and W myocytes respectively, $p< 0.05$ n= 8-11 myocytes from 3-4 rats). An increase in extracellular Ca from 1 to 2.5 mM, which enhanced Ca transient amplitude and accelerated its decay, alleviated global Ca alternans in SHR myocytes (alternans ratio: 0.50±0.03 and 0.04±0.02 at 1 and 2.5 mM Ca, respectively, $p<0.05$, n=8 from 3 rats). Conclusions: The occurrence of subcellularly discordant Ca alternans suggests that local SR Ca release within individual SHR myocytes alternates at multiple sites independently from each other. The suppression of Ca alternans by the enhancement of extracellular Ca, suggests that increasing excitation-contraction coupling efficiency is beneficial for controlling cardiac alternans.

58. (676) **CARDIOVASCULAR CHARACTERIZATION OF THE EFFECT OF THE SILENCING OF THE ELECTRONEUTRAL SODIUM/BICARBONATE COTRANSPORTER (NBCN1)**

Gallo Delfina[1], Di Mattia Romina[1], Ciarrocchi Sofía[1], Blanco

Paula[2], Portiansky Enrique[3], Orlowski Alejandro[1], Aiello E. Alejandro[1].
[1]Centro de Investigaciones Cardiovasculares "Dr. Horacio Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.
[2]Servicio de Cardiología, Facultad de Veterinaria, Universidad Nacional de La Plata. La Plata, Argentina.
[3]Laboratorio de Análisis de Imágenes, Facultad de Veterinaria, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.

Introduction: Cardiac cells depend on specific sarcolemmal ion transporters to assure the correct intracellular pH regulation. The Na^+/HCO_3^- cotransporter (NBC) is one of the mayor alkalinizing transporters. In the heart, two different NBC isoforms have been described: the electroneutral NBCn1 ($1Na^+:1HCO_3^-$) and the electrogenic NBCe1 ($1Na^+:2HCO_3^-$). Aims: Preliminary results from our lab demonstrated that specific cardiovascular downregulation of NBCe1 induced cardiac hypertrophy (CH) without changes in blood pressure (BP). However, no clear reports about the role of the NBCn1 in these cardiovascular parameters are available. Thus, we developed an interference RNA cloned in a cardiotropic adeno-associated vector (AAV9-shNBCn1) to study the effect of the specific inhibition of NBCn1 in CH and BP. Materials and methods: We delivered the virus through a lateral tail vein injection in male 3-4 months old Wistar rats and then performed a series of studies to assess CH, using an AAV9-shControl as control. Data is expressed as means±S.E.M. and was compared with Student's t-test or two-way ANOVA test as was needed. Results: After 30 days of injection, we confirm a significant reduction on NBCn1 ventricular expression in Wistar rats. Furthermore, preliminary data suggest a compensatory increase in NBCe1 expression. Although rats injected with shNBCn1 exhibited a significant increase in systolic blood pressure (shControl: 132.1±3.65, n=6; shNBCn1*: 151.2±4.29, n=4; $p<0.05$ vs. shControl), no changes in left ventricular mass index obtained by echocardiography or cross-sectional area of cardiomyocytes were found. Conclusion: These data suggest that vascular NBCn1 silencing provoked a sustained increase in blood pressure in rats. However, no differences were found in heart size. This apparent contradiction between vascular and cardiac data might be explained by the fact that the hypertension assessed in the model was modest or CH requires more time to develop.

59. (678) **"GENETICALLY ENCODED pH SENSORS TARGETING CELLULAR MICRODOMAINS"**

Sofía Ciarrocchi, Delfina Gallo, Romina A Di Mattia, Ernesto A Aiello, Alejandro Orlowski
Centro de Investigaciones Cardiovasculares "Dr. Horacio Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET. La Plata, Argentina.

Introduction: Intra- and extracellular pH regulation is a key function of all cells and tissues and a prerequisite for normal physiological function. Spatial nonuniformity of intracellular pH is generated due to differential subcellular distribution of Na^+/H^+ exchanger (NHE1) and the Na^+/HCO_3^- cotransporter (NBC).
Aim: To study proton microdomains we generated a proton sensor fluorescent protein (pHluorin2) fused with canonical targeting signals to the endoplasmic reticulum, mitochondrial matrix and junctional cleft of cardiac myocytes (cleft-targeted pH sensor were generated by fusing pHluorin2 to FKBP12.6).
Methods and Results: We initially corroborated the localization of the sensor in HEK cells with confocal microscopy. We used the ammonia-prepulse technique to create an intracellular acid load. pH recoveries were obtained from cytosol and endoplasmic reticulum lumen. NHE inhibition by amiloride drastically reduced the pH recovery suggesting the presence of NHE in reticulum (A.U. cito: 0.144±0.004, n=24; cito+amiloride: 0.066±0.005*, n=23; ER:0.098±0.005 n=24; ER+amiloride: 0.011±0.005* n=24; *$p<0.05$). Cytosolic, mitochondrial, and endoplasmic pH decreased during cytosolic Ca^{2+} elevation trigger by histamine (ΔA.U. Cito: -0.70±0.03, n=5; ER: -0.43±0.028, n=5; Mito:-0.63±0.03, n=5). Next, we generated a cardiotropic adeno-associated virus (AAV9) to express the pH sensors into cardiac ventricular myocytes. AAV9-pHluorin2 and AAV9-pHluorin2-FKBP were injected in 3 months rats, after 28 days cardiac myocytes were isolated and pH sensors expression were corroborated by confocal microscopy. pHluorin2-FKBP12.6 express in a striated pattern with intensity maxima spaced ~2 µm apart, this strongly supports the conclusion that the FKBP12.6-tagged sensors are targeted to cardiac ryanodine receptor (RyR) at the z-line.
Discussion: Proton dynamics in cardiac cells are poorly understood. Thus, it is important to highlight that herein we generated for the first time a fluorescent pH sensor targeting different cellular microdomains as a tool for proton microdomains studies.

CARDIOVASCULAR AND RESPIRATORY II

Saturday, November 19, 9-10:30 hr
Chairs: Silvia Alvarez - Andrea Fellet - Romina Di Mattia

60. (375) **ISOSTEVIOL-MEDIATED CARDIOPROTECTION: RELATIONSHIP BETWEEN MITOCHONDRIAL PRESERVATION AND PROTEIN KINASE B (AKT) ACTIVATION**

Victoria Mestre Cordero[1,2], Julieta Mourglia[1], Debora Vélez[1], Lucia Sánchez[1], María de las Mercedes Fernández Pazos[1], Romina Hermann[1,2], Maria Gabriela Marina Prendes[1,2].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Biológicas. Cátedra de Fisiología. Buenos Aires, Argentina; [2]CONICET- Universidad de Buenos Aires. Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA) Buenos Aires, Argentina.

Isosteviol (I) is a stevioside derivative, obtained from the leaves of *Stevia rebaudiana bertoni.* Recently, several studies have attributed cardioprotective properties to this compound, however, its underlying mechanisms remain to be elucidated. Since mitochondrial dysfunction plays a key role in ischemia-reperfusion (IR) injury, we aimed to investigate the effects of acute preischemic administration of I (5μM) and its relationship with the activation of Akt on several mitochondrial parameters from Langendorff-perfused rat hearts subjected to IR. Hearts from female Wistar rats (200-250g) fed ad libitum were used. Wortmannin (W,100nM), a PI3K/Akt inhibitor was added 15 min before ischemia and 5 min before I. Mitochondrial ultrastructure was analyzed by electron microscopy and mitochondrial isolated function was evaluated by the rate of ATP synthesis, O_2 consumption, membrane potential (ΔΨ) and respiratory complexes I-III, II-III and IV activities. Calcium retention capacity (CRC) was also measured. Western blot was used to study Akt activation profile, considering the ratio p-Akt/total-Akt. ANOVA n=6. At the end of reperfusion, results showed an increase in the rate of mitochondrial ATP synthesis in I group (C 66.4±6.5; CW 56.5±6.1; I 81.0±6.9*; IW 79.6±6.9 nmol/min/mg protein; *$p<0.05$ vs C, CW) and a significant diminution of O2 consumption in W groups. Respiratory complexes I-II and II-III activities showed a 2-fold increase in I and IW groups ($p<0.05$). CRC was significantly higher compared to control groups (C 31.0±4.9, CW 30.7±4.7, I 50.5±5.5*, IW 45.5±6.1* nmol calcium/mg protein; *$p<0.05$ vs C and CW). Likewise, electron micrographs showed better mitochondrial conservation in the group treated with I. Akt presented higher phosphorylation with I treatment that was partially reverted with W. These findings suggest that acute administration of I presents cardioprotective effects due to better mitochondrial preservation and could be partly mediated by Akt activation.

61. (444) **VOLUNTARY WHEEL RUNNING AND TRX-1 OVEREXPRESSION IN MICE SUBJECTED TO ISCHEMIA/REPERFUSION INJURY**

Virginia Perez[1,3], Verena Franco Riveros[1], Georgina Ossani[2,3], Eugenia Godoy[1], Yohana Yanaje[1], Aurora Lee[1], Brian Lee[1], Carla Greco[1], Sofía Gimenez[1], Bruno Buchholz[1,3], Ricardo J Gelpi,[1,3], Verónica D´Annunzio[1,3].
[1] Institute of Cardiovascular Physiopathology (INFICA), Department of Pathology, School of Medicine, University of Buenos Aires. [2] Centre of Experimental and Applied Pathology, Department of Pathology, School of Medicine, University of Buenos Aires. [3] National Council of Scientific and Technical Research (CONICET), Argentina.

Voluntary wheel running and thioredoxin-1 (Trx1) overexpression reduce myocardial injury caused by acute ischemia/reperfusion (I/R). However, Trx1 role in exercise is not fully understood. The aim was to study if Trx1is involved in the exercise cardioprotection mechanism. Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative mutant (DN) of Trx1 were used. Mice were divided in exercise group (E) and sedentary group (S). Mice were placed in cages fitted with running wheels for 4 weeks. After the exercise-training period, mice hearts were subjected to 30min of I and 120min of R (Langendorff technique). The assessment of the infarct size was performed using TTC. Moreover, heart, quadriceps, gastrocnemius, and soleus weight were measured. Besides, body weight, caloric intake and running distance were masured. Data were expressed as mean±SEM and p<0.05 was considered statistically significant. n= 8 each group. As we previously shown, training was confirmed by heart rate variation and voluntary exercise reduced infarct size in Wt mice but not in DN mice. The changes in body weight and running distance at the fourth week of training in transgenic mice were comparable with Wt mice. Nevertheless, caloric intake was significantly higher in E groups compared to S groups (Wt-E: 32.2±1.1; Trx1-E: 33.8±1.2; DN-E: 34.6±0.9; Wt-S: 28.5±1.1; Trx1-S: 29.9±1.2; DN-S:30.4±1.2, Kcal/g). Heart weight increase significantly with exercise (Wt-E: 8.68±0.13; Trx1-E: 8.65±0.22; DN-E: 9.24±0.14; Wt-S: 7.99±0.18; Trx1-S: 7.81±0.19; DN-S: 8.40±0.34, mg/mm). Further, soleus weight showed similar values in S groups (Wt:0.44±0.02; Trx1:0.45±0.02; DN:0.43±0.02, mg/mm) but there was an increase in Wt-E and DN-E groups (Wt: 0,60±0,02; DN:0.55±0.03, mg/mm). There were no differences in quadriceps and gastrocnemius weight between E and S groups. In conclusion, we found that Trx1 could be involved in the cardioprotection mediated by voluntary wheel running.

62. (446) **CARDIOPROTECTION MEDIATED BY ISCHEMIC POST-CONDITIONING AND OVEREXPRESSION OF TRX-1 RESTORE MITOCHONDRIAL FUNCTION IN MICE HEARTS**

Eugenia Godoy[1], Virginia Perez[1,2], Tamara Zaobornyj[2], Verónica Casanova[3] Eliana Cicale[3], Yohanna Yanaje[1], Silvia Álvarez[2],Virginia Vagnasco[2],Tamara Vico[2], Ricardo J. Gelpi[1,2], Verónica D´Annunzio[1,2]

[1] Institute of Cardiovascular Pathophysiology, Department of Pathology, Faculty of Medicine. Buenos Aires University. Buenos Aires. Argentina. [2] Institute of Biochemistry and Molecular Medicine (IBIMOL-UBA-CONICET). [3]Faculty of Veterinary Science, Buenos Aires University, Buenos Aires. Argentina.

Thioredoxin-1 (Trx1) maintains the cellular redox status and decreases the infarct size in ischemia/reperfusion injury (I/R). However, it is not fully understood its role in ischemic postconditioning (PostC) in young (Y) and middle-aged (MA)mice and its relation with mitochondrial function. The aim was to study if Trx1 and the PostC mechanism restore mitochondrial function and if the age can modify this andTrx1 activity. Wild type (Wt), transgenic overexpressing Trx1, and a dominant negative mutant (DN-Trx1) of Trx1 mice hearts were used and divided into young (Y: 4 months) and middle-aged groups (MA: 12 months). Using the Langendorff technique, hearts were subjected to 30 min of ischemia and 120 min of reperfusion(I/R) and PostC after the long ischemia, 6 cycles of R/I (15 sec each) at the beginning of R were performed. We measured infarct size (Triphenyl tetrazolium), mitochondrial function (polarographically) and Trx1 activity (Heuck oxidoreductase assay) Previously we showed that PostC and Trx1 overexpression reduced infarct size in Y, but not in MA mice. No changes in state 4 in MA mice and Y either were detected. In Y groups, state 3 was significantly lower in Wt-I/R than in Wt-Nx (Nx:137±6 vs. I/R:97±14) and in Wt-PostC the value tended to recover normoxic values (127±11). No differences appeared between the Trx1 groups. DN-I/R (117±9) and DN-PostC (113±9) were decreased compared to DN-Nx (135±10). In MA groups, state 3 was lower in Wt-I/R and Wt-PostC than in Wt-Nx (Nx:148±6 vs I/R:120±10 and PostC:106±8). No differences appeared between Trx1 groups and the same was observed in DN-Trx1 groups. Also,Trx1 activity was significantly diminished in all I/R groups compared to basal groups. In conclusion, we found the cardioprotection mediated by PostC and Trx1 overexpression restore mitochondrial function in Y but not in MA mice, mainly related with Trx1 activity.

63. (597) **PRE-ISCHEMIC VAGUS NERVE STIMULATION IMPROVES LONG-TERM LEFT VENTRICULAR FUNCTION AND REDUCES FIBROSIS IN ISCHEMIC HEARTS WITH OR WITHOUT REPERFUSION**

Verena B. Franco-Riveros[1,2], Nahuel Méndez Diodati[1,2], Eduardo Bernatené[1,2], Beatriz González Peña[1], Ignacio Barbieri[1], Eliana Cicale[3], Verónica Casanova[3], María Celina Morales[1], Martín Donato[1,2], Ricardo J. Gelpi[1,2], Bruno Buchholz[1,2]

[1] Universidad de Buenos Aires. Facultad de Medicina. Departamento de Patología. Instituto de Fisiopatología Cardiovascular. [2] Consejo Nacional de Investigaciones Científicas y Técnicas – CONICET. [3] Universidad de Buenos Aires, Facultad de Veterinaria.

The aim was to determine the effects of brief electric vagus nerve stimulation (VS) applied before ischemia on acute myocardial infarction (MI) and its long-term benefits on an experimental ischemia-reperfusion and non-reperfusion model. Mice underwent regional MI for 45min, followed by either 2 hours (2hR) or 28 days of reperfusion (28dR), with or without 10min of pre-ischemic VS. LVF was assessed by catheterization through the right carotid artery and, by echocardiography: EF, SF, IVRT. Participation of muscarinic receptors (MR) was determined by the administration of atropine (ATR) during VS. Infarct size (IS) was measured by TTC stain on acute groups. Histological measurements were also assessed to study myocardial IS and remodeling. VS decreased IS after 2hR, from 65.3±1.7% to 43±2.1% (p<0.05) and ATR reversed the protection (IS:60.4±1.6%; p<0.001 vs VS+IR-2h). VS improved LVF after 28dR evidenced by a lower LVPDF(mmHg) (Sham-28d:3.8±0.2; IR-28d:6.8±0.5; VS+IR-28d:3.7±1; p<0.01), higher EF% (Sham-28d:77.3±11.7%; IR-28d:59.7±2.8%; VS+IR-28d:69.6±2.4%; p<0.05), and lower TRIV (Sham-28d:19.4±1.4; IR-28d:30.3±1.2; VS+IR-28d:25±0.9; p<0.05). ATR didn't reverse VS protective effects on LVF. VS didn't reduce CSAm (Sham-28d:267.5±9um; IR-28d:380,1±34um; VS+IR-28d:374,3±25um; p<0.05), but collagen ventricular fraction (CVF%) on the infarcted (IA) and non-infarcted area (n-IA) was significantly reduced (IA: IR-28d:37.6±6,5%; VS+IR-28d:19.7±3,5%; ATR+VS+IR-28d:16.1±1,4; p<0.05 and, n-IA: IR-28d:5.9±1,7%; VS+IR-28d:2.75±1,4%; ATR+VS+IR-28d:1.26±0,3 p<0.05). Likewise, VS improved LVF after 28d of MI without R (LVDP: I28d:8.4±1mmHg; VS+I28d:3.1±0.8; p<0.05; EF%:I28d: 50.6±4; VS+I28d:69.5±1; p<0.05). VS reduced neither CSAm, nor CVF% on no-reperfusion protocols. In conclusion, short-term pre-ischemic VS reduces acute IS, improves long-term LVF, and reduces fibrosis in ischemic hearts with or without R independently of MR's action or IS.

64. (603) **PHYSIOLOGICAL ATRIAL SYNCHRONY**

Valeria Adriana González [1], Francisco Javier Sánchez [2, 3], Valentina Alonso [4], Natalia Jorgelina Prado [2, 5], Emiliano Raúl Diez [2, 5]

[1] Servicio de Cardiología, Hospital Luis Lagomaggiore
[2] Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina
[3] Clinica de Cuyo, Mendoza, Argentina
[4] Centro Médico Palmares, Penta Cardiología. Mendoza Argentina
[5] IMBECU-UNCuyo-CONICET

Objectives: To characterize inter-atrial synchrony by atrial strain in patients without a cardiovascular disease. Materials and Methods: Patients aged 18-80 years who gave informed consent were included. Clinical data such as age, and body surface area was collected. Patients with a history of any known cardiovascular disease were excluded. Atrial strain and strain rate were analyzed using ESAOTE MyLab tissue tracking software. Reservoir, conduit and atrial contraction were obtained for both atria. For the determination of interatrial synchrony, an adaptation of the atrial strain was made so

that both atrias could be assessed at the same time. The time to maximum deformation of the lateral segments of the right atria (RA) and the lateral segments of the left atria (LA) in a same heartbeat and the time difference between the activation of the walls was determined (Atrial time delta ATD). Measurements carried out were a global measurement of the entire right and left lateral atrial walls. Results: 61 patients were included. The age of the patients was 46.8 ± 19.1 years. 49.2% of the patients were male. Both LA indexed volume and RA area were significantly higher in men than in women (p=0,002). Atrial functional parameters showed no differences between genders. LA reservoir falls significantly over time (p<0.05), as does LA conduit function. An increase in LA and RA contractile function was also observed with age (p<0.05). ATD had an average value of 21 ms (CI95% 21-29). No significant differences were observed between the different ATD by gender (p=0.63). No significant differences were observed when analyzing ATD with respect to age groups (p=0.53) despite of a tendency to increase with age. Conclusion: Atrial size increases with age. Atrial reservoir and conduit values fall over the years, while contractile function increases. Determination of interatrial synchrony did not detect significant differences between sexes and age groups.

65. (675) **EFFECT OF INTRAMYOCARDIAL ADMINISTRATION OF BACULOVIRUS ENCODING THE TRANSCRIPTION FACTOR TBX20 IN SHEEP WITH EXPERIMENTAL ACUTE MYOCARDIAL INFARCTION**

Bauzá María del Rosario[1], Cimbaro Francisco Stefano[1], López Ayelén Emilce[1], Simonin Alejandro Jorge[2], Scharn Agustina[1], Silvestro Cintia[1], Castro Araceli[1], Ferrer Pilar[1], Belaich Mariano Nicolás[2], Crottogini Alberto José[1], Locatelli Paola[1], Olea Fernanda Daniela[1].

1-Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTyB)-Universidad Favaloro-CONICET , 2- Universidad Nacional de Quilmes

Introduction: We have recently designed a TBX20-encoding baculovirus (Bv-Tbx20) to be used as a possible therapeutic vector in myocardial regeneration. The transduction of cardiomyocytes in culture with BvTbx20 increased cell proliferation and their supernatant induced angiogenesis. The aim of the present study was to evaluate the effect of the intramyocardial administration of BvTbx20 in sheep with acute myocardial infarction (AMI). Methods and Results: in sheep with experimental AMI, 10^10 copies of BvTbx20 or BvNull were administrated intramyocardially in the infarct border zone. At 7 days post-treatment, BvTbx20 group showeda) the higher expression of the Tbx20 transgene and mitogenic and angiogenic genes than BvNull group, b) more ki67-labeled cardiomyocytes/mm^2 (14.37±7.22 vs. 6.32±6.69, p<0.05) and c) higher capillary (2329.64±326.65 vs. 1684.50±289.96 capillar/mm2, p<0.05) and arteriolar densities (143.20±45 vs. 92.15±35 arteriola/mm2, p<0.05, Mann Whitney´s test). At 15 days, BvTbx20 group showed higher ejection fraction (55,9±4,3 vs 44,3±4, 7 %, p<0.05, Two way ANOVA-Bonferroni). At 30 days, however, the differences did not reach significance. At 30 days infarct size decreased in BvTbx20 group (9,89±1,92 vs 12,69±1,45%, p<0.05, Student's Test). Conclusions: in sheep with AMI, the administration of Bv-Tbx20 induces angio-arteriogenesis and an increase in Ki67-positive cardiomyocytes at 7 days, decreasing infarct size and improving ventricular function at later follow-up times. These results suggest that gene therapy overexpressing Tbx20 could be potentially useful to induce myocardial regeneration.

66. (704) **AGE-RELATED CARDIAC REMODELING AND APOPTOSIS ARE EXACERBATED IN AGED MICE WITH GENETIC DELETION OF GALECTIN 3**

Fontana Estevez F.s[1.A], Betazza M.c[1], Seropian I.[1], Penas F.[2.B], Kouyoumdzian N[3.A], Silva M.g[4.A], Selser C[1], Villaverde A[1], Touceda V.[1.A.5], Bustos R[1.A], Vidal A[1], Cianciulli T[6], Medina V[7.B], [5]choi M[3.B], Gironacci M[4.B], Miksztowicz V.[1.5.B], González G.e[1.8.B].

1) Universidad Católica Argentina. Facultad de Medicina, Instituto de Investigaciones Biomédicas UCA-CONICET. Buenos Aires, Argentina

2) Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), the Facultad de Farmacia y Bioquímica

3) Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Biológicas. Cátedra de Fisiopatología; Argentina.

4) Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Dpto. Química Biológica, IQUIFIB (UBA-CONICET), Buenos Aires, Argentina.

5) Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal. Buenos Aires, Argentina

6) División Cardiología, Hospital de Agudos "Dr. Cosme Argerich"

7) Pontificia Universidad Católica Argentina, Facultad de Medicina, Instituto de Investigaciones Biomédicas UCA-CONICET, Laboratorio de Biología Tumoral e Inflamación, Buenos Aires, Argentina.

8) Universidad de Buenos Aires, Facultad de Medicina-CONICET, Departamento de Patología. Instituto de Fisiopatología Cardiovascular (IBIMOL), Buenos Aires, Argentina.

[a] Fellow from the National Council of Scientific and Technical Research (CONICET), Argentina

[b] Member of the Investigator Career from the National Council of Scientific and Technical Research (CONICET), Argentina.

Background: We showed that Galectin-3 deficiency (Gal3KO) exacerbated myocardial hypertrophy and fibrosis in aged mice. However, if Gal3KO increases myocardial apoptosis and alters renal function in mice with hypertrophic hearts and kidneys and with normal systolic blood pressure is still unknown. Objective: We aimed to continue investigating the age-associated physiological changes in Gal3KO mice to study if aged hearts exacerbate myocardial apoptosis, and if kidney hypertrophy is linked to renal dysfunction. Methods: Male C57 and Gal3KO mice were followed up for 24 months. After 2 years, animals were isolated in metabolic cages to recollect 24hs diuresis to quantified renal Creatinine and Albumin. After euthanasia, animal tissues were harvested, and cardiac apoptosis were quantified in Tunnel positive cells. With Radioimmunoassay cardiac levels of Angiotensin (Ang) II and Ang 1-7 were quantified. RT q-PCR was performed to quantified cardiac mRNA of TGF B, MMP 9 and Sirtuins 1 and 7 (SIRT). Results: (Media±SEM C57vsGal3KO). At 24 months, Gal3KO mice had exacerbated levels of cardiac Ang II (3±0.5 *vs* 7±1.5 ng/g, p<0.05), and increased in TGF b (1.0 ± 0.2 vs 2.0 ± 0.2 AU, p<0,05) and MMP-9 mRNA levels (2.2±0.3 vs 0.9±0.2 AU, p<0.01). Similar MMP-2 mRNA levels and Ang 1-7 expression were found (p=ns). SIRT-7 mRNA levels were significantly reduced in Gal3KO group (0.6±0.1 vs 1.2±0.1, p<0.05). When evaluating renal function, Gal3KO and C57 aged animals had similar values of urinary Creatinine and Albumin (p=ns). The number of cardiac tunnel positive cells were higher in Gal3KO mice (p<0,05). **Conclusion**: Aged Gal3KO mice with hypertrophic hearts and cardiac fibrosis had higher Ang II and TGF b levels in the absence of hypertension and with higher number of apoptotic cells. Renal hypertrophy was not followed by renal dysfunction. Gal3 may be a critical regulator of age-associated myocardial remodeling and interacts with other related tissues during aging.

67. (785) **REMOTE ISCHEMIC PRECONDITIONING MODIFIES EARLY VENTRICULAR REMODELING POST MYOCARDIAL INFARCTION BY ATTENUATION OF THE INFLAMMATORY RESPONSE AND OXIDATIVE STRESS**

Eliana P Bin[1], Mariana Garces[2], Federico Penas[3], Mariana Armani[1], Camila Marquez Roa[1], Carla Greco[4], Sofia Gimenez[4], Luciana Wilensky[1], Nora Goren [3], Pablo Evelson[2], Ricardo Gelpi[1], Martin Donato[1]

1. Institute of Cardiovascular Pathophysiology, School of Medicine, University of Buenos Aires. 2. Institute for Biochemistry and Molecular Medicine (IBIMOL). 3. Institute of biomedical research in retroviruses and AIDS. 4 Veterinary school, University of Buenos Aires

Introduction: It is known that remote ischemic preconditioning (rIPC)

reduces infarct size in experimental models of myocardial infarction (MI) but the effect of rIPC on post-infarction ventricular remodeling is unknown. The aim of this work is to evaluate the effect of rIPC on early ventricular remodeling, considering the myocardial infarction expansion. Methods: Male FVB mice, 2-6 months old, were underwent to MI by permanent ligation (7 days) of the left anterior coronary artery; In a second group, a rIPC protocol (3 cycles of 5 min ischemia/reperfusion in the left lower limb) was performed prior to MI. A third group was undergoing to left thoracotomy, but without myocardial ischemia (Sham). We evaluated infarct size with triphenyltetrazolium, ventricular function by hemodynamic and echocardiography studies, and MMPs 2 and 9 activity by zymography. Finally, carbonylated proteins and TNF-α levels in serum were measured. Results: There were no significant differences in the risk area and infarct size between groups. MI decreased ejection fraction and area shortening fraction to a value of 55,06±2,6% and 26,76±1,21%; respectively (p≤0,05 vs sham), while rIPC improved systolic function increasing ejection fraction and the area shortening fraction to a value of 67,64%±1,42 and 37,41±2,37%, respectively (p≤0,05 vs IM). The relationship between end-systolic stress (afterload index) and ejection fraction was plotted, observing a significant improvement in the rIPC group, compared to the MI group. On the other hand, rIPC reduced significantly MMP-9 activity in the left ventricle (infarct area) without changes in MMP-2. Finally, rIPC decreased carbonylated proteins and TNF-α in serum. Concluision: rIPC has a beneficial effect on early remodelling, improving ventricular function. These beneficial effects could be related to a reduced oxidative stress and inflammatory response.

68. (798) **LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN-1 EXPRESSION ON MONOCYTES/MACROPHAGES OF APOE-/- MICE**

Gutierrez MV[1], Casale R[2], Chiabrando GA[2], Bonacci G[1].
1. CIBICI-CONICET. Dpto. Bioquímica Clínica. Facultad de Ciencias Químicas. UNC. 2. Centro de Investigación en Medicina Traslacional Severo Raúl Amuchástegui IUCBC-CONICET.
E-mail: victoria.gutierrez@unc.edu.ar

Atherosclerosis is a chronic inflammatory disease associated with imbalance of lipid metabolism and activation of innate immune cells (IICs) expressing low density lipoprotein receptor-related protein 1 (LRP1) such as peripheral blood monocytes (PBM) and macrophages. LRP1 has an active participation in the modulation of inflammatory profile in monocytes/macrophages during atherosclerosis. Recently, our group has demonstrated a decrease in the expression of LRP1 in PBM of individuals with subclinical atherosclerosis. Thus, the aim of the present work is to study the expression level of scavenger receptors, LRP1 and CD36, and intracellular lipid content in PBM at early stage of atherosclerosis disease. To carry out this objective, $ApoE^{-/-}$ and wild type (C57BL/6) mice were fed with a high fat diet (HFD) or normal diet (ND) for 3 months. For flow cytometry, blood samples were obtained by submandibular vein draw at 0, 15, 30 and 75 days of diet from the same animal and labeled with following antibodies LRP1-APC, CD36-APC, CD115-APC Cy7, Bodipy-FITC, Ly6G-PE, CD3-PE and CD19-PE. In addition, aortas and blood (cardiac puncture) for Ficoll isolation of total mononuclear cells were collected at 0, 30, 75 and 90 days for immunofluorescence, Oil Red O for lipid staining and cells for qPCR assays. Our results shown that $ApoE^{-/-}$ mice develop atherosclerotic plaque after 2 months of HFD diet and the expression of LRP1 increase significantly into the atheroma plaque. From the longitudinal analysis of PBM, we observed that LRP1 expression is significantly increased at day 15 compared to wild type mice ($p<0,05$) and CD36 had a similar trend. This group of monocytes showed high intracellular lipid content with correlate with increased expression of CD36. These results suggest that LRP1 expression in PBM changes in early stages of atherosclerotic plaque formation and may be related with the pro-inflammatory stage of circulating monocytes.

69. (831) **THERAPEUTIC APPLICATION OF LIPOIC ACID IN A RAT MODEL OF SUPERIMPOSED PREECLAMPSIA: EFFECT ON MATERNAL SIGNS, PLACENTAL FUNCTION AND FETAL GROWTH**

Gabriela Barrientos[1,✉], Yerko Zlatar[1], Graciela Giardina[1], Ana Uceda[1], Maria Alejandra Sgariglia[1], Elizabeth Robello[3], Silvia I García[1,4], Monica Galleano[3]
[1] Laboratorio de Medicina Experimental, Hospital Alemán - CONICET, Buenos Aires, Argentina [3] Instituto de Bioquímica y Medicina Molecular (IBIMOL), UBA-CONICET, [4] Instituto de Investigaciones Médicas (IDIM), UBA-CONICET, ✉ gbarrientos@hospitalaleman.com

Superimposed preeclampsia (SPE) occurs in 25-40% of pregnancies affected by chronic hypertension and is associated with adverse outcomes. In previous studies, we showed that pregnant Stroke-prone Spontaneously Hypertensive Rat (SHRSP) spontaneously develop SPE-like features worsened hypertension with renal dysfunction, fetal growth restriction and placental insufficiency and oxidative stress. Here, we sought to evaluate the therapeutic potential of lipoic acid (ALA), a potent antioxidant, during early pregnancy in this model. Materials and methods: Congenic timed breedings were established using SHRSP and Wistar Kyoto (WKY) females (10–12 weeks old, 200-250 g body weight, N = 10 animals/day analyzed). Upon vaginal plug detection (gestation day (GD)1), dams were assigned to two groups: ALA treatment, injected 25mg/kg body weight ALA i.p. on GD1, GD8 and GD12 and control, receiving PBS following the same protocol. Volume pressure recording (VPR) was used for recording systolic blood pressure (SBP) profiles throughout pregnancy. On GD20, animals were euthanized and fetoplacental specimens isolated for morphological and molecular analyzes. Statistical analysis was run on GraphPad Prism v 8.0, using 2 way ANOVA. Statistical significance was set at $p^{<}$ 0.05. Results: Treatment with ALA prevented the pregnancy-dependent SBP increase in the SHRSP model (SBP GD14 SHRSP: 169.3±2.1 mmHg vs. SHRSP+ALA: 146.1±3.4, $p^{<}0.001$). Furthermore, fetuses carried by ALA-treated SHRSP displayed increased weights (SHRSP: 1.75±0.05g vs. SHRSP+ALA: 1.99±0.01g, $p^{<}0.05$) on GD20. This was associated with an improved placental function in treated dams, which showed increased numbers of PAS^{+} cells in the junctional zone and an enhanced vessel density on Isolectin $B4^{+}$ staining. Conclusion: ALA administration improved maternal signs, placental function and fetal growth in the SHRSP model, emerging as a novel therapeutic intervention in pregnancies affected by chronic hypertension.

70. (836) **ONSET OF SUPERIMPOSED PREECLAMPSIA IN THE SHRSP MODEL IS ASSOCIATED WITH STRUCTURAL AND FUNCTIONAL ALTERATION OF THE MATERNAL GUT MICROBIOME**

Gabriela Barrientos[1,✉], Moumen M. Alhasan[2], Yerko Zlatar[1], Graciela Giardina[1], Ana Uceda[1], Maria Alejandra Sgariglia[1], Melanie L Conrad[2]
[1] Laboratorio de Medicina Experimental, Hospital Alemán, Buenos Aires - CONICET, [2] Institute of Microbiology, Infectious Diseases and Immunology, Charité Universitätsmedizin Berlin, ✉ gbarrientos@hospitalaleman.com

Superimposed preeclampsia (SPE) occurs in 25-40% of pregnant women with chronic hypertension and is associated with adverse outcomes. Growing evidence suggests a role for dysbiosis of the gut microbiome in the development of hypertension and also in preeclampsia. Our previous studies demonstrated that pregnant Stroke-prone Spontaneously Hypertensive Rat (SHRSP) display a SPE phenotype characterized by worsening hypertension, placental dysfunction and fetal growth restriction. The aim of this study to was to investigate changes in the maternal gut microbiome associated with the onset of SPE in the SHRSP. Materials and methods SHRSP (SPE model) and Wistar Kyoto (WKY, Control) females (10–12 weeks old, 200-250 g body weight, N = 6) were mated to congenic males and checked daily for vaginal plugs, denoted as gestation day (GD)1. On GD14, dams were placed individually in clean empty cages for collection of freshly excreted feces samples. Systolic blood pressure (SBP) was determined using a tail-cuff device. Gut microbiome profiles were assessed by MiSeq 16S rRNA sequenc-

ing. Fecal short-chain fatty acid (SCFA) content was analyzed using GC-FID gas chromatography. Statistical analysis was run on GraphPad Prism v 8.0. Results SPE rats demonstrated a higher index of α-diversity than controls. Differential abundance testing showed that onset of SPE was associated with increased Proteobacteria, Bacteroidota and Actinobacteria phyla and depletion of Firmicutes. Furthermore, we observed a strong positive correlation of SBP and the relative abundance of genera *Bifidobacterium*, *Prevotella* and a negative correlation with the abundances of *Ruminococcus*, *Faecalibacterium* and *Blautia*. Fecal SCFA analysis showed significantly increased levels of acetate and propionate in SPE rats compared to controls. Conclusion Onset of SPE in the SHRSP model is associated with significant structural and functional changes in the maternal gut microbiome, which correlate with maternal signs.

71. (884) **DOXORUBICIN-INDUCED CARDIAC DYSFUNCTION IS AMELIORATED IN GALECTIN-3 KNOCKOUT MICE**

Ignacio M. Seropian[1,2], Florencia S. Fontana Estevez[1], Alejo Villaverde[1], Romina Bustos[1], Vanesa Touceda[1], Carolina Selser[1], Leonardo Cacciagiú[3], Tomás Cianciulli[4], Verónica Cassanova[5], Agustina Vidal[1], Verónica Miksztowicz[1], German E. González[1].

(1) Laboratorio de Patología Cardiovascular experimental e Hipertensión Arterial, BIOMED (UCA-CONICET). (2) Servicio de Hemodinamia y Cardiología Intervencionista, Hospital Italiano de Buenos Aires.(3) Hospital General de Agudos Teodoro Álvarez. Laboratorio Central. Sección Bioquímica,(4) División Cardiología, Hospital General de Agudos "Dr. Cosme Argerich," Buenos Aires, Argentina(5) Universidad de Buenos Aires, Facultad de Veterinarias, Buenos Aires, Argentina

Background: Doxorubicin (DOX) treatment leads to cardiovascular toxicity through a direct effect on cardiomyocytes and inflammation. Objective: We aimed to study the role of Galectin-3 (Gal3) in DOX-induced cardiac toxicity. Methods: Male C57 (N=30) and Gal-3KO (N=32) mice with a single dose administration of DOX (15 mg/kg, IP) or saline solution (S) (0.2 ml, IP). Serum creatine phosphokinase (CPK) at 3 days was measure for cardiac damage. Cardiac remodeling and function studied in vivo by echocardiography (echo) (n=10/group) and catheterization at 7 days (n=5/group). Cardiac fibrosis (%) was measured in picrosirius red-stained sections. Results are shown as Mean±SEM. p<0.05 was considered statistically significant. Results: All variables were similar in both genotypes treated with S. Serum CPK was 3169±573 UI/L in C57+DOX vs 573±54 UI/L in Gal3KO+DOX mice (p<0.05). Lack of Gal3 reduced mortality at 7-days from 18% to 10% compared to C57+DOX (p=NS). Contractile dysfunction by echo was ameliorated in treated Gal3KO mice accompanied by higher fractional shortening (75±4 vs 63±2% in Gal3KO+-DOX and C57+DOX mice respectively; p<0.05). Left ventricular (LV) systolic diameter (mm) was significantly increased in C57+DOX (1.2 ±0.1) vs C57+S (0.4±0.04)(p<0.05) and this increase was prevented in Gal3KO+DOX (0.7±0.3 mm vs C57+DOX; p<0.05). LV-developed pressure was significantly reduced in C57+DOX (61±5 mmHg) vs C57+S (86±6 mmHg; p<0.05), and that reduction was attenuated in Gal3KO+DOX (96±14 mmHg vs C57+DOX). Additionally, diastolic dysfunction was prevented in Gal3KO+DOX since the LV-end diastolic pressure was reduced from 9±2 mmHg in C57+DOX to 2±1 mmHg in Gal3KO+DOX (p<0.05). Myocardial fibrosis was also reduced in Gal3KO+DOX mice 3.5±0.5 % vs 4.6±0.2% in C57+DOX (p<0.05). Conclusion: the genetic deletion of Gal3 ameliorated cardiac dysfunction and fibrosis after DOX treatment. Gal3 inhibition may represent a new treatment to prevent cardiac toxicity of DOX.

72. (925) **THE IMPACT OF AEROBIC TRAINING IN CARDIOVASCULAR ALTERATIONS AND COGNITIVE PROCESSES FOLLOWING OVARIECTOMY: THE KEY ROLE OF IGF1**

Ibañez AM (1); Martínez VR (1); Godoy Coto J (1); Vélez Rueda JO (1); Lofeudo JM (1); Cervellini S (2) Dolcetti FJC (2); ; Bellini MJ (2); Portiansky EL (3); Yeves AM (1); Ennis IL (1); Aiello EA (1); De Giusti VC (1).

(1)Centro de Investigaciones Cardiovasculares (CIC-CONICET-UNLP)

(2) Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP-CONICET-UNLP)

(3) Facultad de Veterinaria, Universidad Nacional de La Plata.

During menopause women are exposed to an increase in cardiovascular risk (CVR), associated with lack of estrogens. Sodium/proton exchanger (NHE) and Sodium/bicarbonate cotransporter (NBC) are regulators of the cardiomyocyte pH. NHE hyperactivity and the reduced activity of the electrogenic isoform of NBC (NBCe1) are correlated with increased CVR. Estrogen deficiency is associated with cognitive changes. . Aerobic exercise exerts beneficial effects on cardiovascular health and cognitive processes. Bilateral ovariectomy was performed in young female Wistar rats (OVX), sham operated animals (Sham) were used as control. Sham operated animals were used as control group. OVX rats were randomly assigned to a sedentary group (OVX_s) or to an aerobic swimming routine (8 weeks/5 days a week) (OVX_e). Another group of OVX rats were intramuscularly injected with a recombinant adenoviral construct harboring the cDNA of human Insulin like growth factor 1 (IGF1). OVX_s cardiomyocytes were cultured and treated with IGF1 (1uM) to measure the impact in ROS production. Aerobic training induces physiological hypertrophy in OVX_e. NHE activity was increased in OVX_s, and both exercise and IGF1 abolished this alteration (JH mmol/min: Sham 1.42±0.24; OVX_s 2.15±0.23*; OVX_e 1.22±0.24; OVX_{IGF1} 0.82±0.15) (JH mmoles/min: Sham 1,42±0,24 OVXs 2,15±0,23*; OVX_e 1,22±0,24; OVX_{IGF1} 0,82±0,15). NBCe1 activity was decreased in OVX_s, and exercise prevented it (DpH: Sham 0.25±0.02; OVX_s 0.09±0.02*; OVX_e 0.14±0.01) (DpH Sham 0,25±0,02 OVXs 0,09±0,22*; OVX_e 0,15±0,1). Antioxidant capacity was impaired in OVX_s, and recovered after the training. OVX_{IGF1} rats showed an improvement in long-term memory and exploratory behavior, both of which were affected by ovariectomy. IGF1 decreased ROS production in OVX_s cultured cardiomyocytes. Our results show that aerobic training has a positive impact in the remodeling of alkalinizing mechanisms observed in OVX_s, as well as an increase in antioxidant capacity, and this is, at least partially, due to IGF1 signaling. Our data also support the involvement of sex hormones in cognitive functions in female rats and suggest a positive effect of transgenic IGF1.

CELL PHYSIOLOGY *Friday, November 18, 14-15:30 hr*
Chairs: Alejandro Orlowski - Ivana Gómez

73. (72) **MOLECULAR INTERACTION BETWEEN THE CRH SYSTEM AND BMP4 SIGNALING IN THE HIPPOCAMPAL NEURONAL CONTEXT**

Micaela Silbermins[1], Mariana Fuertes[1], Eduardo Arzt[1,2], Susana Silberstein[1,2]

[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) – CONICET – Partner Institute of the Max Planck Society, Buenos Aires, Argentina

[2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

The main goal of our work is the identification of cellular mechanisms and molecular components involved in corticotropin-releasing hormone (CRH) system signaling in its context of physiological action. A modulatory role for CRH in neuronal architecture and hippocampal neuron maturation has been documented, but the mechanisms involved are not yet defined. CRH neurogenic activity was suggested to be antagonized by BMP4, a negative regulator of POMC and CRHR1 expression in corticotrophs. We used our developed bioassay based on morphological changes induced by CRH in neuronal hippocampal cells stably expressing CRHR1 (HT22-CRHR1), to analyze BMP4 effects on neurite elongation and cell proliferation. While CRH induces neurite elongation, BMP4 significantly stimulates proliferation. Our transcriptional analyses in HT22-CRHR1 cells indicate expression of BMP4 receptors Bmpr1a,

Bmpr2, Acvr2a and Acvr2b, BMP4 effectors SMAD1 and SMAD5, and the inhibitory SMAD7. BMP4-stimulated HT22-CRHR1 cells activated SMAD1/5 in a time-dependent manner. SMAD1/5 phosphorylation was detected as early as 20 minutes after 5ng/mL BMP4 stimulation. We evaluated BMP4 effect on CRHR1 expression using a Crhr1-LUC promoter reporter. First experiments suggest that 100ng/mL BMP4 treatment reduces luciferase expression, being this effect reverted by 1ug/mL BMP4 antagonist noggin. Elucidating the interaction of BMP-4 and CRH signaling would contribute to develop new therapeutic approaches for pathologies in which these systems play a crucial role, such as depression. The results presented here aim to define BMP4 pathway in the hippocampal neuronal context and constitute the basis to describe the molecular events that drive neuronal balance of maturation and proliferation induced by CRH/BMP4 crosstalk. Supported by ANPCyT and FOCEM (COF 03/11).

74. (200) **ACTIVE ss-CATENIN AND TRPV4 EXPRESSION IN NORMAL AND CANCEROUS KIDNEY CELLS**

Juan José Sterba[1], Gisela Di Giusto[1], Natalia Beltramone[1], Mariel Fanelli[2], Claudia Capurro[1], Valeria Rivarola[1], Paula Ford[1]

[1]Laboratorio de Biomembranas, IFIBIO-HOUSSAY (UBA-CONICET), Facultad de Medicina, UBA. Buenos Aires, Argentina. [2]Laboratorio de Oncología IMBECU, CCT CONICET, Mendoza.

In adults, clear cell Renal Cell Carcinoma (ccRCC) is the most common type of kidney cancer. Many of the physical features that affect cancer progression may be mediated by mechanosensitive ion channels that, through calcium influx, favor the activation of specific downstream pathways. TRPV4 calcium channel (Transient Receptor Potential Vanilloid Channel) is a protein activated by chemical, osmotic and mechanical stimuli. TRPV4 has been proposed as a therapeutic target in cancer treatment. However, their involvement in ccRCC has not been studied. Recently, it has been proposed that TRPV4 activation inhibits β-catenin transcriptional activity. The aim of this study was to evaluate TRPV4 and active β-catenin (Aβ-catenin) expression in normal proximal tubule cells (HK2 cells) and in two cell lines derived from human ccRCC (786-O and CAKI-1 primary and metastatic model) under TRPV4 activation. Previously we found that CAKI-1 cells proliferated faster than 786-O cells and 786-O faster than HK2 cells. Now we performed immunofluorescence experiments. Confocal images were analyzed with Imaris. We found intracellular TRPV4 expression in the three cell lines. The % of active Aβ-catenin in the cell membrane was significantly lower in CAKI cells respect to HK2 cells (Mean ± SEM: HK2: 28.9 ± 6.0, n=3; 786-O: 16.2 ± 3.0, n=4; CAKI-1: 9.2 ± 3.5 n=3, $p < 0.05$ vs HK2). In CAKI-1 cells Aβ-catenin colocalization with the nucleus was greater than in HK2 (Mean ± SEM of Pearson's coefficient: HK2: 0.22 ± 0.03, n=3; 786-O: 0.26 ± 0.04, n=4; CAKI-1: 0,46 ± 0.06, n=3. $p < 0.05$ vs HK2). When TRPV4 was activated (GSK 10 nM) the colocalization between Aβ-catenin and nuclei decreased in 786-O and CAKI-1 cells. In conclusion, the metastatic cells, which have the higher proliferation rate, have more Aβ-catenin in the nuclei and TRPV4 activation decreases this colocalization. Proliferating experiments activating TRPV4 are needed to confirm the consequence of the reduction of nuclear Aβ-catenin.

75. (214) **ULTRAVIOLET B LIGHT INDUCED PREMATURE SENESCENCE IN HUMAN DERMAL PAPILLA CELLS. EFFECT ON EPITHELIAL-MESENCHYMAL INTERACTIONS**

Nahuel Matías Martínez, Karin Hagelin, Florencia Maia Oppenheimer, María Eugenia Balañá, Julieta María Ceruti.
Instituto de Ciencia y Tecnología César Milstein- Fundación Pablo Cassará- CONICET- Buenos Aires- Argentina

The hair follicle (HF) is a mini-organ that supports the cyclical growth of hair starting with differentiation of hair follicle stem cells (HFSC) induced by dermal papilla cells (DPC). Baldness, as consequence of HF miniaturization is a typical aging phenotype. In balding DPC, decrease in proliferation is associated with premature cell senescence. Senescent cells generate a complex mix of signals known as senescence associated secretory phenotype (SASP) that promotes inflammation and tissue damage. The aim of this work was to generate a model of UVB induced-senescence in human DPC grown as spheroids and to study its effect on HFSC differentiation.
DPC irradiated six times with 4 mJ/cm² UVB went into cell cycle arrest and decreased their metabolic activity measured by MTS assay (59%) comparable to cells grown with low serum concentration. UVB damaged DPC also showed high β-galactosidase activity (52% of cells) and higher expression of $p16^{INK4a}$ and $p21^{Cip1}$ mRNAs, markers of cellular senescence, as cells treated with camptothecin (20 nM) or H_2O_2 (50 μM). The percentage of UVB treated cells that suffered apoptosis or necrosis (9,34%) was not significant compared with untreated cells (13,84%), measured as Anexin V binding by flow citometry. HFSC were cultured with conditioned media from DPC spheroids previously irradiated or not. Differentiation of HFSC to hair lineage, determined by the expression of keratin K6hf (K75) was impaired in conditioned medium from UVB induced senescent DPC spheroids (UVB 0,4 vs. C 3,7 fold expression). The expression of SASP components IL-1α, IL-1β, IL-6 and MMP-1 was significantly increased in UVB treated DPC spheroids. Moreover, Dkk-1, an inhibitor of HFSC differentiation was up-regulated, whereas mRNA of Wnt10b, known inducer of differentiation, was downregulated by UVB. We conclude that senescent DPC lose their inductive ability, deregulating epithelial (HFSC)-mesenchymal (DPC) interactions and thus driving hair follicle aging.

76. (287) **ALTERATIONS IN NHE1 OVEREXPRESSING MITOCHONDRIA**

Rayen De Fazio, María C. Ciancio, Alejandro Orlowski, Ernesto A. Aiello, Carolina Jaquenod De Giusti
Centro de Investigaciones Cardiovasculares – CONICET – UNLP – Facultad de Ciencias Médicas

Mitochondria are not only implicated in energy production but also in pathological events. Mitochondrial dysfunction has been related to the development of several pathologies including heart failure and diabetes. The Na+/H+ exchanger 1 (NHE1) is one of the main alkalinizing mechanisms in the heart and its hyperactivity has been observed in several heart conditions, including diabetic cardiomyopathy. In obese and diabetic mice mitochondria NHE1 expression is increased despite similar cardiac levels, together with altered calcium handling reverted in the presence of an NHE1 inhibitor. Aim: understanding the consequences of increased mitochondrial NHE1 expression. Materials and methods: HEK293T cells were transfected with a mitochondrial-targeted NHE1 expression vector (pmitoNHE1). Mitochondrial membrane potential was expressed as JC-1 aggregate/monomer fluorescence. Mitochondrial pH was measured using a mitochondrial-targeted pH sensor. Data are expressed as means ± SEM and are compared with Student's t-test. Results: Mitochondrial NHE1 overexpression resulted in increased mitochondrial membrane potential, similar to our observations in obese and diabetic mitochondria (0,6140 in control vs 0,7561 in pmitoNHE1 cells, $p<0,05$). Cells overexpressing pmitoNHE1 had increased H+ flux after an acid load (0,07798 in control vs 0,1302 -dpH units/min in pmitoNHE1 cells, $p<0,05$) and reduced mitochondrial pH (7,062 in control vs 6,946 in pmitoNHE1 cells, $p<0,05$) confirming its H+ pumping activity into the mitochondrial matrix. However, pmitoNHE1 overexpressing cells had increased H+ flux into mitochondria after CCCP treatment (1,265 in control vs 2,213 in pmitoNHE1 cells, $p<0,05$), probably due to the increased membrane potential. Conclusions: our results show a correlation between increased mitochondrial NHE1 expression with altered mitochondrial potential and pH control. Measuring mitochondrial Ca2+ content is essential to understanding the role of mitochondrial NHE1.

77. (343) **GALECTIN-1 MODIFIES CELL VOLUME REGULATION AND CHEMORESISTANCE THROUGH INTEGRINS PATHWAYS IN HUMAN HEPATOCELLULAR CARCINOMA**

P Carabias, L Sarrias, F Sarti, MF Troncoso, MV Espelt.
Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) (UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA.

Galectin-1 (Gal1) is a β-galactoside-binding protein overexpressed

in many cancers. In a recent work we demonstrated that Gal1 induced chemoresistance when it was overexpressed in a human hepatocellular carcinoma (HCC) cell line. Also, it was shown that Gal1 binds to β_1-integrin (INTb1) and that both proteins through their interaction induces chemoresistance. This integrin is also an osmosensor in hepatic cells. So, the aim of this work was to describe Gal1 overexpression (Gal1o) effect on INTb1 activation and localization, and cell volume regulation (CVR) in HCC cells. We also investigated if Gal1o effect on CVR and chemoresistance depend on integrin signaling pathway. We worked with the HCC cell line HepG2. Immunofluorescence experiments showed that Gal1o induced plasma membrane expression of active INTb1 while in control cells (CC) its expression was mainly intracellular. Active INTb1 localization at plasma membrane in CC was promoted by incubation with hypotonic medium while Gal1o did not further modify active UNTb1 localization. In bright field microscopy (planimetry) experiments, we quantified volume regulatory capability of cells incubated in hypotonic medium. We used RVD40 parameter (% of the maximum volume reached after 40 min of incubation). Gal1o reduced the ability of cells to regulate their volume respect to CC (RVD40=35.46±1.06% vs 93.03±3.05% respectively, $p<0.05$). The inhibition of integrin signaling pathway with PP2 (Src family kinase inhibitor, 10 μM) prevented this effect (RVD40=93.9±4.42%). Furthermore, Gal1o induced higher cell viability than the observed in CC (75.5±7.63% vs 48.6±5.19%, $p<0.05$, MTT assay) after treatment with the chemotherapeutic drug doxorubicin (DOX, 1 μM). When cells were incubated with PP2+DOX, Gal1o produced a similar percentage of viability than the observed in CC. Our results suggest that Gal1o effect on cell volume regulation and chemoresistance could be mediated by integrin signaling pathway, particularly INTb1.

78. (434) **OSTEOPONTIN REGULATES CELL VOLUME HOMEOSTASIS IN HUMAN MÜLLER CELLS**

Azul Cocca, Nicolás Cutrera, Gisela Di Giusto, Paula Ford, Claudia Capurro, Vanina Netti

Laboratorio de Biomembranas, Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

During the intense neuronal activity in the retina, Müller cells are exposed to a hypotonic environment leading to cell swelling and consequently to a regulatory volume decrease (RVD) response, which depends on the activation of Aquaporin-4 (AQP4) and Transient receptor potential vanilloid-4 channel (TRPV4) to induce the efflux of solutes and water. It was recently reported that Osteopontin (OPN), a pro-inflammatory molecule, may modulate the RVD of Müller cells. OPN also participates in cell survival and migration, as well as in neuronal regeneration after injury. Since proliferation, migration and differentiation of Müller cells into different retinal cell types are neuroprotective and cell volume dependent, OPN may also impact on these processes. Then, the aim of this work was to study the putative crosstalk of OPN with AQP4 and/or TRPV4 in cell volume regulation and consequently to proliferation and migration of Müller cells. Cell volume, osmotic water permeability (P*f*), intracellular Ca^{2+} levels and membrane potential (Vm) during an osmotic swelling were measured by fluorescent videomicroscopy. AQP4 expression was evaluated by immunocitochemistry. Cell proliferation was evaluated by cell count and cell migration by wound healing assay. We observed that OPN induced an isosmotic cell swelling and Vm depolarization, indicating the activation of ionic channels. During hypotonic shock, OPN induced a reduction of P*f*, RVD and an increase in intracellular Ca^{2+} levels. These effects were prevented by TRPV4 inhibitor HC067047. OPN also induced a reduction in AQP4 expression and significant changes in cell shape. Finally, cell proliferation was increased by OPN treatment during serum starvation, but cell migration was reduced. We propose that OPN modulates cell volume regulation of Müller cells by TRPV4 activation and AQP4 downregulation. Changes in cell proliferation and migration induced by OPN may affect retinal tissue repair *in vivo* during inflammatory conditions.

79. (456) **AQP4 AND TRPV4 PARTICIPATE IN CELL MIGRATION IN HUMAN RETINAL MÜLLER CELLS**

Nicolás Cutrera, Vanina Netti, Paula Ford, Claudia Capurro, Gisela Di Giusto

Laboratorio de Biomembranas, Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Medicina, Universidad de Buenos Aires.

Retinal neural activity alters osmotic gradients favoring cell swelling in glial Müller cells, which is followed by a regulatory volume decrease (RVD) response. We previously showed that Aquaporin-4 (AQP4) determines RVD efficiency while Transient receptor potential channel 4 (TRPV4) modulates membrane potential changes during RVD of Müller cells. It was reported that AQP4 and TRPV4 synergistically regulate cell volume and calcium homeostasis in Müller cells and multiple studies in astrocytes show that AQP4 facilitates volume-dependent processes such as proliferation and migration. However, the role of AQP4 or TRPV4 in cell migration has not been studied in Müller cells. Considering that within the retina, the activation of these cells during injury leads to their proliferation, migration and differentiation into different cell types to restore tissue function, the aim of this study was to investigate if AQP4 and TRPV4 participate in cell migration. Cell volume changes and osmotic water permeability (P*f*) were evaluated during hypotonic shock by fluorescent videomicroscopy and cell migration by wound healing assays in a human Müller cell line (MIO-M1). AQP4 inhibition by 0.5 μM TGN-020 decreased P*f* and RVD as expected and also decreased cell migration (% migration, control vs. TGN: 32 ± 8 vs. 23 ± 5, n=4, $p<0.01$). TRPV4 inhibition by 1 μM HC067047 increased swelling amplitude, without changes in P*f*, and also reduced RVD. TRPV4 inhibition also decreased cell migration (% migration, control vs. HC: 32 ± 8 vs. 26 ± 5, n=4, $p<0.05$). However, simultaneous inhibition of AQP4 and TRPV4 induced a greater reduction in RVD without changes in Pf or cell migration (% migration, control vs. TGN+HC: 32 ± 8 vs. 31 ± 7, n=4, ns). Our results propose for the first time that both AQP4 and TRPV4 participate in the migration of Müller cells. This knowledge is essential to understand the physiopathology of retinal diseases and the development of new therapeutic strategies.

80. (465) **ACYL-COA SYNTHETASE 4 MODULATES GLYCOLYTIC FUNCTION AND MITOCHONDRIAL METABOLISM IN BREAST CANCER CELLS**

Yanina Benzo, Jesica G. Prada, Melina A. Dattilo, Paula F. López, Ana F. Castillo, M. Mercedes Mori Sequeiros García, Cecilia Poderoso, Paula M. Maloberti.

INBIOMED (UBA-CONICET), Dpto. de Bioquímica Humana, Facultad de Medicina, UBA

The relationship between cancer and mitochondrial function has been widely demonstrated. Mitochondrial dysfunction is associated with oncogenesis and cancer development mainly by the prevalence of anaerobic glycolysis even under normoxia (Warburg effect). In breast cancer, Acyl-CoA synthetase 4 (ACSL4), an enzyme involved in arachidonic acid metabolism, promotes tumor aggressiveness. Previously, we demonstrated in MCF-7 breast cancer cells that stable overexpression of ACSL4 significantly increases the levels of mitochondrial markers, mitochondrial activity and respiratory parameters such as proton leak, maximal respiration, and spare respiratory capacity ($p^{<}$ 0.05, $p^{<}0.01$) respective to control cells. The aim of this work is to determine whether glycolytic function and mitochondrial metabolism are modulated by ACSL4 in breast cancer cells. For this purpose, Seahorse XF Glycolysis Stress Test was carried out to measure extra acidification rate (ECAR) and glycolytic parameters. Our results showed a significant increase in glycolytic function, non- glycolytic acidification, and glycolysis ($p^{<}0.05$, $p^{<}0.01$) and an increase in glycolytic capacity in MCF-7 overexpressing ACSL4. Furthermore, ACSL4 induced a significant decrease in the percentage of glycolytic reserve ($p^{<}$ 0.05) relative to control cells. These results corroborate a role of ACSL4 in glycolytic function in MCF-7 breast cancer cells. Also, we observed a significant increase ($p^{<}$ 0.05) in mRNA levels of mitochondrial genes related to mitochondrial function such as NRF-1/2, UCP2 and ANT1 in MCF-7

overexpressing ACSL4 cells ($p<0.05$). In conclusion, our work suggests that ACSL4 could confer an adaptive advantage to tumor cells by inducing glycolytic metabolism favoring tumor development and could protect mitochondria by promoting the expression of biogenesis genes. These results expand our knowledge of the role of ACSL4 in glycolytic function and metabolism of mitochondria in breast cancer cells.

81. (541) **ROLE OF ANGIOTENSIN TYPE 2 RECEPTOR (AT2R) IN MICROTUBULE RESPONSE TO ISCHEMIC INJURY**

Tomás Rivabella-Maknis[1], María Fernanda Fussi[2], Alejandro Pariani[1], Cristian Favre[1], Sara María Molinas[2], María Cecilia Larocca[1].
[1]IFISE-CONICET. [2]Área Farmacología, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. E-mail: maknis@ifise-conicet.gov.ar

Ischemic acute kidney injury (IAKI) leads to alterations of the microtubule cytoskeleton and loss of epithelial integrity in proximal tubular cells. AT2R pre-stimulation elicits renoprotective effects in IAKI, preserving tubular cell polarity. Primary cilia are microtubule based sensory organelles whose stability correlates with their acetylated-alpha tubulin levels (ac-tub). Because microtubules and, specifically, the primary cilia modulate cell homeostasis, we used a cell-model of IAKI to examine AT2R role in microtubule and primary cilia response to ischemic injury. Methods: polarized MDCK cells were incubated in serum free media containing antimycin A 10 µM and 2-deoxyglucose 10 mM by 90 min (Ischemia, I) and re-incubated with full media for 2 h (I-reperfusion, IR) at 37 °C. AT2R was stimulated with CGP42112A 0.1 µM (CG) beginning 24h before damage. Cell viability was tested by Trypan Blue exclusion. Microtubule integrity and cilia morphology were analyzed by confocal microscopy and relative ac-tub by western blot. Results are expressed as media +/- SE. *p<0.05 with basal (B), #p<0.05 with I, &p<0.05 with non treated (C) cells. Results: I and IR induced a significant decrease in cell viability (B 98+/-0; I 88+/-1*; IR 80+/-4*, n=3) which, in IR, was partially prevented by AT2R stimulation (CG-B: 95+/-2, CG-I: 88+/-2*, CG-IR: 87+/-4, n=3). I and IR damaged the microtubule network in both groups. I increased relative ac-tub, which partially reverted in IR, whereas AT2R stimulation per se increased ac-tub, preventing further I-induced effect (% of C-B: C-I 155 +/- 17*; C-IR 126 +/-19#; CG-B 145 +/- 18&; CG-I 151 +/- 18; CG-IR 118+/-7#, n=5). Our preliminary data indicates that % of ciliated cells and cilia length increase during I and decrease in IR, and that those changes are prevented by CG. Overall, our results suggest that AT2R stimulation may induce a preconditioning effect by increasing ac-tub levels and, therefore, may improve cilia function during IR damage.

82. (544) **TRPV4 CALCIUM CHANNEL IS INVOLVED IN AQUAPORIN 2-DEPENDENT RENAL CELL MIGRATION**

Alexis Breunig, María Florencia Rivera, Oriana Giovannini, Nicolás Cutrera, Natalia Beltramone, Valeria Rivarola, Paula Ford, Claudia Capurro, Gisela Di Giusto.
Laboratorio de Biomembranas, IFIBIO-HOUSSAY (UBA-CONICET), Facultad de Medicina, UBA, Buenos Aires, Argentina.

Water channel Aquaporin-2 (AQP2) promotes renal cell migration. Considering that we have demonstrated a physical and functional interaction between AQP2 and Transient receptor potential vanilloid-4 channel (TRPV4), the aim of our work was to investigate the role of TRPV4 in AQP2-dependent renal cell migration. We used two renal cell models: one not expressing AQPs (WT) and another one expressing AQP2 (AQP2). TRPV4 involvement in cell migration was evaluated with wound healing and cell tracking assays in the presence of the selective inhibitor HC-067047 (HC, 10 µM) and the specific agonist GSK1016790A (GSK, 10 nM). Cell viability was checked with trypan blue staining. TRPV4 localization was analyzed with immunofluorescence assays. Our results showed that both, inhibition and activation of TRPV4, reduced cell migration (%; WT: Control 22 ± 1, n=25; HC 22 ± 1, n=8, ns; GSK 19 ± 1, n=9, ns; AQP2: Control 30 ± 1, n=39; HC 25 ± 1, n=20, p<0,001; GSK 16 ± 2, n=9, p<0,001) and directionality index (D, WT: Control 0.850 ± 0.012, n=60; HC 0.851±0.011, n=61, ns; GSK 0.853±0.015, n=21, ns; AQP2: Control 0.921±0.007, n=54; HC 0.804±0.017, n=51, p<0.001; GSK 0.666±0.028 n=63, p<0.001) only in AQP2-expressing cells. Viability assays demonstrated that decreased cell migration is not due to an increment in cell death. We have already shown that lamellipodia of AQP2-expressing cells have higher TRPV4 expression than WT-ones. After HC treatment we found that TRPV4 expression is diminished and after GSK treatment it almost disappears from lamellipodia of these cells (Lamellipodia Intensity Mean, AQP2: Control 1371 ± 79 n=74; HC 472 ± 30 n=5 p<0.001, GSK 192 ± 18, n=5, p<0.001). Our results demonstrate that in the presence of AQP2, TRPV4 is in an active state and an additional stimulation with the agonist GSK leads to its endocytosis probably preserving cells of calcium excess. We propose that AQP2/TRPV4 interplay contributes to mechanisms involved in migratory AQP2-dependent behavior.

83. (607) **FRACTAL STUDY OF THE BONE-MUSCLE BINOMIAL BEHAVIOUR AT THE UPPER FEMORAL EPIPHYSIS AND ITS POTENTIAL ASSOCIATION WITH THE FEMORAL NECK ANTEVERSION, WITH PROGNOSTIC PURPOSES**

María Eugenia Cabral, Camila Luciana Chiorazo, Gina Garuti, Mateo Iván Vásquez, Mario Pedro Túrich, Matías Mariano Martin, Sara Feldman
LABOATEM. Laboratorio de Biología Osteoarticular, Ingeniería Tisular y Terapias Emergentes. Cátedra de Anatomía Normal. Área Clínica Pediátrica. Facultad de Ciencias Médicas. Universidad Nacional de Rosario.

Femoral neck anteversion(FNA) twists due to muscular action during skeletal alignment of the Lower Limb(LL). The bone-muscle binomial(BMB) can be analysed by fractal algorithms that stablish fractal dimension(FD) and coefficient of determination(R^2). FD is the area occupied by BMB and R^2 would indicate BMB´s response aptitude towards demands. The purpose of this study is to analyse BMB behaviour at the upper femoral epiphysis(UFE) with Box Counting Algorithm(BCA) and its association with the FNA. 71 randomly selected computed tomography images at the level of UFE were studied, with 76,27% of them belonging to women and 23,73% belonging to men, the mean age was 16.6±5.57 years. The BCA was applied to UFE, Adduction Muscles(ADM) and Abduction Muscles(ABM), FD and R^2 from each one of them was also determined. The FNA were measured by using GIMP software. Right(R) and Left(L) LL were separately analysed. Mean(M) and Standard Deviation(±) were obtained. Pearson correlation coefficient between BMB´s and FNA´s R^2 was stablished, with a statistical significance of p=<0,05. Results: R(LL): UFE: FD:M=1,18 ±0,15, R^2:M=0,95±0,02; ABM: FD:M=1,44±0,1, R^2:M=0,98±0,02; ADM: FD:M= 1,1±0,14, R^2:M=0,92±0,04; FNA(I):M=21,45°± 10,8°; L(LL): UFE: FD:M=1,15 ±0,18, R^2:M=0,94±0,01; ABM: FD:M:1,43±0,16, R^2:M=0,98±0,02; ADM: FD:M= 1,44±0,17, R^2:M=0,98±0,02, FNA: M=20,75 °±8,78°. Right LL Pearson Coefficients were FNA vs R^2 (UFE): r=0,31 (p=<0,4), FNA vs R^2 (ADM): r=0,03 (p=<0,98), FNA vs R^2 (ABM): r=0,23 (p= 0,8). Left LL Pearson Coefficients were FNA vs R^2 (UFE): r=0,84 (p=<0,001), FNA vs R^2 (ADM): r=0,93 (p= 0,001), AVF vs R^2 (ABM): r=0,98 (p=<0,0001). The applied BCA reveals BMB´s capacity to adapt. Left LL BMB´s muscular components interact with each other depending on demands, this phenomenon is not shown in Right LL BMB analysis, regardless its potential structural possibility to achieve it. The BCA could reveal asymmetries before they are clinically expressed

84. (780) **CELLS DERIVED FROM CLEAR RENAL CELL CARCINOMA HAVE A DIFFERENT NHE1 CELL LOCALIZATION THAN CELLS DERIVED FROM HEALTHY RENAL PROXIMAL EPITHELIA**

Ana Mechali, Victor Van Balen, Martin Nahuel Amabile, Natalia Beltramone, Claudia Capurro, Gisela Di Giusto, Paula Ford, Valeria Rivarola.
Laboratorio de Biomembranas, IFIBIO Houssay UBA-CONICET, Facultad de Medicina, UBA. Buenos Aires, Argentina

Even though pH homeostasis is critical for cell survival, extracellular

acidosis is a hallmark of cancers. It has been described that the NHE1 isoform of the Na^+/H^+ exchanger would be associated with the adaptation of cancer cells to an acid extracellular environment. However, our previous studies in healthy and clear cell cancer-derived proximal cells, showed that total NHE1 expression was higher in healthy renal proximal epithelial than in cancer cells. This study aimed to investigate whether NHE1 cell localization or its function was different in cancer cells as compared to healthy ones. We use three renal cell models: HK2, derived from normal human proximal epithelial cells; 786-O and Caki-1 cells, derived from human renal clear cell carcinoma. We analyzed NHE1 cell localization with immunofluorescence followed by confocal microscopy using two NHE1 antibodies: one directed to an extracellular epitope (NHE1 plasmatic membrane expression) and the other to an intracellular one (total NHE1 expression). We also analyzed the NHE1 activity with ammonium pulse pHi experiments using BCECF cell fluorescence. Results showed that even though HK2 cells have high total NHE1 expression, most of it was localized inside the cell. On the contrary, both 786-O and Caki-1 cells have high NHE1 both, inside the cells and in their plasma membrane. Moreover, NHE1 function correlated with plasma membrane expression as NHE1-associated ammonium pulse recovery was higher in cancer-derived cells than in HK2 cells (NHE1 recovery, 10^{-5} pH units. s^{-1}, HK2: 63 ± 7; 786-O: 175 ± 11; Caki-1: 99 ± 9. HK2 vs 786-O $p<0.001$ n=300. HK2 vs Caki-1 $p<0.01$ n=84). Then, NHE1 activity is higher in cancer-derived cells than in healthy renal epithelial-derived cells. So, it is likely that a higher NHE1 function is participating in the adaptation of cancer-derived cells to an acidic extracellular media. More studies are needed to elucidate this hypothesis.

85. (892) **HRAS reduces store-operated calcium entry (SOCE) in RAS-less MEF cells**

Julieta Mansilla Ricartti, Lutz Birnbaumer, and Sebastian Susperreguy

Institute of Biomedical Research (BIOMED UCA-CONICET), Catholic University of Argentina, Buenos Aires C1107AFF, CABA

Background and puropose: The store-operated calcium entry (SOCE) is the major Ca2+ entry pathway in nonexcitable cells, that is activated as a cellular response to refill the Ca2+ stores when reticulum endoplasmic (ER)-Ca2+ store are depleted. The depletion of ER Ca2+ results in STIM1 translocation to ER-plasma membrane junctions where they bind and activate Orai1, the pore subunit of the Ca2+ release-activated Ca2+ (CRAC) channel. Ras GTPases are molecular switches localized at plasma membrane that cycle between an inactive GDP-bound state and an activate GTP- bound state that engages effector proteins to regulate multiple signal transduction pathways. In humans, three RAS genes encode four distinct isoforms: HRAS, NRAS, and the two splice variants of KRAS gene, KRAS4a and KRAS4b, containing exons 4a and 4b, respectively. Our work has focused on elucidating whether RAS has a role in the SOCE mechanism. Experimental approach: To determine whether the protooncogene RAS is involved in the mechanism of SOCE, we used a unique cell model of mouse embryonic fibroblast devoid of RAS proteins (RAS-less MEFs). First, we analyzed the Ras-less MEF and RAS-less clones generated by stable transfection of human cDNAs of HRAS or KRAS4A (RAS-less MEF HRAS+, RAS-less MEF KRAS4A+) by Tg-evoked SOCE using the radiometric fura2 assay. Next, we transfected the clone of MEF devoid of RAS proteins with EYFP-tagged H-,N- or KRAS and analyzed the change of SOCE. Finally, we used pharmacological inhibitors of MEK-ERK, PI3K and PKC to investigate the intracellular signaling pathways involved in the effects of RAS Key results: We found Tg-evoked SOCE was reduced in RAS-less MEFs by more than 50%, whereas in the clone that stable express HRAS (RAS-less MEF HRAS+) SOCE was reduced by almost 90%. Moreover, HRAS transfected into RAS-less MEFs reduced Tg-induced SOCE in a dose-dependent manner. PKC blockades with GF109203X increased SOCE in SOCE RAS-less MEF BRAF by almost 30%, whereas blockade of MEK/ERK with UO126 or PI3-K with LY294002 had no effect. Finally, HRAS transfected still reduced SOCE in RAS-less MEFs when the intracellular signaling pathways MEK/ERK or PI3-K were blocked, whereas HRAS transfected did not reduce SOCE when PKC was blocked. Conclusions and implications: In conclusion, the results suggest HRAS reduces SOCE without using MEK/ERK and PI3-K, the major intracellular signaling pathways activated by GTP-bound RAS

CLINICAL IMMUNOLOGY

Thursday, November 17, 9-10:30 hr

Chairs: Daniela Di Giovanni - Patricia Carabajal - Pablo Fernández - Ana Ceballos

86. (31) **PERSISTENT SYMPTOMS AFTER COVID-19 IN CHILDREN AND ADOLESCENTS FROM ARGENTINA**

Vanesa Seery[1], Silvina Raiden[2], Juan Martin Gómez Penedo[3], Mauricio Borda[4], Largión Herrera[5], Macarena Uranga[6], Constanza Erramuspe[7], María Marcó del Pont[6], Melisa Lenoir[6], Carolina Davenport[2], Alexsa Alarcón Flores[4], Constanza Russo[1], Inés Sananez[1], Natalia Laufer[1], Roberto Muiños[3], Fernando Ferrero[2], Jorge Geffner[1], Lourdes Arruvito[1].

[1] Instituto de Investigaciones Biomédicas en Retrovirus y SIDA. UBA-CONICET, CABA, Argentina. [2] Hospital General de Niños Pedro de Elizalde. CABA, Argentina. [3] Facultad de Psicología. UBA-CONICET. CABA, Argentina. [4] Hospital Pediátrico Juan Pablo II. Corrientes, Argentina. [5] Hospital Dr. Salvador Mazza. Chaco, Argentina. [6] Hospital Universitario Austral. Buenos Aires, Argentina. [7] Policlínico Regional Juan Domingo Perón. San Luis, Argentina

Background: While long COVID-19 is widely recognized in adults, its existence in children is more controversial. Data are limited by the lack of appropriate definition and small size of cohorts. We aimed to study the long-term symptoms and associated risk factors for persistent symptoms beyond 3 months of COVID-19 in the pediatric population. Methods: This observational study includes 639 Argentinian children aged 1 to 17 years with previous SARS-CoV-2 infection and 577 uninfected children during June 2020-June 2021. Parents completed a survey about the acute COVID-19 and persistent or new symptoms that their child had for more than 3 months after diagnostic. Results: A high proportion of parents of previously infected children (34%, n=219) reported that their children had at least one persistent or new symptom in comparison with the perception of parents of healthy children (13%, n=74, $p<0.0001$). SARS-CoV-2 infection increased 3 to 6 folds the risk of having headache, dizziness, loss of taste, dyspnea, cough, fatigue, muscle pain and loss of weight. Loss of smell was only reported in cases. Furthermore, more than 50% of children had multisystem impacts. Finally, older age, symptomatic COVID-19 and comorbidities were predictor variables significantly associated with a higher risk of developing long-term symptoms. Conclusion: A third of children experienced persistent symptoms months after acute COVID-19. Older age, symptomatic COVID-19 and underlying disease were associated with higher risk of persistent symptoms.

87. (38) **LEVELS OF MONOCYTE SUBSETS IN HIV-INFECTED CHILDREN AND CHANGES ASSOCIATED TO ANTIRETROVIRAL THERAPY ADHERENCE**

CeresV, Quiroz H, Cortopassi G, Candi M, BarboniG, Balbaryski J, Gaddi E

Introduction. Persistent viral replication and continuous immune activation during HIV infection are associated with alterations in the homeostasis of monocytes subsets (MS) and conditioned to a correct antiretroviral treatment (ART) adherence. Aim. To evaluate levels of MS in HIV-infected children and to monitore the changes associated to ART adherence. Materials and methods. MS percentage levels were studied in 39 mother-to child HIV-infected patients and 10 healthy controls (Co). To evaluate the association between frequency of MS and ART adherence, 13 of the 39 patients were prospectively evaluated, in average, during a 12-month follow-up period (t0, t1). CD14++CD16- (classical C), CD14++CD16+ (intermediate I) and CD14+CD16++ (non-classical NC) MS levels, were measured by flow cytometry. Clinical status and viral load (VL, log), at both

points of follow-up, were also studied. Results. When patients were divided according to immunosuppression degree (CD4+T cells ≥ 25%, group A, n= 21 or < 25% group B, n= 18), percentage levels of NC, and I MS, presented significant increase, p<0.05, respect to Co group, meanwhile a significant decrease in C MS, was observed. To compare HIV-infected groups, only in NC MS, significant differences was recorded, (NC A: 5.05±4.07 vs NC B: 8.53±5.32, p: 0.026). During follow-up period, in patients with correct adherence to ARV (n=6) (VL t0: 2.31±1.0, VL t1: <1.70), non-significant decreases in the NC and I MS, were verified, on the contrary, a significant increase, (p: 0.031), in levels of C monocytes, was observed. No significant changes in levels of MS in patients with deficient adherence (n=7) (VL t0: 2.93±1.21, VL t1: 3.40±1.18), at the end of follow-up period, were recorded. Conclusion. The observed increase in CD16+ MS, would be associated with a decrease in CD4+ T cells levels as a consequence of a poor rate of adherence. Quantitative normalization of classic population would be correlated with an improvement in ART adherence.

88. (51) **EFFECT OF CONVALESCENT PLASMA IN A SERIES OF HOSPITALIZED PATIENTS WITH COVID-19 IN SANTA FE PROVINCE, ARGENTINA**

Mario Perichon[1], Andrea Acosta[2], Liliana Di Tulio[2], Stella Pezzotto[3], Oscar Bottasso[3], Esteban Nannini[3].
[1]Centro Único de Donación, Ablación e Implante de Órganos, Ministerio de Salud de la provincia de Santa Fe
[2]Centro Regional de Hemoterapia Sur, Ministerio de Salud de la provincia de Santa Fe
[3]Instituto de Inmunología Clínica y Experimental de Rosario (UNR-CONICET)

After the outbreak of COVID-19 in Santa Fe province, the Ministry of Health recommended the use of convalescent plasma transfusion (CPT) obtained from recovered patients. This is a retrospective analysis of 790 patients hospitalized with COVID-19 treated with CPT, between 06/2020 and 10/2020. The median age was 61 years (interquartile range 51-70) and 67.5% were men. More than 50% were in the general ward (n=446) and 43% (n=344) in the intensive care unit. A single CPT infusion was given in 92% of the patients, 73.8%, and 95.5% before day-4 and day-9 of hospitalization, respectively. The day 28 simplified WHO ordinal clinical scale showed improvement in 57.4% of patients (1 or ≥2 points in 310 and 144 cases, respectively), and an unchanged or lower scale in 336 (42.5%). At that time, 284 (36%) were discharged and 315 (40%) remained hospitalized: 161(20%) without O_2, 22 (3%) with O_2, and 132 (17%) on mechanical ventilation (MV), whereas 24.2% died. Multivariate analysis showed an age-related increase in fatal outcomes, with the risk of dying increasing by 28% for each added comorbidity. Patients who required MV were 5.8 times more likely to die. There were no differences in the anti-spike antibody titers (UI/ml, anti-SARS-COV-2 chemiluminescence quantitative assay, ®Roche) of CPT administered to survivors (76.2 ± 52.5) or non-survivors (76.3 ± 55.4). The 28-day mortality was higher in those receiving CPT beyond day 5 of admission compared with those given CPT <day 3 (logistic regression, OR: 3.5; 95% CI: 2.27-5.43, p<0.0001). Present findings of CPT for severe Covid-19 disease in the real world may imply an early antiviral neutralizing activity of CPT and the lack of its effect when given later. Despite not detecting any impact on patients' outcomes according to the antibody levels present in administered CTP, a seemingly favorable effect was seen among those receiving CPT sooner. The observational design hinders drawing stronger conclusions.

89. (63) **LACKING ACUTE-PHASE INFLAMMATORY RESPONSE AND SUSCEPTIBILITY TO *STAPHYLOCCOCUS AUREUS* DUE TO A PHENOCOPY OF INBORN ERROR OF THE IL6 PATHWAY: FIRST SOUTH AMERICAN CASE REPORT**

Fernanda Quinteros[1], Matías Oleastro[2], Laura Perez[1]
[1] Laboratorio de Inmunología Humoral, Hospital de Pediatría "J. P. Garrahan", [2] Servicio de Inmunología, Hospital de Pediatría "J. P. Garrahan"

Inborn errors of immunity (IEI) were thought to predispose various infectious diseases in individual patients until 1996, from which some IEI were found to underlie a single, specific infectious disease. IE of the IL6 pathway suggests that IL6 is crucial for immunity to bacterial diseases, including staphylococcal infection in particular, and for acute-phase inflammatory responses. Neutralizing auto-antibodies (auto-Abs) against cytokines underlie phenocopies of IE of the corresponding cytokine or response pathway. Four patients with high levels of auto-Abs neutralizing IL6 have been reported since 2008. We present here the immunological work-up of a patient suspected to have IRAK4/MyD88 deficiency, whom blood serum showed anti-IL6 activity. Case report: a previously healthy Argentinean 5-year-old boy developed a severe community-acquired pneumonia with pleuro-pulmonary suppuration requiring drainage of pleural cavity. No fever was registered. No eczema, non-syndromic phenotype or other findings related to primary immunodeficiencies were found. Culture performed on pleural fluid specimen was positive for *Staphyloccocus aureus*. Acute phase reactants were 4 mm/h for erythrocyte sedimentation rate (ESR) and < 0.6 mg/l for C-reactive protein (CRP). Four months later, the patient (Pt) was re-admitted with a necrotizing pneumonia. As before, no fever was registered. 1) Whole blood (WB) and PBMC assays were conducted to evaluate the production of IL6 by ELISA in response to LPS. 2) Healthy control (HC) PBMC were incubated with LPS with and without medium containing 10% plasma from the Pt for 48 h. IL6 concentrations in supernatants were measured by ELISA. Production of IL6 (pg/10*6CMNT): 1) Pt WB: 20, Pt PBMC: 45590; HC WB: 20827, HC PBMC: 36375. 2) HC PBMC. LPS: 36375; LPS+Pt's plasma: 850.
Conclusion: we present the first South American patient with a phenocopy of an inborn error of the IL6 pathway.

90. (125) **ABNORMALITIES IN T AND B CELL SUBSETS IN PATIENTS WITH MUTATIONS IN PI3K PATHWAY**

María Soledad Caldirola[1], Agustín Bernacchia[2], Andrea Gomez Raccio[2], Analía Gisela Seminario[2], Antonella Invernizzi[2], María Paula Martinez[2], Ana Luz García[2], Daniela Di Giovanni2, María Isabel Gaillard[2].
[1] Inmunología- Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas
(IMIPP- CONICET-GCBA)- Hospital de Niños "Dr. Ricardo Gutiérrez"; Buenos Aires, Argentina.
[2] Servicio de Inmunología-Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires, Argentina.

Introduction: Phosphatidylinositol 3–kinases (PI3Ks) represent a family of lipid kinases, among which class IA PI3Ks have a critical role in the immune system in mammals. Class IA PI3Ks form mandatory heterodimers comprised of a catalytic subunit and a regulatory subunit. The PI3K signaling pathway is involved in a broad range of cellular processes, including cell growth, metabolism, proliferation, and survival. PI3Kδ is comprised of the p110δ catalytic and the p85α regulatory subunits, encoded by *PIK3CD* and *PIK3R1*, respectively. *"Activated PI3Kδ Syndrome"* (APDS) has recently been described in patents with immunodeficiency due to germline heterozygous gain-of-function mutations in *PIK3CD* and loss-of-function mutations in *PIK3R1*. Aim: describe clinical features and abnormalities in T and B cell subsets of a patient with *PIK3CD* c.3061G>A (E1021K) (P1) and *PIK3R1* (1425+1G>A) (P2) variant. Results: both patients presented early-onset recurrent sinopulmonary infections, growth impairment, microcephaly (P1), lymphoproliferation, autoimmunity (P2). Immunological findings revealed hypogammaglobulinemia (IgG) (P1) and IgA (P2), poor polysaccharide and proteic response, lymphopenia ($CD4^+$ T and $CD19^+$ B cells), CD4/CD8 inversion, low naive T-cells with expanded memory and activated phenotype (HLA-DR). Expanded terminal effector $CD8^+$ T cells with high expression of senescence markers (CD57 and PD-1) and lower proliferation response to mitogens (PHA). Increased $CD4^+$ circulating follicular T cells with a skew towards a Tfh1 profile. Impaired B-cell subsets showed markedly high frequencies of transitional/immature B cells with concomitant low memory-switched B cells. Conclusion: patients with APDS have increased susceptibility to infections, combined immunodeficiency, and immune dysregulation. Deep immune-phenotype analysis in patients with inborn errors

of immunity provided mechanistic insights into the role of PI3K in T and B cells differentiation.

91. (128) **THE JAK INHIBITOR TOFACITINIB ACTIVATES IMMUNOSENESCENCE PATHWAYS AND LIMITS ACTIVATION AND FUNCTION OF T LYMPHOCYTES**

*Luisina Onofrio[1,2], *Vanina Alejandra Alamino[1,2], Paola Ferrero[1], Cristina Acosta[1], Marina Werner[1], Laura Beatriz Onetti[1], Isaac Ignacio Cadile[1], Eduardo Daniel Mussano[1], Carolina Montes[2], Adriana Gruppi [2], Eva Virginia Acosta- Rodriguez[2].
*[1]Hospital Nacional de Clínicas (HNC), Universidad Nacional de Córdoba (UNC). [2]Facultad de Ciencias Químicas, UNC - CIBICI, CONICET. *equal contribution*

Tofacitinib (Tofa) is a Jak1/3 inhibitor that blocks the intracellular signaling of inflammatory cytokines and is used for treatment in Rheumatoid Arthritis (RA). Tofa is very effective to achieve disease remission but it is associated to higher herpes zoster incidence and tuberculosis reactivation likely due to immune alterations. While several studies have evaluated the effects of Tofa on the immune system, knowledge about its impact on activation and differentiation of T lymphocytes (TL) is scarce. Our previous results showed that TL from Tofa-treated RA patients exhibit a phenotype of terminal differentiation and immunosenescence. In this context, we aimed at assessing the impact of Tofa *in vitro* on TL activation and function focusing on possible differences according to naive vs. memory TL differentiation status. To this end, we activated TL sorted from healthy donors PBMCs with a-CD3/a-CD28 for 3 days in presence or absence of Tofa (1 to 10 uM). Tofa significantly reduced TL activation as evidenced by a decrease in the frequency of $CD25^+$, T-bet^+ and Ki-67^+ cells. These effects were dose-dependent and observed in all the subsets but stronger in memory, particularly $CD8^+$ TL. Reduced TL activation was associated with the upregulation of KLRG1, a pre-senescence marker, only in CD8 memory cells. In addition, Tofa reduced the effector function of all TL subpopulations evaluated as highlighted by the decreased frequency of cells expressing IL-2, IFNy and granzyme B. Interestingly, Tofa increased the expression of markers associated to cellular senescence like p-ATM and γH2AX, two kinases involved in the earliest stage of cellular response to DNA Double-Strand Break formation. The maximal effect size was detected in CD8 memory TL. Altogether, our findings suggest that Tofa trigger immunosenescence pathways in TL that could underlie its biological effects in RA but also be involved in its side effects by restraining the activity of memory TL involved in microbial control.

92. (205) **FACTORS ASSOCIATED WITH THE PRESENTATION OF COMPLICATIONS IN PATIENTS UNDERGOING HIGH-RISK SURGERY**

Juan Carlos Pendino[1,2], Juan Ignacio Ibarzabal[3], Lisandro Bettini[2], Pablo Depaoli[3], Daniel Devuono[4], Natalia Santucci[5], Oscar Bottasso[5]
[1]1° Cátedra Clínica Médica, Facultad de Ciencias Médicas UNR
[2]Unidad de Terapia Intensiva Hospital Provincial del Centenario, Rosario.
[3]Servicio de Cirugía General, Hospital Provincial del Centenario, Rosario.
[4]Jefe del Laboratorio Central Hospital Provincial del Centenario Rosario; Facultad de Ciencias Bioquímicas y Farmacéuticas UNR
[5]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER, UNR-CONICET)

The purpose of this consecutive prospective observational study was to assess whether some laboratory variables could be related to the development of complications in patients undergoing surgical operations at a School Hospital in Rosario city between May 2019 and January 2021 (18 women and 25 men, mean age 58 ± 9 years -SD-). A group of sex- and age-matched control individuals (n=10) was included to compare biochemical data. Evaluations were made on 0, 1-, 2-, 3-, and 7-days post-operation (PO). Hematological, hepatic, renal, acid-base profiles, and acute phase reactants, as well as pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-10, IL-17-A, IFNγ, and TNFα), were measured. There were complications in 31 patients between days 1 and 18 of the PO (mostly in the first days). By employing a non-parametric multiple comparison approach, complicated patients showed significantly higher levels of leukocytes, procalcitonin, troponin, and urea within the first 3 days of PO, even more in those with a higher number of complications. The pH was statistically lower in patients with more severe complications (vs. those who did not have complications or were less severe). There was no difference between groups either in the presence of immunosuppression-related factors such as cytomegalovirus infection or salivary cortisol levels, but on day 7, the latter was found to increase in the complicated ones ($p<0.035$). In all cases, a significant increase in IL-6 and IL-10 was seen in the first 2 days of PO with no differences between complicated or uncomplicated patients. By day 3, IL-6 values decreased significantly ($p<0.01$). IL-10 increased initially and began to decrease after 48 h of PO ($p<0.001$), showing no between-group differences. The development of complications coexists (and in some cases is preceded) by changes in some acute phase reactants, a very transitory modification of pro- and anti-inflammatory cytokines, and a relative delay in the adrenal hormonal response.

93. (315) **COMMON VARIABLE IMMUNODEFICIENCY IN ADULTS - A PUBLIC HOSPITAL EXPERIENCE**

Roberts, J; Leon, C ; Lopez, AL ; Quirno Costa, ME; Ramos, G; Guevara, R; Novoa, V.
Sección Inmunología - División Laboratorio - Hospital Carlos G. Durand, Buenos Aires, Argentina.

Introduction: Common variable immunodeficiency (CVID) is a heterogeneous disease characterized by defects in B Lymphocyte (BL) differentiation and low or absent levels of IgG (<2SD from the mean), IgA and/or IgM. The spectrum of clinical manifestations is: recurrent infections (RI), autoimmunity (AI), malignancy (M), and granulomas (G). Moreover, defects in T Lymphocytes (TL) differentiation have also been described. Aim: To describe and analyze the clinical manifestations, and the B and T cells immunophenotype in CVID patients. Material and Methods: Retrospective analysis of medical records and laboratory results of 31 CVID patients (p). Lymphocytes subpopulations were analyzed by flow cytometry and immunoglobulins by immunoturbidimetry. Results: 31p (20p women), $\bar{x}$ age at diagnosis: 32.5 years old (yo) (10-67yo), $\bar{x}$ age at onset: 15yo (1-53yo). 23p were diagnosed in adulthood, $\bar{x}$ time between onset and diagnosis: 17.9yo (1-52yo). 26p consulted for RI, 2p for hypogammaglobulinemia, 2p for M and 1p for AI. The most frequent infections were: pneumonia 27p, rhinitis 25p, diarrhea 21p, and otitis 9p. In addition to RI 24p presented AI, M, lymphoproliferation and G. During the follow up 17p presented complications such as bronchiectasis, gastritis and hearing loss. $\bar{x}$ TL $CD4^+$ naive, $CD8^+$ naive and switched memory BL was decreased compared to age-matched reference values (RV). $\bar{x}$ TL $CD4^+$ central memory, TL $CD4^+$ effector memory, TL $CD8^+$ effector memory (EM), TL $CD8^+$ terminal effector cells, BL $CD21^{low}$ and transitional BL was increased compared to age-matched RV. Patients with post diagnosis complications showed an increased number of TL $CD8^+$ and EM $CD8^+$ compared to those who did not present them. ($p<0.05$). Conclusions: CVID patients present a dysregulated immunophenotype profile, indicating that this pathology is more than a humoral deficiency. Finally, the long time gap between onset and diagnosis shows that it is not only a rare disease but also a forgotten one.

94. (383) **CLINICAL, LABORATORY FEATURES AND FOLLOW-UP IN SELECTIVE IGA DEFICIENCY: A SINGLE PAEDIATRIC CENTRE REPORT**

Espantoso, Daiana Natalí[1]; Natoli, Veronica[1]; Gaillard, María Isabel[1]; Gomez Raccio, Andrea[1]; Di Giovanni, Daniela[1]; Bernacchia, Agustin[1]; Caldirola, María Soledad[1,2]; Martinez, María Paula[1]; Carabajal, Patricia[1].
[1]Servicio de Inmunología - Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires, Argentina.
[2]Inmunología - Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP- CONICET-GCBA) - Hospital de Niños "Dr. Ricardo Gutiérrez"; Buenos Aires, Argentina.

Introduction: Selective IgA deficiency (SIgAD) is the most common inborn error of immunity (IEI). According to ESID Criteria the diagnosis is made after 4 years old (yo) and defined by serum IgA levels <7 mg/dl, measured in two opportunities, with normal IgG and IgM and adequate antibody response having ruled out other causes of hypogammaglobulinemia. Some patients may be asymptomatic or present respiratory/gastrointestinal infections, allergy and autoimmunity. Aim**.** To describe clinical and laboratory features in a cohort of SIgAD paediatric patients (pts). Methods: Retrospective study of 211 medical records from 1995 to 2022 with SIgAD and follow-up more than 12 months (mo). Results: We registered 211 pts; 111 males and 100 females, median age at first consult 6,3 yo (r:0.9- 24.5), follow up median 12 years (r:1-17). 41/211 pts presented hypoIgA before 4yo and were diagnosed SIgAD at 4yo. Common clinical manifestations were: upper and lower respiratory tract (82%), infections (42%), gastrointestinal disorders (18%) and allergy (12%). Immunological results: 100% normal or elevated IgG-IgM and 67% high IgE. 15/182 pts presented specific antibody deficiency (SAD) and 10/111 pts showed low levels of serum IgG subclasses (IgGsub). 129/170 (76%) lymphocyte populations and 50/118 (42%) B subsets evaluated were normal. During the follow up 48/211 pts (23%) modified their initial diagnosis: 27 hypoIgA, 11 SAD, 6 IgGsub and 4 IgGsub with SAD. About 67% of pts had family history of allergy, autoimmunity and malignancies. IgA levels were below 2SD in an 8% of evaluated parents and siblings. Discussion: Our results highlight the importance of long-term clinical and laboratory follow-up in order to reclassify and/or detect new abnormalities that will modify the clinical management. Likewise, it is important to carry out familial screening to detect a possible SIgAD or another IEI.

95. (554) **OSSEOUS LANGERHANS HISTIOCYTOSIS AND INDOMETHACIN EFFICACY BASED ON HOMING GENE EXPRESSION**

María Catalina Lava*[1], Denise Mariel Risnik*[1], Cinthia Mariel Olexen*[2], Diego Alfredo Rosso[1,3#], Eugenio Antonio Carrera Silva[2#] and Andrea Emilse Errasti[1#]
**Equal contribution*
[#]Senior authors
1-Instituto de Farmacología, Facultad de Medicina, Univerdidad de Buenos Aires, Argentina
2-IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina
3-Hospital de Clínicas, José de San Martín, Facultad de Medicina, Univerdidad de Buenos Aires, Argentina

Langerhans cell Histiocytosis (LCH) is a rare inflammatory and myeloid proliferative disorder, leading to the accumulation of CD1a+/CD207+ cells into different tissues causing a wide range of lesions and compromise. Indomethacin, a non-selective COX inhibitor, has proven to be effective and well accepted for patients with bone affection, but its specific mechanism has not yet been established. We hypothesize that Indomethacin affects the homing of pathogenic Langerhans cells (LC) or their precursors to the bone. We retrospectively (2018-2022), evaluate the outcome of patients with bone LCH under indomethacin treatment analyzing bone healing, clinical improvement, presence of circulating CD1a+/CD207+ cells, and the expression of homing and migration molecules in sorted circulating mononuclear myeloid cells. The effect of indomethacin and ibuprofen on the expression of homing genes was evaluated *in vitro* in a inflammatory LC-like model (IL-4 plus GM-CSF plus TGFβ plus TNFα plus dexamethasone) by qPCR and flow cytometry. We have found 21 patients with bone compromise and indomethacin treatment, and curiously the clinical improvement still showed circulating CD1a+/CD207+ cells suggesting a potential restraining mechanism to the bone. AXL, a tyrosine kinase receptor involved in cancer cell migration, is increased in patients with LCH (N=13, P=0.01) and particularly higher in bone compromise (N=6, P<0.001). Furthermore, the chemokine receptor CCR6 (N=6) was also increased in the myeloid cells of these patients. The *in vitro* inflammatory LC-like cells were characterized by high levels of AXL (N=6, P=0.03), CXCR4 and CCR6, compared to conventional dendritic cells and a reduction of AXL receptor (N=7, P=0.01) was found with indomethacin treatment but not when treated with ibuprofen, that unlike showed higher levels of CXCR4 and CCR2. Our results suggest that indomethacin could be affecting the homing of LCH precursors to the bone and consequently reducing tissue damage.

96. (559) **CIRCULATING CD207+ CD1A+ MYELOID CELLS DELINEATE A NEW CELLULAR SCORE IN LANGERHANS CELL HISTIOCYTOSIS**

Cinthia Mariel Olexen[1], Diego Alfredo Rosso[2,3,*], Wanda Nowak[2], Daniela Fortunati[3], Guido Luis Dalla Vecchia[1], Andrea Emilse Errasti[2*], and Eugenio Antonio Carrera Silva[1*].
**Co-senior authors and contributed equally to this work.*
1-Instituto de Medicina Experimental (IMEX), Academia Nacional de Medicina-CONICET, Buenos Aires (1425), Argentina
2-Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires (1121), Argentina
3-Hospital de Clínicas General San Martin, Departamento de Pediatría, Universidad de Buenos Aires, Buenos Aires (1121), Argentina

Langerhans cell histiocytosis (LCH) is a neoplasm inflammatory disorder characterized by accumulation of CD207+ and CD1a+ cells in almost any tissue with putative myeloid precursors circulating in the blood. Currently, there is a lack of prognostic markers to follow-up patients, monitor disease reactivation or treatment response. Our aim was to set a standardized method to screen circulating CD207+ and CD1a+ cells in a drop of blood of patients with LCH and monitor them during follow up. Employing flow cytometry, 202 independent blood sampling including follow up from patients with LCH and 23 controls were examined. A standardized cellular score was defined by measuring CD207+ and CD1a+ expression in monocytes and dendritic cells, based on CD11b, CD14, CD11c, and CD1c subsets, giving a unique value for each sample. To test the trajectory of the cellular score associate with biochemical parameters and clinical outcome we examine some patients with at least 6 independent sampling including bone, multisystem and skin compromise. A ROC curve was used to validate the scoring system (AUC: 0.849) with a threshold value obtained with Youden´s test (Y index: 14), defining the presence of circulating CD207+CD1a+ cells. A positive correlation between the cellular score and sCD40L, sIL-2Ra, and CXCL12 molecules in plasma was also found. During the follow-up, circulating CD207+CD1a+ cells were evidenced just before the clinical manifestation in bone compromise. The patient with multisystem involvement showed circulating cells just before bone reactivation and during the clinical manifestation. The patient with skin involvement, have also circulating cells before and/or during at least one of the clinical reactivations. We have set a cellular score based on the presence of circulating CD207 and CD1a cells that could help with prognostic accuracy, early reactivation, and follow-up with minimal invasiveness.

97. (689) **NOVEL QUANTITATIVE ENZYME LINKED IMMUNOSORBENT ASSAY TO DETECT RBD AND SPIKE PROTEIN OF SARS-COV-2 USEFUL FOR DIAGNOSIS OF COVID-19**

Juan Ignacio Marfía[1], Adriana Victoria Sabljic[1], Silvina Sonia Bombicino[1], Aldana Trabucchi[1], Ruben Francisco Iacono[1], Ignacio Smith[2], Gregorio Juan Mc callum[2], Federico Javier Wolman[2], Alexandra Marisa Targovnik[2], Joaquín Poodts[2], Matías Fingermann[3], Adolfo De Roodt[3], Marcelo Rodriguez Fermepin[4], Lucía Gallo Vaulet[4], Leonardo Gabriel Alonso[2], María Victoria Miranda[2], Silvina Noemí Valdez[1]
[1]Universidad de Buenos Aires (UBA), Facultad de Farmacia y Bioquímica (FFyB), Cátedra de Inmunología e Instituto de Estudios de la Inmunidad Humoral, Prof. Ricardo A. Margni (IDEHU) UBA-CONICET, [2]Universidad de Buenos Aires (UBA), Facultad de Farmacia y Bioquímica (FFyB), Cátedra de Biotecnología e Instituto de Nanobiotecnología (NANOBIOTEC) UBA-CONICET, [3]Instituto Nacional de Producción de Biológicos (INPB), ANLIS "Dr. Carlos G. Malbrán", Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, [4]Universidad de Buenos Aires (UBA), Facultad de Farmacia y Bioquímica (FFyB),

Departamento de Bioquímica Clínica, Cátedra de Microbiología Clínica e Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), UBA.

Herein we describe a novel enzyme-linked immunosorbent assay (ELISA) for SARS-CoV-2 antigen detection employing a high affinity polyclonal serum to recombinant Spike (S) protein, which was produced at high scale in horse. This assay is aimed for diagnosis of COVID-19 and quantification of RBD and S protein from any production process. Materials and methods: Equine polyclonal anti-S antibodies were obtained by immunization of one mixed-breed 4 to 10 years-old, 300 to 450 kg horse. The purified antibodies were incubated with a 20-fold molar excess of sulfo-NHS-biotin. Free biotin was removed on a PD-10 desalting column. The ELISA was based on the capture of the antigen present in samples or calibration curve by equine anti-S antibodies immobilized in the solid phase. Bound RBD or S protein was detected by the addition of antibodies anti-S-biotin followed by Streptavidin-Horseradish Peroxidase. Twenty human samples from the respiratory tract were analyzed in parallel by rRT-PCR and by ELISA. Additionally, recombinant S protein or RBD both expressed in baculovirus/ insect larvae were detected and quantified. Results: Out of the 20 patient samples, 15 were positive for rRT-PCR and 5 were negative. When a cut-off value of 0.1 OD was established, 10 out of the 15 positive samples were also positive by our developed ELISA (sensitivity: 67%). Moreover, the test showed a lineal response for concentrations of 2.4 to $35x10^{-11}$ M and 1 to $20x10^{-11}$ M for RBD and S protein, respectively. So, this assay allows the quantification not only in biological samples but also in production processes of both recombinant proteins. Conclusion: Our results demonstrate that the method developed herein, based on detection of RBD and S protein of SARS-CoV-2, is useful as an alternative and rapid screening method for diagnosis of COVID-19 in low or medium complexity laboratories. Moreover, this assay can also be applied for the quantification of recombinant RBD or S protein.

98. (867) **IMMUNOLOGICAL ABNORMALITIES IN CHILDREN WITH DOWN SYNDROME. A SINGLE-CENTER EXPERIENCE**

Bernacchia Agustin[1], Di Giovanni Daniela[1], Caldirola María Soledad[1,2], Espantoso Daiana[1], Martinez María Paula[1], Natoli Verónica[1], Comas Dorina[1], Carabajal Patricia[1], Gaillard María Isabel[1]

1-Grupo de Inmunología, Hospital de Niños Ricardo Gutiérrez, Ciudad de Buenos Aires, Argentina

2-Inmunología- Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP- CONICET-GCBA)

Introduction: Down syndrome (DS) is a multi-system condition associated to developmental impairment and congenital malformations, particularly, congenital heart disease that usually requires cardiac surgery (CS). Children with DS have susceptibility to recurrent/severe infections, autoimmunity and malignancies. Aim: To describe clinical and immunological features of children with DS. Material and methods: Immunoglobulin levels, polysaccharide response, autoantibodies, lymphocytes, T and B cells subsets and proliferative response were retrospectively collected from medical records. Mann Whitney U test was used($p<0.05$). Results: 28 DS children were evaluated(median age: 8yo [1,6-23 yo]). Median age at first evaluation was 4yo, being recurrent and/or severe respiratory infections the most common signs at presentation (81%), followed by lymphopenia (17%), arthritis (4%) and autoimmunity (4%). As consequence of CS, 42% of DS children had incidental thymectomy before 1 yo. Follow-up: 17/28 developed autoimmunity. Laboratory: 15/27 had low IgM. 5/18 had IgG subclass deficiency, and 11/18 defective polysaccharide response. 14/27 presented autoantibodies. 4/28 had severe T cell Lymphopenia. Almost all showed a skew towards central memory in CD4+, and memory effector/terminal effector in CD8+. All evaluated children(11) showed normal proliferative response. 13/28 showed B lymphopenia with decreased total memory(11/24) and elevated CD21low, transitional and plasmablast B cells. DS children with CS, associated even lower T cells counts($p<0.001$), naive CD4+ T cells($p<0.001$) and recent thymic emigrants($p<0.05$). Discussion: We found profound immunological T and B phenotypic abnormalities in children with DS. Cardiac surgery worsens T-cellular immunity and may predispose to immune dysregulation and severe infections. Further studies including other cardiopathies with similar surgical procedures are needed to evaluate the impact on T cell compartment and its clinical implications.

ENDOCRINOLOGY I

Wednesday, November 16, 14-15:30 hr

Chairs: Gabriela Guercio - María Andrea Camilletti - Ana de Paul

99. (45) **RELATIONSHIP BETWEEN *GHR* GENE POLYMORPHISM, GONADAL FUNCTION, AND PRENATAL GROWTH IN A NONSPECIFIC 46,XY DSD COHORT**

María Celeste Mattone[1,4], Natalia Perez Garrido[1], Mariana Costanzo[1], Lorena Hidalgo[2], Malena Berger[3], Luciana Zoff[4], María Sonia Baquedano[4], Pablo Ramirez[1], Esperanza Berensztein[1], Marta Ciaccio[1], Roxana Marino[1], Alicia Belgorosky[1,4], Gabriela Guercio[1,4]._

[1]Hospital de Pediatría Garrahan, Endocrinology Department; [2]Hospital Pedro de Elizalde, Endocrinology Department; [3]Hospital Universitario Austral; [4]CONICET. Buenos Aires, Argentina

Background: A higher frequency of being born small for gestational age (SGA) in non-dysgenetic 46,XY DSD patients without molecular diagnosis has been reported, but the underlying mechanism is unknown. The GH-IGF system is crucial for sex differentiation in mice and humans, and members of this system were detected in embryonic and fetal gonads. Moreover, *GHR* gene polymorphism (*GHRd3*) has been associated with decreased fetal growth and lower birth weight. Aims: To analyze the genotypic frequency of *GHRd3* in nonspecific 46,XY DSD according to fetal restriction and its relationship with gonadal function in minipuberty. Methods: A cohort of 46,XY DSD patients followed at a tertiary pediatric center was evaluated (n=187). SGA was defined based on gestational age. In nonspecific 46,XY DSD, *GHR* genotypes (*GHRfl* and *GHRd3*) were analyzed according to fetal restriction and compared with control subjects (n=159). Serum LH, FSH, testosterone, and AMH levels were analyzed according to fetal restriction and the genotypic variants of the *GHR* gene. Results: Molecular diagnosis was achieved in 38% of patients. SGA was found in 25.4% with a higher frequency in nonspecific 46,XY DSD ($p<0.05$). In this group, no difference was found in the frequency of *GHRd3* among SGA and non-SGA, and neither in the control group. No difference was found in minipubertal gonadal function according to *GHR* genotypes and SGA condition. Conclusions: The frequency of being born SGA in 46,XY DSD was higher in those patients with no specific disorders of undermasculinization. In this group, the allelic frequency of the *GHRd3* polymorphism was similar to controls regardless of being born SGA. Furthermore, testicular function during minipuberty seems to be unrelated to the *GHR* genotype and fetal restriction. More patients and further studies are needed to evaluate these associations, and to clarify the role of other factors involved in early embryonic growth and development, and in gonadal differentiation.

100. (113) **NUR77 AND OCT-4 CROSSTALK ON POMC TRANSCRIPTION IN CORTICOTROPH CELLS**

Josefina Rosmino[1], Mariana Fuertes[1], Eduardo Arzt[1,2]

[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society, Buenos Aires, Argentina, [2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Cushing disease is an endocrine disorder characterized by an excessive adrenocorticotropic hormone (ACTH) production by the anterior pituitary, leading to the release of an excess of cortisol from the adrenal glands. Pro-opiomelanocortin (POMC) is a precursor protein of a variety of peptide hormones including ACTH. ACTH biosynthesis is coordinately controlled by different transcription fac-

tors at Pomc promoter. Corticotropin releasing hormone (CRH), a stress mediator in the hypothalamic–pituitary–adrenal axis induces the expression of the nuclear receptors Nur77 and stimulates the transcriptional activity of Pomc promotor, leading to an increase in ACTH production. In previous experiments, using a murine pituitary corticotrope cell experimental model (AtT-20) we demonstrated, that octamer-binding transcription factor 4 (OCT-4) has a dose dependent inhibitory effect on Pomc transcription. In this work we studied the crosstalk between Nur77 and OCT-4 on Pomc transcription and its impact on hormone regulation. In AtT-20 cells by reporter assays with a complete Pomc-LUC construct, we observed that the stimulation of Nurr77 on Pomc is blocked in the presence of OCT-4, while OCT-4 continues inhibiting despite the presence of Nurr77. Using a reporter deleted of the Nurr site (PomcΔNurr-LUC) we found that OCT-4 loses completely its inhibitory action. OCT-4 action in relation to Nur77 depends on the DNA site more than the interaction with the protein. We observed that OCT-4 inhibits not only the constitutive transcription of the Pomc promoter, but also stimulated with CRH (inhibition 22%, $p< 0.05$). Furthermore, OCT-4 blocks the known inhibitory action of dexamethasone on Pomc transcription. Knowing the actions of different factors involved on the Pomc promoter allows to understand the pathophysiology of pituitary ACTH adenomas and open new posible future therapeutic strategic lines. Supported by Fundación Fiorini, ANPCyT and FOCEM (COF 03/11).

101. (114) **CONTRIBUTION OF OVARIAN FACTORS IN THE REPRODUCTIVE AXIS REACTIVATION OF PREGNANT VIZCACHAS**

Proietto S[1,2], Corso MC[1,2], Cortasa S[1,2], Schmidt AR[1,3], Di Giorgio NP[2,3], Lux-Lantos V[2,3], Vitullo AD[1,2], Dorfman VB[1,2], Halperin J[1,2]

[1]Centro de Estudios Biomédicos Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina.

[2]CONICET, Argentina.

[3]Instituto de Biología y Medicina Experimental (IByME), Buenos Aires, Argentina.

Unlike most mammals, pregnant vizcachas exhibit a reactivation of the hypothalamic-pituitary axis at mid-pregnancy showing gonadotropin and steroid hormones profiles similar to those described during the estrous cycle. In order to analyze the modulation of gonadotropin release during HHO axis reactivation, serum FSH and LH levels; ovarian FSHR and LHR; steroidogenic enzymes as 3 β-HSD, 17 β-HSD and CYP19A1; and inhibinβ-A were characterized by both Western-blot and immunohistochemistry. Serum estradiol and FSH levels were measured by ELISA and serum LH by RIA. Pregnant vizcachas ovaries before and after the reactivation of the HHO axis (P2 and P3, respectively) were used. P2 females showed higher serum FSH levels and lower LH levels in comparison to P3 females. P3 ovaries presented significant changes in the analyzed markers compare to P2, showing lower expression of FSHR and higher expression of inhibinβ-A and LHR. Inhibinβ-A immunolabeling was more noticeable in mature follicles than in corpora lutea. Regarding the expression of steroidogenic enzymes, 3 β-HSD showed no differences, meanwhile a significantly higher expression of 17 β-HSD and lower expression of CYP19A1 were observed in ovaries of P2 than that of P3. These results suggest that, during pregnancy, inhibinβ-A would control both the pituitary FSH secretion and the ovarian sensitivity to this hormone by a negative feedback mechanism and by inhibition of its ovarian receptor respectively. Furthermore, the augmented estradiol serum levels recorded in the P3 group would be related to the key role of 17 β-HSD on androgens synthesis prior axis reactivation which would be converted into estradiol by CYP19A1 after reactivation. Moreover, this would cooperate in the negative feedback on pituitary FSH release. These findings suggest that inhibinβ-A and estradiol would exert negative feedback on FSH but not on LH release during reactivation of the reproductive axis in pregnant vizcachas. FCFF, PIP11220200100036.

102. (276) **LEYDIG CELL TUMOURS: UNCOVERING THE MECHANISMS UNDERLYING CURCUMIN ANTITUMOR EFFECT**

Trinidad Raices1,2, María Luisa Varela1, Adriana María Belén Abiuso1, Elba Nora Pereyra1, Carolina Mondillo1, María Fernanda Riera2, Omar Pedro Pignataro1.

1Instituto de Biología y Medicina Experimental (IBYME-CONICET), 2Centro de Investigaciones Endocrinológicas, Hospital de Niños Ricardo Gutiérrez (CEDIE-CONICET)

Curcumin has been ascribed with countless therapeutic effects, but its impact on testicular function has been scarcely researched. Leydig cells comprise the androgen-secreting population of the testis and may give rise to Leydig cell tumours (LCT). Due to their steroid-secreting nature, LCT entail endocrine, reproductive, and psychological disorders. Approximately 10% are malignant and do not respond to chemotherapy and radiotherapy. Our group has described the antitumoral effect of curcumin in an ectopic allograft murine model of MA-10 Leydig cell tumours. The aim of this study was to assess the mechanisms underlying this effect, focusing on two of the main cancer hallmarks: angiogenesis and proliferation. Firstly, we examined curcumin's effect on MA-10 tumour cells proangiogenic potential. The cells were incubated for 24 h with 40 μM curcumin and conditioned media were obtained. The effect on angiogenesis was evaluated by the chorioallantoic membrane assay. Surprisingly, curcumin showed a stimulatory effect on MA-10 cells proangiogenic capacity. Then, we evaluated curcumin's effect on VEGF expression in MA-10 Leydig cell tumours *in vivo* through RT-qPCR. In this case, there were no differences between animals treated with vehicle and 20 mg/kg curcumin. Furthermore, having observed that curcumin arrests cell cycle progression, we analysed its effect on cell cycle regulators expression. Through RT-qPCR we observed that 60 μM curcumin stimulated p21 expression (1.5 average increase, $p<0.05$), while having a negative effect on cyclin D1 mRNA levels (0.3 average reduction, $p<0.05$). No effect was observed on p27 expression. To sum up, curcumin's antitumor effect appears not to be mediated by antiangiogenic mechanisms. Instead, it might involve an inhibitory effect on proliferation through the modulation of cell cycle regulators. Further *in vivo* and *in vitro* studies are necessary to complete the description of curcumin's effects on Leydig cell tumours.

103. (428) **EFFECTS OF ENDOCRINE DISRUPTORS ON AUTOPHAGY MARKERS GENE EXPRESSION IN MATURE AND IMMATURE GNRH NEURONS**

Juan Riaño Gómez, Eleonora Sorianello, Victoria Lux-Lantos y Marina Fernandez.

Instituto de Biología y Medicina Experimental

Email: juanrianog@gmail.com

Benzophenones (BP) and Bisphenol A (BPA) are endocrine disrupting chemicals (EDC). Previously, we showed that the in-vitro exposure to EDC inhibited Kiss-induced GnRH gene expression, and altered expression of inflammatory markers in GT1-7 and GN11 cells (mature and immature GnRH neurons, donated by Dr. Mellon, UCSD). In this study, the effects of the in-vitro exposure, for 12 or 24 hours, to BPA, BP2 or BP3 ($1x10^{-9}$M, Sigma), or DMSO as control, were evaluated in GT1-7 and GN11 cells. RNA was obtained (Tri-Reagent, Molecular Research Center, Inc), reverse-transcribed, and gene expression was analyzed by qPCR, using *Ppib* as control gene. Results were presented as Media±SE and analyzed by ANOVA (Statistica, StatSoft Inc, USA). In GT1-7 cells, *p62* expression was significantly increased by 12-h exposure to BP2 and BP3 [DMSO-24h: 1.19±0.02, DMSO-12h: 0.95± 0.09, BPA-24h: 0.61±0.21, BPA-12h: 0.55±0.16, BP2-24h: 0.79±0.39, BP2-12h: 2.69±0.39, BP3-24h: 0.61±0.24, BP3-12h: 2.67±0.95, Repeated Measures ANOVA: $p<0.05$, BP2-12h and BP3-12h vs DMSO-12h, $p<0.05$, n=4]. *Lamp2* expression was increased after 24 h exposure to BP2 [DMSO-24h: 1.18±0.06, DMSO-12h: 1.25± 0.12, BPA-24h: 2.68±0.45, BPA-12h: 2.43±0.79, BP2-24h: 4.44±1.31, BP2-12h: 1.84±0.48, BP3-24h: 1.62±0.15, BP3-12h: 2.35±0.19, Repeated Measures ANOVA: $p<0.05$, BP2-24h vs DMSO-24h, $p<0.05$, n=4]. *Ulk1* gene expression was increased after 12 and 24 h exposure to BP3 in GN11 cells [DMSO-24h: 0.93±0.21, DMSO-12h: 0.73± 0.19, BPA-24h: 1.78±0.31, BPA-12h: 1.47±0.33, BP2-24h: 1.51±0.49, BP2-12h: 1.06±0.23, BP3-24h: 2.41±0.89, BP3-12h: 2.45±0.82, Repeated Measures ANOVA: Main effect "TREATMENT" $p<0.05$, BP3

vs DMSO, p<0.05, n=4]. Our results reinforce the notion that the exposure to EDC have different effects depending on the experimental model and time of exposure, with developing cells being more susceptible to disruption that mature cells. Funding: CONICET, ANPCYT, F. Williams, F. R. Barón.

104. (497) **FILAMIN A MODULATES CELL GROWTH AND EGFR/ERK SIGNALING IN PITUITARY TUMORAL CELLS**

Jonathan Toledo [1,2], Pablo Aníbal Pérez[1,2], Ana Lucía De Paul[1,2], Silvina Gutiérrez [1,2]

1. Centro de Microscopia Electrónica, Facultad de Ciencias Médicas, UNC.

2. Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET).

Filamin A (FLNA) interacts with signaling molecules and membrane receptors to affect cell signal transduction and function. Epidermal Growth Factor Receptor (EGFR) is a major player modulating cell proliferation and hormone secretion in pituitary tumors, however, whether FLNA regulates EGFR expression affecting pituitary tumoral growth remains to be elucidated.The aim of this study was to evaluate the impact of FLNA on proliferation, viability and hormone secretion in tumoral pituitary cells and establish if these alterations are mediated by EGFR signaling. Lactosomatotrophic GH3 cells were transfected for FLNA overexpression (GH3F+) and stimulated for 5, 10, 30 min and 24h with EGF (10 ng/ml). Protein expression was analyzed by western blot and immunocitochemistry (ICC) and quantified by flow cytometry, protein subcellular localization was observed by indirect immunofluorescence (IFI), cell cycle progression was determined by cellular DNA content with flow cytometry and cell viability was analyzed by number of colonies formed in a clonogenic assay. The statistical analysis used was ANOVA-Tukey. FLNA overexpression induced a significant decreased of Ki67-labelled cells, reduced S/G2-M cell percentage, decreased cell viability, and inhibited PRL secretion. In order to elucidate the mechanism through FLNA overexpression impacts on these parameters, we evaluate membrane EGFR expression and EGF-induced signaling pathway activation. Plasmatic membrane EGFR was significantly reduced under FLNA overexpression in GH3 cells, with further decrease with EGF stimulation. Also, a consequent declined in ERK1/2 phosphorilation in response to EGF was observed in GH3F+ vs. GH3. The results showed that FLNA overexpression decreased lactosomatotroph cell growth and membrane EGFR localization and activity, suggesting that FLNA may inhibit EGF-induced cell proliferation in pituitary tumor cells.

105. (533) **UTERINE ENDOCRINE ENVIRONMENT IN A RAT PCOS MODEL**

María Virginia Acosta, Gisela Soledad Bracho, Gabriela Anahí Altamirano, Laura Kass, Verónica Lis Bosquiazzo

Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

This study aimed to investigate whether the development of lesions found in the uterus of rats with polycystic ovary syndrome (PCOS) is associated with changes in the local endocrine environment. Female Wistar rats were treated subcutaneously with sesame oil (CONTROL) or dehydroepiandrosterone 6mg/100g of body weight (PCOS) from 21 to 40 days of age. After 24 hours, blood and uterine horns were collected. To analyze the uterine endocrine environment in these animals, the levels of steroid hormones [estrone (E1), estradiol (E2), progesterone (P4), and testosterone (T)] were measured in uterine tissue and serum. Also, the main steroidogenic proteins [steroidogenic acute regulatory protein (StAR), 17b-hydroxysteroid dehydrogenase isoform 2 (17b-HSD2), 5a-reductase isoform 1 (SRD5A1) and aromatase (P450arom)] and the expression of estrogen alpha (ESR1), progesterone (PR) and androgen (AR) receptors were studied in the uterus. The PCOS group showed no difference in serum levels of E2 and P4 compared to CONTROL, however, an increase in T levels was observed. In uterine tissue, E1, E2, and P4 were detected in all CONTROL animals. In the PCOS group, E2 was detected in 100%, E1 in 75%, and P4 in only 50% of the rats. Notably, T was detected in 66.7% of CONTROL, however was not found in PCOS animals. In addition, we observed decreased mRNA levels of StAR and increased expression of 17b-HSD2, SRD5A1, and P450arom in the uterus of PCOS rats. Regarding steroid receptors, only AR expression was modified, increasing in the subepithelial stroma and myometrium of the PCOS group. Our results suggest that, in the uterus of PCOS rats, androgens and estrogens come from systemic hormonal metabolism, not from *de novo* synthesis since StAR was decreased. Also, the undetectable T levels, the increase in P450arom, together with a lower P4 detection, suggest that P4 would not sufficiently counteract estrogenic stimulation, which could promote the development of uterine lesions.

106. (536) **EFFECT OF CLIMATE FACTORS ON THE REPRODUCTIVE ENDOCRINOLOGY. A STUDY IN THE FEMALE PLAINS VIZCACHA, *LAGOSTOMUS MAXIMUS***

Beraldo Pissinis Priscila Stephanie[1,4], Proietto Sofìa[1,4], De Winne Catalina[3,4], Calcagno Javier[2,4], Corso Marìa Clara[1,4], Schmidt Alejandro Raúl[1,4], Becu-Villalobos Damasia[3,4], Vitullo Alfredo Daniel[1,4], Halperin Julia[1,4], Dorfman Verónica Berta[1,4].

[1] Laboratorio de Neuroendocrinología de la Reproducción, Centro de Estudios Biomédicos Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides

[2] Laboratorio de Biología Marina, Centro de Ciencias Naturales, Ambientales y Antropológicas (CCNAA), Universidad Maimónides

[3] Laboratorio de Regulación Hipofisaria, Instituto de Biología y Medicina Experimental (IByME) – CONICET.

[4] CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas.

Variations in climate factors affect reproductive conditions of species, being seasonal breeding a reproductive strategy among mammals. The South American plains vizcacha shows seasonal breeding, with two gestation periods per year (150-days long) and pseudo-ovulation at mid-pregnancy. The aim was to study the influence of climate factors on the endocrinology of the female vizcacha. We developed a retrospective study using stored serum and ovaries of animals that were captured between 2008 and 2019. The study was focused on the two non-pregnancy months (March, n=14; September, n=18) and on the pseudo-ovulatory period of gestation (June-July, n=32). Using Principal Components Analysis, we determined the years between 2008 and 2019 with extreme temperature, rainfall and humidity conditions. Serum luteinizing hormone (LH) was studied by RIA, progesterone (P4) and estradiol (E2) by ELISA, and the ovary dynamics was evaluated in H&E stained ovary sections. t-Test for non-parametric values was used to determine significant differences between groups. In March, a significant decrease in P4 and E2 was determined in the hottest and highest rainfall years (HHR years) compared to the coldest and least rainfall years, and that was correlated with a significant decrease in the antral follicles and accessory corpora lutea (CL) density (p<0.03). Similar results were obtained in September, with a significant decrease in P4, E2, and LH in the HHR years, and correlated with a significant decrease in the primary CL density (p<0.03). Finally, during the pseudo-ovulatory period of gestation, a significant decrease in LH was determined in the coldest and highest rainfall years (p<0.005), without significant effects on the ovary dynamics. These results suggest that both pituitary activity and follicular development are favored by low rainfall and temperatures; however, during pregnancy endocrinology is not so affected by climate conditions (FCFF and PIP 11220200100036CO).

107. (625) **THE EPIGENETIC EZH2/H3K27me3 AXIS MODULATES LACTOTROPH TUMOUR CELL PROLIFERATION**

Natacha Zlocowski, Liliana del Valle Sosa, Carolina Beatriz Guido, Jorge Humberto Mukdsi, Alicia Inés Torres, Juan Pablo Petiti.

Centro de Microscopía Electrónica, INICSA-CONICET-Facultad de Ciencias Médicas de la Universidad Nacional de Córdoba.

Pituitary Neuroendocrine tumours (PitNETs) genes could be inactivated via epigenetic modifications, suggesting an important role

of epigenetic regulatory factors in PitNET tumorigenesis. However, the inhibition of these factors in order to propose new therapeutic strategies remain poorly understood. The objective of the present study was to examine the participation of the EZH2/H3K27me3 axis in the proliferation of lactotroph tumour cells. The experimental prolactin (PRL) tumour model in adult Fisher rats and the pituitary tumoral PRL-secreting cell GH3 were used. EZH2 and H3K27me3 expression was analysed by Western Blot and Immunohistochemistry. The H3K27me3/PRL co-expression was performed by immunofluorescence and immunoelectron labelling. mRNA levels of *p21, p53, Cdk4 and Cyclin D1* were determined by qPCR. GH3 cell line was treated with specific EZH2 inhibitor GSK343 (5-10uM) for 72h and then MTT assay and BrdU uptake were carried out. Statistic: ANOVA-Tukey or t-test. EZH2 and H3K27me3 expression increased with a higher number of positive nuclear marks, in experimental PRL tumours respect to control pituitaries. In addition, H3K27me3/PRL co-expression increased in tumoral glands respect to normal glands ($p<0.05$). In contrast, the *p21* mRNA levels significant decreased, whereas the mRNA levels of *p53, Cdk4* and *Cyclin D1* did not reveal any significant differences ($p<0.05$) in PRL tumour vs normal glands. *In vitro* assays revealed that the EZH2/H3K27me3 axis inhibition by GSK343 significantly decreased the cell viability and BrdU uptake in GH3 tumour cells ($p<0.05$). These is the first time that H3K27me3 is studied on a prolactinoma context and these findings suggest that an increase of EZH2 and H3K27me3 levels on prolactin tumour have a direct impact on cellular proliferation, whereby the EZH2/H3K27me3 axis could be an attractive therapeutic target for prolactin PitNETs.

108. (664) **EVIDENCE OF DAMAGE RESPONSE ACTIVATION IN HUMAN PITUITARY NEUROENDOCRINE TUMOURS**

[1]Fernández S; [1]Bertetti C; [1]Venier AC; [2]Bernhardt C; [3]Pautasso M; [1]Sosa LdV; [1]Gutiérrez S;
[1]Grondona E; [1]De Paul AL.
[1]Centro de Microscopia Electrónica, Facultad de Ciencias Médicas. INICSA-CONICET-UNC.
[2]Servicio de Patología, Clínica Universitaria Reina Fabiola, Córdoba.
[3]Servicio de Endocrinología, Clínica Universitaria Reina Fabiola, Córdoba.

Senescence is considered to be a stress response characterized by permanent cell cycle arrest, which can be triggered by different factors including DNA damage, oxidative stress. This program may constitute a plausible explanation for the benign nature of pituitary neuroendocrine tumours (PitNETs). We analysed the oxidative stress response activation, as part of the senescent process, in human somatotroph and lactotroph PitNETs. Samples from female and male patients (n=15) were supplied by Clínica Reina Fabiola and Sanatorio Allende, Córdoba. Clinical records and GH, PRL and Ki-67 immunostaining were also provided. The 8-hydroxy-2'-deoxyguanosine (8OhdG) expression, a marker of oxidative damage in DNA, and Nuclear factor erythroid 2-related factor 2 (Nrf2), indicative of antioxidant response activation, were evaluated by immunohistochemistry. Five randomly chosen fields were selected (400x) and 1000 cells were counted. Data are expressed as percentages of nuclear 8OdHG positive cells in relation to total cell number. The cytoplasmic Nrf2 expression intensity was classified as weak/strong and focal/diffuse regarding its distribution. Statics analysis: t-Test.
In somatotroph PitNETs, the positive cell number exhibiting 8OhdG nuclear expression was significantly higher when compared to lactotroph PitNETs (13.6% vs 5.4% respectively). Furthermore, in the first group, the cytosolic Nrf2 expression was predominantly strong and diffuse whereas in lactotroph PitNETs this signal showed a pattern exclusively weak and focal. In somatotroph and lactotroph PitNETs, there would be a disparity in the cellular stress response. Differences in marker expressions detected in human PitNETs expose the specificity of this cellular phenomenon to the tumour subtype. The highest antioxidant response, detected somatotrophs PitNET, to face the DNA oxidative damage, may suggest that cellular senescence could be a significant mediator to avoid unregulated cell proliferation in these tumours.

109. (756) **"GESTATIONAL AND LACTATIONAL EXPOSURE TO DEHP ALTERS PITUITARY ANDROGEN RECEPTOR EXPRESSION AND GONADOTROPH CELL GROWTH"**

Pablo A. Pérez[12], Jonathan Toledo[12], Amado A. Quintar[12], Ana L. De Paul[12], Silvina Gutiérrez[12]
1. Instituto de investigaciones en Ciencias de la Salud (INICSA-CONICET).
2. Centro de microscopía electrónica. Facultad de Ciencias Médicas. Universidad Nacional de Córdoba. Argentina.

Di-(2-ethylhexyl)phthalate (DEHP), an endocrine disruptor used in a variety of commercial products, crosses the placental barrier, causing reproductive alterations. The impact on pituitary gland, and in particular, the molecular mechanisms involved are poorly known. We focused on the DEHP effects on pituitary androgen receptor (AR) expression in male and female rats, and explored their influence on gonadotroph cell growth. For *in vivo* model, pregnant rats were assigned to 2 groups: DEHP (200 μg/kg body weight/day) and control (corn oil), administrated by gavage until weaning. The offspring were euthanized at postnatal day (PND) 21 (prepubertal) or PND75 (adult) and the pituitary glands were used in this study.
For *in vitro* model, primary pituitary cell cultures were stimulated for 72 h with DEHP (1, 10 or 100 nM). The percentage of LH positive cells was quantified by flow cytometry (FC) and the gonadotroph cell proliferation was determined by double immunostaining for LH and Ki67. ANOVA Fisher ($P<0.05$). In male rats, DEHP perinatal exposure induced a significant increase of AR expression at PND21 (78.6±1.5% DEHP *vs.* 54.8±7.8% controls), and at PND75 (50.3±4.3% DEHP *vs.* 37.81±3.34% controls). In contrast, in female rats DEHP increased pituitary cells expressing AR at PND21 (74.6±1.8% DEHP *vs.* 45±5.2% control), but decreased it in the adulthood (26.4±4.5% DEHP *vs.* 44±4.3% controls). The gonadotroph cell percentage was significantly decreased in the exposed adult female rats (8.9±1.2%) *vs.* control (13±1.2), showing no differences in adult male rats. Finally, DEHP *in vitro* significantly decreased LH-Ki67 cells in male and female pituitary cultures. These results showed that DEHP exposure during developmental stages induces changes in pituitary AR expression in a sex dependent manner, differentially manifested in the adulthood, affecting the gonadotroph proliferation. These findings contribute to understand the impact of environmental contaminants on the pituitary gland.

110. (759) **MODULATION OF THE ANTI-PROLIFERATIVE EFFECT OF OCTREOTIDE IN SOMATOTROPH TUMORS. ROLE OF FGFR4 AND SHP2**

Facundo Garcia Barberá1, Liliana Sosa1, Florencia Picech1, Juan De Batista2, Laura Cecenarro1, Jorge Mukdsi1, Juan Pablo Petiti1
1Centro de Microscopía Electrónica-INICSA-CONICET. Facultad de Medicina-Universidad Nacional de Córdoba, Córdoba-Argentina. 2Hospital Privado Universitario de Córdoba, Córdoba-Argentina

Octreotide (OCT), somatostatin analog that binds with high affinity to receptor SSTR2, is widely used to inhibit GH secretion and cell proliferation in GH-secreting tumors. It has been reported that the phosphatase SHP2 is a key mediator in the signal triggered by SSTR2 and FGFR4, but its role in pituitary tumors is still unknown. The objective was to analyze whether the anti-proliferative effect of OCT is modulated by SHP2 and FGFR4. We determined the protein expression of SHP2 and FGFR4 in 39 PitNETs (12 GH-,3 PRL-, 7 ACTH-secreting and 17 non-functioning tumors) and in human silent GH tumors developed in nude mice treated with OCT for 11d by IHC or/and WB. GH3 cells were treated with OCT (100 nM), SHP2 inhibitor SHP099 (15 μM), and FGFR4 inhibitors Blu99931 or Roblitinib (50-100 nM). Protein levels of pSTAT3 and p-ERK 1/2 were determined by WB. Localization of pSTAT3 and FGFR4 were analyzed by IF and cell viability by MTT assay. Statics: ANOVA (Fisher) or t-test. In GH- tumors we observed a higher SHP2 expression in associated with high ki67% without remarkable FGFR4 expression in comparison to other tumors subtypes. The *in vivo* assays showed that the anti-proliferative effect of OCT was associated with a decrease of SHP2 expression and increase of FGFR4 and pERK1/2.

In GH3 cells, the treatment with SHP099 decreased the cell viability, increasing STAT3 phosphorylation and nuclear translocation. Both FGFR4 inhibitors reduced significantly the cell viability and pSTAT3 levels, and the OCT effect was potentiated in presence of Blu99931. These results indicate that SHP2 and FGFR4 inhibition are important for strengthening the antiproliferative effects of OCT, being STAT3 a molecular hub integrating the receptors signaling pathways, possibly being part of a counterbalance mechanisms.

111. (854) **AGE-DEPENDENT AND SEX-SPECIFIC CHANGES OF DNA METHYLATION WITHIN GROWTH HORMONE RECEPTOR (GHR), INSULIN-LIKE GROWTH FACTOR TYPE 1 RECEPTOR (IGF1R) AND INSULIN RECEPTOR (IR) GENE PROMOTERS IN PERIPHERAL BLOOD LEUKOCYTES FROM HEALTHY PREPUBERTAL AND PUBERTAL CHILDREN**

Luciana Zoff1,2, Francisco M Garcia2, María Florencia Fernandez2, Aschettino Giovanna2, Gabriel Veneruzzo2, Carolina Pepe2, Celeste Mattone1,2, Natalia Perez Garrido2, Matías Juanes1,2, Nora Saraco1,2, Cristina Alonso2, Gabriela Guercio1,2, Alicia Belgorosky1,2, María Sonia Baquedano1,2.

1 CONICET

2 Hospital de Pediatría Garrahan

Growth proceeds in a predictable pattern characterized by a constant growth deceleration between childhood and adolescence. This growth deceleration is coordinated in tissues and organs to maintain body proportions. GH/IGF1 axis and insulin are crucial growth stimulators. However, their levels do not change in a pattern that would explain the prepubertal decline in growth rate. We hypothesize that the postnatal growth pattern could be orchestrated by epigenetic mechanisms. To analyze age-related physiological changes, we evaluated promoter methylation in the GHR, IGF1R and INSR genes in peripheral blood from 40 healthy children (females (F) n=21 and males (M) n=19), between 3 and 15 years by targeted deep-amplicon bisulfite sequencing. 4 promoter regions for GHR, 3 for IGF1R and 5 for INSR (104, 103 and 141 CpG sites, respectively) were analyzed for mean methylation values of all CpGs. Results suggest a sexual dimorphism in the epigenetic regulation of GHR, IGF1R and INSR gene expression. A significant negative correlation between GHR mean methylation and age was observed in both sexes, but in different promoter regions. Specifically, a decrease in mean methylation levels with age were detected in GHRP1P2 amplicon (p=0.04) and in 3 of its CpGs (CpG+183, CpG+243 and CpG+304, p<0.05) in F, and in the GHRP3 amplicon (p=0,03) including CpG+343, CpG+391 and CpG+487 (p<0.05) in M. In contrast, in IGF1R, a positive correlation was observed exclusively in F, between mean methylation levels and age for the IGF1R PR3 amplicon (p=0.039), as well as in CpG-51, CpG-47, CpG-43, CpG-41, CpG-23, CpG-17, CpG-15, CpG-13, CpG-11, and CpG-9 (p<0.05). No significant changes were detected in INSR methylation except for a male-specific positive correlation between methylation values in some specific CpGs and age (CpG-768, CpG-567, CpG-431, CpG+712, CpG+770 and CpG+784, p<0.05). Our findings are in agreement with the hypothesis that age-associated DNA promoter methylation changes could be involved in the physiological growth pattern. Finally, the observed sexual dimorphism suggests age- and sex-dependent regulatory mechanisms for normal growth.

112. (877) **FROM INDUCED PLURIPOTENT STEM CELLS TO LHX3+ PITUITARY CELLS: A PROMISING DIFFERENTIATION PROTOCOL FOR THE STUDY OF HORMONAL DEFICIENCIES OF GENETIC ORIGIN**

Chirino Felker Gonzalo Tomas[2], Romano Florit Carolina[2], Amin Guadalupe[2], Castañeda Sheila[2], Waisman Ariel[2], Miriuka Santiago Gabriel[2], Perez Millan María Inés[1], Moro Lucia Natalia[2], Camilletti María Andrea[1,2]

1 Instituto de Biociencias, Biotecnología y Biología translacional (iB3), Depto Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales (FBMC, FCEyN-UBA-CONICET) 2 Laboratorio de Investigación Aplicada a Neurociencias (LIAN), Instituto de Neurociencias (INEU, FLENI-CONICET)

Generation of patient-derived pituitary cells is a promising approach in modeling hormonal deficiencies. Since their discovery,human induced pluripotent stem cells (iPSCs) obtained by cell reprogramming of adult somatic cells,have revolutionized biomedical research. To date,only two approaches (two and three-dimensional) have been published for obtaining functional secretory pituitary cells from iPSCs with an efficiency of about 20-30%. In this study,we adapted the two-dimensional protocol for differentiation of iPSCs to pituitary cells. Our previous results showed that culturing iPSCs in a cocktail of morphogens (BMP4, SAG and bFGF) induces increased expression of pituitary-specific genes and loss of pluripotency markers after 15 days compared to control iPSCs (without stimulation). Based on these findings, iPSCs cultures were incubated for additional days (up to 40 days) and samples corresponding to several days (0,4,15,30 and 40) were collected for their characterization by qRT-PCR and immunostaining. We observed a robust up-regulation of pituitary-like progenitor markers (*PITX1,PITX2* and SIX6) and pituitary placode marker *SIX1,*accompanied by a downregulation of pluripotency markers *OCT4* and *NANOG,* in treated cultures compared to control iPSCs,from day 4 to 40 (N=4). Transcript levels of early pituitary markers *HESX1* and *OTX2* were found to increase at day 4 and then decreased by day 15. Induction of other cell lineages was evaluated, however,no differences were found among the cultures,as determined by mesoderm (*TBXT*,*TBX6*),ectoderm (*NESS*,*TUBB3*) and endoderm (*SOX17*) lineage markers expression. Finally,immunodetection of the transcription factor LIM Homeobox 3 (LHX3),an early marker of pituitary lineage committed cells,revealed nuclear LHX3 expression in clusters in treated iPSCs at day 40. In summary,our findings suggest this methodology can be used to obtain pituitary progenitor LHX3+ cells *in vitro* from iPSCs,and has the potential to,in the near future,serve as a model for human pituitary development. This is particularly relevant for hormonal deficiencies of genetic origin,as it will provide a possible tool to evaluate variants in early genes involved in the formation of the anterior pituitary gland.

ENDOCRINOLOGY II

Saturday, November 19, 9-10:30 hr

Chairs: Fabiana Cornejo Maciel - Maria Sonia Baquedano - Andrés Giovambattista

113. (8) **PROTECTIVE EFFECT OF NARINGIN AGAINST BONE ALTERATIONS CAUSED BY THE METABOLIC SYNDROME AND STRESS**

Valeria Andrea Rodríguez[1], María Angélica Rivoira[1], María Angélica Rivarola[2], Gabriela Picotto[1], Gabriela Díaz de Barboza[1], Lucas Brun[3], Franco Mir[2], Marta Suárez[2], Nori Tolosa de Talamoni[1]

[1]Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-UNC), [2]Cátedra de Fisiología Animal, FCEFyN, INICSA (CONICET-UNC), [3]Laboratorio de Biología Ósea, Facultad de Ciencias Médicas, UNR, Argentina

We evaluated the effect of Naringin (NAR) on the alterations in long bones caused by a high-fructose diet (HFD) in rats exposed or not to environmental chronic stress (CS). Male Wistar rats were divided: 1) Rats with metabolic syndrome (MS): fed 10% HFD (w/v) in drinking water for 30 days, 2) MS rats exposed to CS, 3) MS rats treated with NAR (40 mg NAR/kg b.w.) and 4) rats with MS and CS treated with NAR. CS was variable, random, and unpredictable. Systemic biochemical parameters, body weight and waist circumference were measured. Histomorphometric parameters were measured in the proximal tibia. Bone mineral density (BMD) was determined in femur and tibia. Oxidative stress in femur bone marrow was evaluated. Adipocytes and osteocytes were counted in tibia slices. ANOVA/Bonferroni was used for statistical analysis. MS+CS rats had lower body weight and higher serum corticosterone levels compared to MS rats; NAR treatment normalized these parameters. Serum osteocalcin and proximal tibial bone volume were lower in MS rats compared to the other groups. MS+CS rats showed an increase in trabecular separation. NAR treatment increased trabecular thickness in both

MS and MS+CS rats, and prevented increased trabecular separation in MS+CS rats. Tibia BMD of MS rats was lower than that of the other groups. NAR increased femur BMD in MS and MS+CS rats. Stress increased the number of adipocytes in tibias of MS rats. NAR decreased adipocytes in MS rats and in MS+CS rats. The number of osteocytes was lower in MS rats compared to the other groups. In bone marrow of MS+CS rats, total GSH content was lower than that of MS rats. NAR increased total GSH and decreased $\dot{O}_2^-$ content in MS and MS+CS rats. Catalase activity in MS rats was lower compared to the other groups. In conclusion, NAR treatment partially prevents bone deterioration and oxidative stress in bone marrow caused by MS in the absence and presence of CS.

114. (9) **COMBINED METFORMIN AND NARINGIN REVERSES THE METABOLIC ALTERATIONS OF THE NONALCOHOLIC FATTY LIVER DISEASE IN EXPERIMENTAL METABOLIC SYNDROME**

María Agustina Rizzi[1], Tamara Magalí Mazo[2], María Eugenia Pasqualini[2], Nori Tolosa de Talamoni[1], Valeria Andrea Rodríguez[1]

[1]Laboratorio "Dr. Cañas", Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba);[2] Biología Celular, Histología y Embriología, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba)

The fructose rich diet (FRD) causes metabolic alterations that resemble metabolic syndrome (MS), which are associated with non-alcoholic fatty liver disease (NAFLD). Metformin (Met), is a medicine of first line for the treatment of insulin resistance associated with MS. However, it presents adverse reactions and side effects. Naringin (NAR) is a flavonoid with antioxidant, antiapoptotic and anti-inflammatory properties. The aim of this work was to know the effect of Met+NAR on the metabolic systemic variables and histological alterations in the liver of animals with MS. Male Wistar rats were divided in 5 groups: 1) controls; 2) rats fed FRD 10% (w/v) in drinking water for 60 days; 3) FRD rats treated with Met (100 mg/kg b.w.); 4) FRD rats treated with NAR (40 mg/kg b.w.); 5) FRD rats treated with Met+NAR. Treatments started from day 21 of FRD administration. Body weight, waist circumference and biochemical parameters were measured. The adiposity and hepatosomatic index (HSI), and histomorphometric studies were accomplished in liver. ANOVA/ Bonferroni was used for statistical analysis. The body weight and waist circumference s were significantly higher in FRD rats compared to those from the control rats, while all treatments decreased waist circumference. Adiposity and HSI increased in FRD rats as compared to those from control rats; however, they were reversed with Met+NAR. The increase in the serum values of triglycerides and AST of FRD rats was completely abrogated by NAR and Met+NAR treatments. FRD rats showed ballooning of hepatocytes with steatosis and dilatation of the portal space and fibrosis. Only the combined treatment reversed these alterations. In conclusion, FRD causes systemic metabolic changes, and body and histological alterations. Since the combined treatment reverses the biochemical parameters and histological alterations as well as the liver fibrosis, it constitutes a new therapeutic alternative for NAFLD.

115. (62) **MORPHOLOGICAL ALTERATIONS IN THE ADULT FROG, GENERATED BY EXPOSURE TO NITRATES AS ENDOCRINE DISRUPTORS DURING HER LARVAL DEVELOPMENT**

Modarelli María Fernanda, Bilbao Rodrigo Miguel, Ponzo Osvaldo Juan.

Endocrinology Laboratory, Academic Unit 2, Department of Physiology, Faculty of Medicine, UBA.

The exposure of amphibians to endocrine disruptors during metamorphosis process affects the thyroid axis, conducing to morphological alterations of the larvae. Objectives: evaluate the consequences of exposure to nitrates as endocrine disruptor, during the larval stage of Xenopus Laevis, on the morphology of the adult frog. Materials and method: Xenopus Laevis larvae at T: 22ºC ± 2ºC, pH: 7 to 7.8 and cycle light-dark: 12h-12h, immersed in: a) filtered potable water (control group: C, n=6), b) ground water with nitrate at concentration of 40-83 mg/l (exposed: E, n=6) and c) filtered potable water plus 0.007 mg/l potassium perchlorate (positive control: PC, n=6). Histological and western blot analyses were performed. Statistical method: ANOVA with Tukey Kramer and Bonferroni post test. Results: during 58NF/60NF stage of metamorphosis the length of the hind legs was C=E>PC ($p<0.01$) and in stage 62NF was C>E<CP ($p<0.001$). In turn, during stages 58NF/60NF, glandular hyperplasia grade 2 was observed in E and grade 3 in PC ($p<0.001$); in stage 62NF grade 3 in E and PC. Also it was observed hypertrophy of the follicular epithelium E>C>PC ($p<0.001$) in 58NF, and PC>C>E ($p<0.001$) in 60NF; vacuolization degree of colloid E>C=PC only in stage 58NF ($p<0.05$). The NIS transporter expression in 58/60NF stages increased E>PC>C ($p<0.05$). However, at stage 62NF NIS transporter expression decreased C>PC>E ($p<0.05$). At stage 66NF the size of the young frog and the length of the hind legs were: E<C ($p<0.0001$). Two-year follow-up of these larvae showed morphological alterations in eyes, mouth, spine and lower limbs in 85.7% of the adult female frogs ($p<0.0001$). Nevertheless, in adult male frogs there were no morphological alterations observed. Conclusion: amphibians exposed during their larval stage to the endocrine disrupting action of nitrates, generate malformations in adult female frogs. Being these events associated with thyroid alteration and perhaps with apoptosis mechanism.

116. (163) **ESTROGENS PROTECT UTERINE AGAINST OXIDATIVE STRESS INDUCED BY HYPERGLYCEMIA AND OBESITY**

María Ivone Valle, Adrián Campelo, Virginia Massheimer

Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), CONICET-UNS, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.

Obesity and diabetes prompt oxidative stress and an inflammatory environment that alter the homeostasis of several tissues, including uterine tissue. Estrogens are the main regulators of the endometrial function through the interaction with estrogen receptors. In this work we studied the effect of estradiol (E_2) and estrone (E_1) on the uterine oxidative stress induced by hyperglycemia; inflammation or obesity. To that end uterine slices isolated from female bilaterally ovariectomized (OVX) obese (Ob) and non-obese (nOb) Wistar rats were in vitro incubated with E_2 or E_1 in the presence of high glucose (25mM) or the pro-inflammatory agent LPS (5μg/mL). Oxidative-non oxidative stress was determined measuring the release to the incubation medium of the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2, fluorescence assay) or nitric oxide (NO, Griess technique) respectively. Short interval treatment (10-20 min) with E_2 or E_1 (0.1-10 nM) increased NO production (2.20 ±0.22; 4.3 ± 0.64; 3.2 ±0.45 nmolNO/mg prot E_2; E_1; control, $p< 0.01$); effect detected either in young and adult OVX rats (3-14 month old age). The stimulatory action of both estrogens on NO synthesis was also evidenced in Ob rats (38; 33% a/c E_2; E_1 $p< 0.05$). H_2O_2 measurements showed that diabetic rats exhibited a 0.6 fold increase in ROS production respect to control group. Similar results were obtained in Ob rats (2656 ±632 vs 1595±287 nmol H_2O_2/mg prot, Ob vs nOb, $p< 0.02$). When uterine slices were exposed to E_2 in the presence of LPS, a markedly reduction in ROS synthesis induced by LPS was observed (50 vs 12 % a/c, LPS vs LPS+E_2, $p< 0.05$). Indeed, the steroid completely blunted the enhancement of H_2O_2 production induced by high glucose. Interestingly, E_1 elicited similar action than E_2. The results presented suggest that estrogens counteracted uterine oxidative stress induced by diabetes or obesity. A point to highlight is that the forgotten estrone exhibits comparable action than estradiol.

117. (209) **THERMOGENIC REGULATION OF BROWN ADIPOSE TISSUE IN RESPONSE TO HIGH FAT DIET OR COLD STRESS IS AFFECTED BY HYPERPROLACTINEMIA**

Pascual Florencia[1], De Winne Catalina [1], Lopez Vicchi Felicitas[1], Ornstein Ana Maria[1], Toneatto Judith[1], Sorianello Eleonora[1], Becu-Villalobos, Damasia[1]

[1] Instituto de Biología y Medicina Experimental, CONICET, Vuelta de Obligado 2490. Buenos Aires 1428 Argentina

In addition to its role in lactation and reproduction, prolactin has a crucial impact on many aspects of metabolism acting on key players involved in the control of energy balance, such as adipose tissue. However, the association between high prolactin levels and brown adipose tissue thermogenesis and autophagy regulation has not been studied in detail. We used LacDrd2KO females with lifelong severe hyperprolactinemia, due dopamine-D2 receptor deletion from lactotropes, and their respective controls (*Drd2*$^{loxP/loxP}$). The first group of mice was fed with a High Fat (HFD) or a Control Diet (CD) for 8 weeks. A second group (10 month-old) was challenged with a 4 hour exposure to 4ºC. At the end of the protocols brown adipose tissue was excised and weighed. Histochemical analysis was performed and thermogenic, lipogenic and autophagic markers were studied, using real time PCR *(Ucp1, Cidea, Pgc1a, Lpl, Adiponectin*, *Prlr, Ulk1*, *Beclin*, *Lamp2*) or immunohistochemistry (UCP1). We found that chronic high prolactin levels aggravate body weight gain and metabolic effects induced by HFD. Furthermore, hyperprolactinemia affected brown adipose tissue accretion and function. Marked tissue whitening and a pronounced decrease in the levels of expression of thermogenic markers *Ucp1*, *Pgc1a* and the angiogenic marker *Vegf* were observed, regardless of the diet. Interestingly there was a marked tendency for increased expression of the inflammatory marker *Il1b* and a decreased expression of the autophagic marker *Ulk1* in BAT of the hyperprolactinemic mice. A 4-hour cold exposure (4ºC) increased the thermogenic markers *Ucp1*, *Pgc1a*, and *Vegf* and decreased *Cidea* expression levels in BAT of control animals. Conversely, in hyperprolactinemic mice there was an impairment in *Pgc1a* and *Vegf* cold-induced increase, and *Ucp1* levels were lower in this genotype. Our results show that pathological hyperprolactinemia has a strong impact in brown adipose tissue. In two opposite settings of BAT activation high prolactin levels have a concordant effect, lowering the thermogenic capacity of BAT.

118. (349) **CONGENITAL HYPOTHYROIDISM IS ASSOCIATED WITH STRUCTURAL AND FUNCTIONAL HEART ALTERATIONS IN MALE SPRAGUE-DAWLEY RATS DURING ADULTHOOD**

Candela Martinez[1,2], Valentina D'Angelo [1,2], Rocío Tavernise[1,2], Sandra Hope[1,2], Noelia Arreche[1,2], Mónica Navarro[1,2], Celeste Ferrari[1,2], Ana María Balaszczuk[1,2], Andrea Fellet[1,2]
[1] Department of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.[2] IQUIMEFA, CONICET.

The goal of this study was to investigate whether congenital hypothyroidism induced by methimazole during three different stages of life (intrauterine period, lactation and perinatal) would affect the growth of animals as well as cardiac function. In this study, pregnant rats were divided in three groups: Group G(free access to water containing 0,02% (w/v) methimazole from day 9 until parturition), Group GL(free access to water containing 0,02% (w/v) methimazole from day 9 until 21 days after parturition), Group C(free access to water without methimazole). All male pups maintained in groups of up to three/cage until 90 days. During all this time, systolic arterial pressure, body mass, length of tibia and tail were evaluated. After 90 days, left ventricular function was evaluated by echocardiography. GL group showed a decrease in FS *(% ,GL:49±6*vs.C:65±16)*, similar to was observed in hypo group *(% ,hypo:47±3*vs.C:65±16).* GL and hypo animals presented lowered EF *(% ,GL:79±6*, hypo: 83±2*vs.C:89±9)*. AWT was diminished in systole and diastole in groups G and hypo (*AWTs, mm,G:2,9±0,6*,hypo:2,5±0,7*vs.C:3,8±0,6; AWTd, mm,G:1,5±0,5*,hypo: 1,4±0,5*vs.C:1,9±0,3).* When analyzing PWT, only hypo group presented lowered results in systole and diastole *(PWTs, mm,hypo: 2,5±0,4*vs.C:3,8±0,6; PWTd, mm, hypo:1,5±0,2*vs.C:2,5±0,33).* Groups GL and hypo showed an increase in the LVID in systole and diastole(*LVIDs, mm,GL:3,5±0,6*,hypo: 2,87±0,1*vs.C:1,8±0,1; LVIDd, mm,GL:6,9±0,5*,hypo:6,0±0,2*vs.C:5,2±0,2).**P<0,05 vs. control group. All statistical procedures were performed using the SPSS statistical software package version 23. In conclusion, this study suggests that hypothyroidism induced in early stages of life conditions heart function during adulthood and results in impairment of heart function, which is likely be involved in structural cardiac alterations. These findings highlight the importance of understanding the effects of an altered thyroid hormone status during intrauterine.

119. (399) **"SPEXIN MODULATE INGUINAL WHITE ADIPOSE TISSUE THERMOGENESIS UPON COLD EXPOSURE"**

Gambaro Se[1,2], Zubiria Mg[1,2], Giordano A[1,2], Harnichar E[1], Castro P[1], Giovambattista A[1,2]
[1] Instituto Multidisciplinario de Biología Celular (IMBICE), CONICET-CIC-UNLP; [2] Facultad de Ciencias Exactas, UNLP

Spexin is an anorexigenic adipokine that has multiple physiological function such as anti-obesogenic properties, anxiolytic, among others but there are not current information about SPX role on white adipose tissue (AT) thermogenesis. So, male mice were treated or not (CTR) with SPX for 10 days (29ug/kg/day; SPX) and at day 3 some animals were kept at RT and other at 4°C during 7 days (CTR-C; SPX-C) for thermogenesis induction. In this study, Inguinal AT was collected and processed for different determinations. Metabolic profile was previously reported. mRNA expression of UCP1, SPX and its receptor GALR2 showed interaction between variables (Two-way-anova; Cold x SPX). UCP1 post test showed an increase in CTR-C group vs. CTR and decreased in SPX-C vs. CTR-C (P<0,001). For SPX we found a reduced expression in all groups vs. CTR (P<0,01). On the other hand, GALR2 expression was reduced in SPX and CTR-C groups vs. CTR (P<0,001), while SPX-C showed an intermediate value between CTR and CTR-C and significantly higher compared to SPX. UCP1 protein analysis was like mRNA, its levels have an increase in CTR-C vs. CTR (P<0,05) and was reduced in SPX-C vs CTR-C (P<0,05). IHQ for UCP1 in paraffin section was evaluated, and UCP1^{+} area was evidenced in Cold exposed group but marked reduction in SPX-C. Mitochondrial DNA content was evaluated and we observed an interaction between variables. Post test showed an increased in CTR-C group vs. CTR (P<0,01) and for SPX-C resulted in an intermediate content. Finally, we evaluate if SPX was able to reduce beige preadipocytes population. Mice were treated with SPX for 3 days and flow cytometry was performed (PDGFRα and EBF2; doble positive population). In this case, we did not observed differences between groups. In conclusion, we demonstrate that SPX treatment reduced UCP1 mRNA and protein expression and mitochondrial DNA content upon cold stimulated conditions. However, SPX did not modulate beige preadipocyte pool. PICT2019-2787/1851.

120. (462) **TRIIODOTHYRONINE (T3) TRIGGERS A METABOLIC REPROGRAMMING ON MICE DENDRITIC CELLS (DC)**

Antonella Blanco[1], Dana María Negretti-Borga[1], Elida Nahir Puentes[1], Mariana Pires Teixeira[1], Ana Carolina Donadio[1], María del Mar Montesinos[1], Claudia Gabriela Pellizas[1].
[1] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

Our group previously demonstrated that T3 triggers adaptive pro-inflammatory and cytotoxic responses through DC activation, restraining regulatory signals. These results were successfully exploited in T3-stimulated DC (T3-DC)-based antitumor vaccines against melanoma and colon carcinoma in mice. It is known that metabolic reprogramming of DC supports its TLR-driven maturation, favoring glycolysis over oxidative phosphorylation (OXPHOS), and is controlled by different time-dependent pathways. Sustained commitment to glycolysis relays on OXPHOS inhibition caused by nitric oxide (NO) produced by inducible NO Synthase (iNOS). On this basis, and given the potential of T3-DC vaccines, our aim was to assess T3 effects on DC's metabolic programming. Immature bone marrow derived DC (iDC) were obtained from C57BL/6 mice and stimulated (or not) with T3 (10nM) for different time points. Glucose and lactate were measured in culture's supernatants (SN) from iDC and T3-DC with commercial kits. Glucose uptake was evaluated using the glucose analog fluorescent dye 2-NBDG by FACS. Glucose transporter 1 (Glut-1) and iNOS expression were analyzed by Western Blot. Nitrite levels in SN were measured by the Griess reaction. Statistical

analysis: t test or t test with Welch's correction, $p<0.05$ was considered statistically significant. Compared to iDC, T3-DC showed a significant time-dependent increase in glucose consumption. Lactate production was also augmented in T3-DC ($p<0.01$). Besides, T3-DC exhibited a significant increase in glucose uptake and higher Glut-1 expression than iDC. T3-DC expressed high levels of iNOS ($p<0.01$), but it was not detected in iDC. Nitrite levels, indicative of NO production, were increased in T3-DC vs iDC ($p<0.05$). This study gives the first insights into the impact of T3 on DC's metabolism, focusing on the glycolytic pathway. Further research is under course to go in-depth with the understanding of the DC´s metabolic reprogramming induced by T3.

121. (514) **CORTISOL IN BREAST MILK IS ASSOCIATED WITH INFANT WEIGHT AND ADIPOSITY AT 6 MONTHS OF AGE**

Ana Luz Kruger[1,3], Agustina Malpeli[1], Laura Orellano[2], Lucía Mazziota[2], Daniela Rocha[2], Lucrecia Fotia[1], Ignacio Méndez[1], Marisa Sala[1], Andrea Tournier[2], Liliana Disalvo[1], Ana Varea[1], María Florencia Andreoli[1,3]

1Instituto de Desarrollo e Investigaciones Pediátricas (IDIP). HIAEP Sor María Ludovica de La Plata - CIC-PBA, La Plata, Argentina, 2HIAEP Sor María Ludovica de La Plata, Argentina, 3Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Objective: to search associations between plasma and breast milk cortisol and anthropometric variables in mothers with pregestational normal-weight (NW) or overweight/obesity (OW/OB) and their children at 3 and 6 months postpartum. *Material and Methods*: Two cross-sectional studies were conducted. Mother-child dyads were recruited at 3 (18 NW and 16 OW/OB mothers) and 6 (35 NW and 33 OW/OB mothers) months postpartum. In mothers, anthropometric measurements and cortisol levels in plasma and milk were assessed at 3 and 6 months postpartum. In children, cortisol was assessed in saliva at 3 and in plasma at 6 months postpartum, as well as anthropometric measurements. Normality of the variables was tested using Shapiro-Wilks test. The differences in plasma and breast milk cortisol levels between NW and OW/OB mothers were assessed with Student's or Mann–Whitney–Wilcoxon test. Pearson or Spearman correlations were performed, considering significant a value of $p<0.05$. *Findings*: No difference was found in milk or plasma cortisol levels between NW and OW/OB mothers, and a positive correlation between maternal plasma and milk cortisol was observed at 3 and 6 months postpartum ($r=0.76$, $p<0.001$ and $r=0.57$, $p<0.001$, respectively). At 3 months postpartum a positive correlation between maternal plasma and child saliva cortisol ($r=0.49$, $p=0.004$) was observed. At 6 months postpartum a positive correlation between milk and child plasma cortisol ($r=0.36$, $p=0.003$) was observed as well as a negative correlation between plasma cortisol and maternal waist ($r=-0.42$, $p=0.012$) and hip circumferences ($r=-0.43$, $p=0.009$) only in NW mothers. A negative correlation between milk cortisol and children body weight ($r=-0.45$, $p=0.008$), triceps ($r=-0.39$, $p=0.024$), and Subscapular ($r=-0.36$, $p=0.041$) skinfolds z-scores was observed only in OW/OB mothers. *Conclusions*: Breast milk cortisol conditions child´s growth, adiposity and cortisol levels, especially in children from mothers with OW/OB.

122. (517) **ROLE OF GAS IN PANCREATIC B-CELL DEVELOPMENT AND FUNCTION**

Juan Ignacio Burgos[1,2*], Dana Steffen[3], Silvio A. Traba[1,2], Agustin Romero[1,2], J. Silvio Gutkind[3,4], Santiago Rodriguez-Seguí[1,2*].

*[1] CONICET-Universidad de Buenos Aires, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Buenos Aires, Argentina; [2] Universidad de Ciencias Exactas y Naturales, Departamento de Fisiología y Biología Molecular y Celular, Buenos Aires, Argentina; [3] University of California San Diego, Moores Cancer Center, La Jolla, California, USA; [4] Department of Pharmacology, University of California, San Diego, USA. *Email: juanignacioburgos@gmail.com srodriguez@fbmc.fcen.uba.ar*

In type 1 diabetes there is a massive destruction of the insulin-producing β-cells of the pancreas. Transplantation of artificially-generated insulin-producing cells could reverse the disease. The aim of our study is to investigate the function of the Gαs-mediated GPCR signaling pathway in the control of the β-cell differentiation program. Our hypothesis is that the signaling integrated by Gαs could dynamically regulate the nuclear localization of the YAP coactivator, a master factor in the control of pancreatic progenitor cell differentiation. A more detailed knowledge of these mechanisms could be used to facilitate the in vitro production of functional β-cells. In order to explore the role of Gαs in pancreatic development, we use transgenic mice with conditional suppression of Gnas in the pancreas, by crossing Pdx1Cre with Gnas fl/fl mice. Our results reveal that mice with deletion of Gαs in the pancreatic compartment, from the beginning of pancreas specification, are hyperglycemic (600 and 199±5.86 mg/dL, regarding to control mice, N=6) from 8 weeks of age, and present defects in the endocrine cell type composition of pancreatic islets, which have a larger proportion of α-cells at the expense of the β-cell population (34.63±2.39 and 18.71±3.55%; N=4). These mice also exhibited an impairment of exocrine architecture in the acinar compartment and an enlarged ductal compartment, assessed by H&E staining on histologic sections. Interestingly, Gαs deletion could promote upregulated YAP signaling since it leads to an increase of nuclear YAP localization in the exocrine tissue of the adult pancreas (69.33±2.96 and 33.34±1.86 %; N=3), evaluated by immunofluorescence. The effect of the absence of Gαs will be further evaluated at the transcriptional level by RT-qPCR in pancreatic buds and islets. Preliminary results suggest that, the Gαs cascade may have a potential role in the differential localization of Yap in acinar tissue and may be critical for pancreatic growth.

123. (522) **DEXAMETHASONE INHIBITS BOTH RETROPERITONEAL AND SUBCUTANEOUS WHITE ADIPOSE TISSUE BROWNING**

Alejandra Giordano[1], María Guillermina Zubiría[1], Ezequiel Harnichar[1], Patricia Castro[1], Andrés Giovambattista[1].

[1] Instituto Multidisciplinario de Biología Molecular (IMBICE), CONICET-CICPBA-UNLP.

Two mechanisms are described for browning of White Adipose Tissue (WAT): trans-differentiation from white to beige adipocytes or Adipocyte Precursor Cells (APCs) differentiation. Glucocorticoids (GCs) inhibit WAT browning, but remains unknown if they affect mature beige adipocytes, APCs or both. Our aim was to study Dexamethasone (DXM) effect on browning markers in whole WAT and in isolated Stromal Vascular Fraction (SVF) cells from Retroperitoneal and Inguinal AT (RPAT and IAT, respectively). Male rats were divided into four groups: control (CTR) and DXM (sc. Injected, 0,03mg/Kg/d for 7 days) housed at room temperature (RT), and CTR and DXM housed under cold (C) stimulus (4°C for 7 days, CTR-C and DXM-C, respectively). Two-way ANOVA test was used for statistical analysis. Firstly, we evaluated the DXM effect in whole WAT. RPAT and IAT pads were dissected and processed for quantification of thermogenic genes (qRT-PCR). We found that DXM inhibited the expression of different thermogenic markers. In RPAT, Ucp-1, pgc1a and dio2 levels was lower in C than RT (Interaction DXMxC $p<0,05$) and rcb3 was decreased in both conditions (DXM $p<0,05$). In IAT, only ucp-1 showed interaction ($p<0,05$), but dio2 and rcb3 diminished in both conditions (DXM $p<0,05$). UCP1 protein levels was also evaluated. We found that UCP-1 levels were increased in both depots from CTR-C rats, but they were only diminished by DXM in RPAT. To evaluate the DXM effect in APCs, we measured gene expression of general APC´s marker (pdgfra) and specific beige markers (ebf2 and prdm16) in SVF cells from both depots. In RPAT, pdgfra was decreased (DXM $p<0,05$) and we found interaction in IAT APCs ($p<0,05$). On the other hand, DXM diminished ebf2 levels in both RPAT and IAT APCs ($p<0,05$) and showed a strong tendency to decrease prdm16 levels in IAT APCs. Overall, our results showed that DXM inhibits browning in whole RPAT and IAT, in part by affecting the expression of beige precursor markers. PICT 2017-2314

124. (747) **IMMUNOMODULATORY ROLE OF SPHINGOSINE KINASE 1 (SK1) ON TRIIODOTHYRONINE (T3)-MATURED DENDRITIC CELLS (DC) AND THE DRIVEN ADAPTIVE**

RESPONSE

Dana M. Negretti-Borga[1], Antonella Blanco [1], Mariana P. Teixeira [1], Vanina A. Alamino [1], Florencia Soler[1], Elida N. Puentes[1], Ana C. Donadio [1], Christopher J. Clarke[2], María del Mar Montesinos [1], Yusuf A. Hannun [2], Claudia G. Pellizas [1]

[1] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba, Argentina.

[2] Stony Brook Cancer Center. Department of Medicine, Stony Brook University. Stony Brook, New York, United States of America.

Our group demonstrated that T3 generates adaptive proinflammatory and cytotoxic responses through DC activation, restraining regulatory signals. This protocol was successfully exploited in T3-stimulated DC (T3-DC)-based antitumor vaccines. T3 effects are mainly triggered by non-genomic mechanisms involving thyroid hormone receptor, Akt and NF-kB. Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid produced by SK1 and 2. Although this pathway is involved in many proinflammatory conditions, little is known about its role in innate immune cells. We aim to evaluate the role of SK1 in T3-DC, and the driven adaptive immunity. Bone marrow DC were obtained from C57BL/6 WT or SK1-KO mice and stimulated (or not) with T3 (10nM). PF-543 (SK1 inhibitor, 100nM) was added, and 30 min later the T3 stimuli (PF-T3-DC). After 30 min, p-Akt/total Akt was analyzed by Western Blot. Allogenic splenocytes isolated from BALB/c mice were co-cultured with T3-DC or PF-T3-DC (exposed to T3 for 18h), for 3 days. Viability and proliferation were evaluated by FACS. Cytokines were measured by FACS and ELISA. Statistical analysis: Two-way ANOVA/Tukey test, and paired t test. $p<0.05$, statistically significant. Results showed that intracellular IL-12 production was increased in T3-DC (18h T3 exposure) from SK1-KO mice (vs WT, $p< 0.0001$). Accordingly, IL-12 secretion was higher in PF-T3-DC (vs T3-DC, $p< 0.005$). Of note, DC viability was not modified by PF-543. In turn, SK1 inhibition reduced p-Akt in T3-DC ($p< 0.005$). IFN-γ and IL-17 production and secretion, as well as splenocytes proliferation, were modified in the co-culture with PF-T3-DC (vs. T3-DC, $p<0.05$). Our results revealed for the first time that the Sphingolipid intracellular pathway is involved in T3-DC activation. The immunomodulation exerted by SK1 on T3-DC and the driven adaptive response disclose a novel role of Sphingolipids in the immune-endocrine crosstalk. Further research would unveil the intimate signaling process.

125. (786) **RAMAN SPECTROSCOPY UNCOVERS BIOLOGICAL CHANGES IN EXTRACELLULAR VESICLES GENERATED BY THYROID TUMOR CELL-FIBROBLAST INTERPLAY**

Mónica Beatriz Gilardoni[1], Graciela Adriana Borioli[2], Esteban Druetta[3], Gabriela Inés Lacconi[3], María del Mar Montesinos[1], Claudia Gabriela Pellizas[1], Ana Carolina Donadio[1].

[1]Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, CIBICI-CONICET, Córdoba, Argentina; [2]Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, CIQUIBIC-CONICET, Córdoba, Argentina; [3]Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, INFIQC-CONICET, Córdoba, Argentina.

Extracellular vesicles (EVs) are a heterogeneous population of nanoparticles involved in cell-cell communication. Carcinoma is a complex society of interacting cells, with tumor-stroma crosstalk leading to tumor progression. We demonstrated that thyroid tumor cell-fibroblast (Fb) interaction induces the secretion of metalloproteinases (MMPs) to culture supernatants (CMs) and promotes a migratory phenotype in the tumor cells. Thyroid tumor cell-Fb crosstalk liberates EVs that are involved in extracellular matrix remodelling. Raman spectroscopy is a well-established label-free and non-destructive method for determining chemical characteristics in biological samples. We utilized Raman Confocal Microspectroscopy (Raman-CM) to explore individual secreted-EVs and to describe the impact of tumor-stroma interplay in their chemical make-up. Thyroid tumor cells (TPC-1) or thyroid non-tumor cells (NThyOri) were co-cultured with normal human Fb as a simulation of the thyroid tumor microenvironment (TM). EVs obtained by ultracentrifugation of thyroid cell, Fb and thyroid cell-Fb CMs were characterised by Raman-CM. The Raman spectra of the EVs secreted by thyroid cells either isolated or co-cultured with Fb were compared in order to distinguish biochemical patterns and their association with EVs potential functionality.

Our results show a differential spectral pattern between EVs obtained from isolated and co-cultured TPC-1 cells. Characteristic patterns in the 1000–1550 cm^{-1} and 2150-2700 cm^{-1} of the Raman spectra of EVs from isolated TPC-1 cells are absent in EVs from co-cultured TPC-1 cells. A set of vibrational signals in the region 2800-3000 cm^{-1} only appear in Fb-TPC-1 derived EVs. There are no changes in the spectra of EVs originated by isolated or co-cultured NThyOri cells.

Taken together these findings suggest an important role of Fbs in EV-phenotype from thyroid tumor. They also open a new approach to study the thyroid TM and its role in thyroid tumor progression.

126. (806) **CHRONIC STRESS EXPOSURE INCREASES DIABETES INCIDENCE AND ALTERS GUT MICROBIOTA IN NOD/SHILTJ MICE.**

Rubinstein MR[1], Wydra L[1], Bianchi MS[2], Wald MR[1], and Genaro AM[1]

1. Instituto de investigaciones Biomédicas (UCA-CONICET).

2. Instituto de Biología y Medicina Experimental (CONICET).

Type 1 diabetes (T1D) is characterized by impaired insulin secretion and it has been recognized the contribution of psychosocial factors (including chronic stress exposure) in T1D. Gut microbiota is the group of microorganisms (commensal, symbiotic and pathogenic) that we find in our gut. It participates in multiple functions and an association between unbalanced microbiota and several diseases, including diabetes, has been reported. NOD/ShiLtJ mice are a model for autoimmune type 1 diabetes. The aim of the present work is to study the effect of chronic stress in diabetes development and to characterize the microbiota alterations in NOD/ShiLtJ mice. For this purpose, the animals were subject to chronic stress (CS) by the application of aleatory and unpredictable stressors. NOD/ShiLtJ mice exposed to CS (NOD + CS) showed an increase in diabetes incidence (Long-rank test, $p<0.05$) and higher glycemia levels (t test, $p<0.001$). Serum samples were collected and the titter for autoantibodies against insulin was measured by ELISA. NOD + CS had a lower antibody titer (t test, $p<0.05$). To determine microbiota alterations, fecal samples were collected and genomic DNA was extracted. 16s total bacteria, 16s Bacteriodetes and 16s Firmicutes (the most abundant components of the microbiota) were measured by qPCR using specific primers. No changes were detected in 16s total bacteria and 16s Firmicutes but a decrease in 16s Bacteroidetes was found in NOD + CS (t test $p<0.05$). These results show that CS has a role in type 1 diabetes development and alters gut microbiota composition, and they could suggest that the decrease in 16s bacteroidetes may participate in type 1 diabetes development.

GASTROENTEROLOGY

Friday, November 18, 9-10:30 hr

Chairs: Daniel Francés – Luján Álvarez

127. (7) **IMPAIRMENT OF ENDOGENOUS BILIRUBIN GENERATION AGGRAVATES BILE-DUCT LIGATION INJURY IN RATS: ROLE OF BILIRUBIN ANTIOXIDANT EFFECTS**

Pamela L. Martín[1], María V. Razori[1], María L. Corbo[2], Guillermina B. Harvey[3], Enrique J. Sánchez Pozzi[1], Marcelo G. Roma[1], Cecilia L. Basiglio[1, 4]

[1]Instituto de Fisiología Experimental (IFISE - CONICET), [2]Servicio de Anatomopatología, Fac. Ciencias Médicas. [3]Escuela de Estadística, Fac. Ciencias Exactas y Estadística. [4]Area Bioquímica Clínica; Fac. Ciencias Bioquímicas y Farmacéuticas. UNR, Rosario, Argentina

We showed that hemeoxygenase-1 (HO1) induction, and consequent bilirubin (BR) elevation, protects the liver from oxidative stress

(OS)-induced cholestasis *in vivo*. Here, we evaluated the effect of HO1 inhibition on the endogenous levels of BR and its protective action in obstructive cholestasis. Male Wistar rats were subjected to 7-day bile-duct ligation (BDL, *n*=7) or sham surgery (Sh, *n*=5). HO1 was inhibited with Zn(II) protoporphyrin IX (PP, 25 mg/Kg b.w., i.p.), 24h before BDL (PP+BDL, *n*=10) or Sh (PP+Sh, *n*=6). Results are expressed as media±SD and analyzed by Kruskal-Wallis' test. After BDL, plasma BR levels (mg/dl) were higher in BDL (8.46±1.70[a]) than in PP+BDL (3.90±2.80[b]), confirming HO1 inhibition ([a]p<0.01 *vs* Sh and PP+Sh; [b]p<0.01 *vs* BDL). Plasma bile acids (µmol/L) increased in BDL (116±14[a]) due to the obstructive process; this increase was milder in PP+BDL (67±25[b], [a]p<0.01 *vs* Sh and PP+Sh; [b]p<0.05 *vs* BDL), likely due to the lower BR-mediated induction of the sinusoidal export pump Mrp3. Histopathological analysis of liver samples evidenced the obstructive injury (portal and parenchymal inflammation, ductular injury, canalicular proliferation, proliferation of hepatic stellate cells and fibrosis), which were more noticeable in PP+BDL than in BDL, indicating that BR-generation impairment aggravates obstructive injury. Hepatic lipid peroxidation (nmol MDA /mg prot) was higher in PP+BDL than in BDL (7.1±1.8 *vs.* 5.5±0.7, p<0.05) and total antioxidant status (*µ*mol Trolox™ equivalents/mg prot) was lower in BDL (0.11±0.03[a]) than in control groups, while PP+BDL showed the lowest values (0.08±0.02[b], [a]p<0.05 *vs* Sh and PP+Sh; [b]p<0.05); in cholestasis, hepatic oxidative damage is more severe and antioxidant defenses are weakened when BR production is inhibited. We conclude that the vicious circle by which OS induce inflammation and *vice versa*, a major cause of progressive cellular damage, is significantly attenuated by the accumulation of antioxidant BR in cholestasis.

128. (108) **TRANSIENT ROS PRODUCTION INVOLVED IN TAUROLITHOCHOLATE-INDUCED IMPAIRMENT OF MRP2 FUNCTION IS MAINLY DERIVED FROM HEPATOCYTE NOX ACTIVATION**

Romina B. Andermatten, Nadia Ciriaci, Virginia S. Schuck, Agustina Fazzi, Ismael R. Barosso, Enrique J. Sánchez Pozzi.
Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

Cholestatic agents, such as the bile salt taurolithocholate (TLC), affect bile flow, in part, by impairing the activity of the canalicular transporters, among them Mrp2 (Multidrug Resistance-associated Protein 2). We have recently demonstrated that TLC induces a rapid and transient increase in intracellular reactive oxygen species (ROS) levels, which mediate the alteration of Mrp2 function. *In vitro* assays with antioxidants prevented this effect, suggesting a key role of these species in TLC-induced cholestasis. Our **aim** was to characterize the source of ROS production triggered by TLC. Hence, we decided to study the involvement of NADPH oxidase (NOX) and to elucidate its relationship with other proteins implicated in TLC-activated signaling pathways. Methodology: Isolated rat hepatocytes (IRH) were pretreated with NOX inhibitor apocynin (Apo 300 *µ*M, 30 min) followed by treatment with TLC (2.5 *µ*M) for 5 min. After washing, 2-7-dichlorofluorescein diacetate (5 *µ*M, 30 min) was added and the fluorescence signal produced by 2-7-dichlorofluorescein (DCF) was determined. In another set of experiments, treatments were done in presence of inhibitors for proteins implicated in TLC cholestasis model, such as S1PR2 antagonist (JTE-013, 10 *µ*M) and p38 MAPK and PKC inhibitors (SB203580, SB 1 µM) and bisindolylmaleimide, Bis 100 nM, respectively). Results: (% of Control±SEM; n=3) As previously reported, TLC treatment increased intracellular ROS production at 5 min in IRH (130±3[a]). Pretreatment with Apo completely prevented this effect (105±5[b]). On the other hand, the rise of ROS levels induced by TLC was also prevented in cells treated with SB (98±2[b]) or Bis (94±8[b]), whereas in presence of JTE-013 no change was observed (127±15[a]). [a]p<0.05 vs Control, [b]p<0.05 vs TLC. Conclusion: Transient and rapid ROS production by TLC treatment is mainly mediated by NOX activity. Additionally, this process seems to depend on PKC and p38 MAPK, but it is independent of the S1PR2 pathway.

129. (210) **ORIGIN OF REACTIVE OXYGEN SPECIES INVOLVED IN THE DECREASE IN MRP2 FUNCTION PRODUCED BY IL-1ss**

Virginia Schuck[1], Romina Andermatten[1], Nadia Ciriaci[1], Agustina Fazzi[1], Gimena Salas[1], Anabela Medeot[1], Ismael Barosso[1], Enrique Sánchez Pozzi[1].
[1]*Instituto de Fisiología Experimental. IFISE-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.*

Inflammatory cytokines produce alterations in the location and function of canalicular transporters and could mediate the biliary secretory failure observed in inflammatory-associated pathologies such as sepsis. This action of cytokines is mediated by activation of signaling proteins. We have previously demonstrated that IL-1ss impairment of hepatocanalicular transporters activity, such as that of multidrug resistance-associated protein 2 (Mrp2) is mediated by reactive oxygen species (ROS). We proposed to evaluate the involvement of NADPH oxidase (NOX) in the production of ROS by IL-1 ss. Methodology: Isolated rat hepatocytes (HAR) were treated with IL-1ss (10ng/ml) for 5, 10, 15 and 20. Then, cells were washed, 2-7-dichlorofluorescein diacetate (DCFH, 5 *µ*M, 30 min) was added and finally the fluorescence signal produced by its oxidized product 2-7-dichlorofluorescein (DCF) was determined at 498 nm excitation and 522 nm emission. The fluorescence intensity of DCF is proportional to the intracellular ROS production. To evaluate the origin of IL-1ss-dependent ROS production, cytokine treatment was repeated for 20 min in the presence of the NOX inhibitor apocynin (Apo 300 *µ*M) and the antioxidant vitamin C (Vit C 100 *µ*M) Result: (% of Control ± SEM; n=3-4) IL -1ss increased intracellular ROS production in hepatocytes, at least up to 20 min (148.7±14a). At this time (20 min of IL-1ss treatment), the co-incubation with APO (APO+IL: 89.5±5b) and VITC (Vit C+IL: 91.2±5b) prevented this increment of ROS. *a different from control, b different from IL-1ss* Conclusion: IL-1ss-induced ROS production is mediated by NOX. This oxidase may play a key role in the cellular signaling responsible for IL-1ss-induced cholestasis. Previous studies in different models of cholestasis showed that reactive oxygen species (ROS)/oxidative stress mediate the internalization of the hepatocanalicular transporters, such as multidrug resistance-associated protein 2 (Mrp2). Taurolithocholate (TLC) is known to be the most pro-oxidative bile salt. However, there is no direct evidence that ROS production derived from TLC action is a mechanism involved in cholestasis pathogenesis. Herein, we evaluated a possible role of ROS in the TLC- induced impairment of Mrp2 activity. Methodology: ROS production was measured by the 2´,7´-dichlorofluorescin-diacetate (DCFH-DA) assay in primary culture rat hepatocytes. Cells were exposed to TLC (2.5 *µ*M) at different times (5, 10, 15 and 20 min) followed by incubation with DCFH-DA (5 µM). On the other hand, isolated rat hepatocyte couplets (IRHC) were co-treated with TLC (2.5 *µ*M) and antioxidants: vitamin C (VitC 100 *µ*M) or mannitol (Man 60 mM) for 20 min. To analyze the TLC-induced ROS involvement in Mrp2 activity impairment, functional studies was carried out by assessing the canalicular vacuolar accumulation of its substrate glutathione methylfluorescein (GMF). Results: (% of Control±SEM; n=3-5): TLC increased intracellular ROS production in hepatocytes, reaching the maximum peak at 5 min (133±7 a) and rapidly returning to control levels at 10 min (102±4). This transient production suggests the participation of ROS as signaling molecules. Pre-treatment of IRCH with both antioxidants prevented TLC-induced impairment of canalicular accumulation of GMF: TLC (67±6 a), TLC+VitC (92±3 b), TLC+Man (94±7 b), pointing out ROS as possible modulators of Mrp2 internalization. a p<0.05 vs Control, b p<0.05 vs TLC. Conclusion: TLC treatment induced transient rise in ROS levels, which could be a key signaling component that contributes to the altered Mrp2 function found in cholestasis.

130. (255) **DECREASED PROTEIN EXPRESSION AND ACTIVITY OF INTESTINAL MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) IN A MOUSE MODEL OF HIGH FAT DIET-INDUCED OBESITY: POTENTIAL ROLE OF OXIDATIVE STRESS (OS)**

Felipe Zecchinati[1], María Manuela Barranco[2], Laura Ricardi[1],

Maite Rocío Arana[1], Virginia Gabriela Perdomo[3,4], Marcelo Gabriel Luquita[1], Aldo Domingo Mottino[1], Fabiana García[2,4], Silvina Stella Maris Villanueva[1]
[1]Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [2]Laboratorio de Fisiología Metabólica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [3]Cátedra de Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [4]CONICET, Rosario, Santa Fe, Argentina.

Consumption of a high fat diet is a key factor in the development of pathophysiological alterations leading to metabolic disorders such as insulin resistance and obesity. Mrp2, an ABC transporter acting as intestinal biochemical barrier, strongly influence the bioavailability, and hence the efficacy and safety, of orally administered drugs. Interestingly, several of these drugs are prescribed to patients with such alterations. We here evaluated the effect of standard diet enriched with 40% kcal from bovine fat (HFD) to C57BL/6J male mice for 8 weeks on jejunal Mrp2 expression, by western blot, and on its transport activity, by using the everted intestinal sacs model. Control mice (C) received standard diet. Statistical analyses were performed using *t*-student's test and results were expressed as % of C. Fat consumption led to high plasma triglycerides and glycemia levels, as well as insulin resistance and a marked increase of epididymal fat in HFD vs C ($p<0.05$, n=6). A significant decrease in Mrp2 protein expression was observed in HFD (-60%) respect to C ($p<0.05$, n=6). In line with this, efflux of the Mrp2 substrate dinitrophenyl-S-glutathione (DNP-SG), generated from its precursor 1-chloro-2,4-dinitrobenzene (CDNB), decreased in fat animals respect to controls (HFD: -70% respect to C, $p<0.05$, n=6). Concomitantly, HFD induced alterations in oxidative stress (OS) parameters by decreasing the activity of antioxidant enzyme catalase (-40%), as well as increasing lipid peroxidation end products (+166%) and oxidized glutathione (+50%) ($p<0.05$, n=6). Conclusion: The present study demonstrated that HFD decreased the protein expression of jejunal Mrp2 and consequently deteriorated its function of biochemical barrier against the absorption and toxicity of food contaminants and therapeutic drugs, thus altering their bioavailability. Our study also suggests that OS may be an important mediator of the Mrp2 down-regulation.

131. (259) **IN VIVO HEPATIC GENE TRANSFER OF HUMAN AQUAPORIN-8 IMPROVES AMMONIA DETOXIFICATION TO UREA**

Alejo Matías Capiglioni[1,2], María Celeste Capitani[1,2]; Julieta Marrone[1,2], Raúl Alberto Marinelli[1,2]
1. Instituto de Fisiología Experimental (IFISE), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) 2. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Santa Fe, Argentina.

The aquaporin-8 (AQP8) channel protein exhibits permeability to small uncharged molecules, including ammonia. In previous studies using cultured hepatocytes, we demonstrated a key role of mitochondrial AQP8 in ammonia-derived ureagenesis and, in addition, that the mitochondrial expression of human AQP8 (hAQP8) is able to increase detoxification of ammonia to urea. Here, our AIM is to study whether the *in vivo* hepatic gene transfer of hAQP8 can improve ammonia detoxification to urea. A recombinant adenoviral vector encoding hAQP8 (AdhAQP8), or a control vector was administered to C57BL/6 mice by retrograde bile ductal infusion in order to transduce mostly periportal hepatocytes. After 72 h, mitochondrial subfractionation followed by immunoblotting confirmed that hAQP8 was rightly expressed at mitochondrial level without affecting the expression of the urea cycle mitochondrial enzymes, CPS1 and OTC. We challenged hepatic ureagenesis by inducing acute and transient hyperammonemia through the i.p. administration of ammonium acetate. hAQP8-transduced mice showed better ammonia detoxification by increasing hepatic urea synthesis by around 25 % ($p< 0.05$) over controls. On the other hand, mice with defective ammonia detoxification induced by thiocetamide (TAA) were transduced with AdhAQP8 or control adenovector. Data of hepatic ureagenesis were: Controls (not TAA-treated): 100%; TAA: 80 ± 1%*; TAA+hAQP8: 94 ± 3%; data are means ± SEM; n=4; *$p<0.05$ from control or TAA+hAQP8. These results were confirmed from initial Nuclear Magnetic Resonance studies using ^{15}N-labeled ammonia and assessing the hepatic ^{15}N-labeled urea synthesis. Conclusion: Our data suggest that in vivo hepatic gene transfer of hAQP8 improves ammonia detoxification to urea, a finding that might have potential therapeutic implications for hyperammonemia disorders.

132. (273) **EFFECTS OF COFFEE CONSUMPTION ON SALIVARY CORTISOL AND ALPHA AMYLASE IN HEALTHY YOUNG ADULTS**

Rolando Pablo Alejandro Juárez, Armando Cesar Celía
Laboratorio de Investigaciones Científicas, Facultad de Odontología, Universidad Nacional del Nordeste, República Argentina.

The purpose of this study was to determine the effects of coffee consumption on salivary cortisol (sCort) and alpha amylase (sAA) in healthy young adults. Sixty university students, habitual coffee consumers, with no history of physical or psychiatric disorders, participated in this experimental study under free-living conditions. Participants were divided into three groups: G1 low consumption coffee users (≤ 2 cups of coffee per day, n = 20), G2 moderate consumption coffee users (2-5 cups of coffee per day, n = 20), and G3 high consumption coffee users (> 5 cups of coffee per day, n = 20). Participants were instructed to collect unstimulated whole saliva samples at home, using passive salivation. Self-collection was morning (6:30-7:30 AM) and evening (08:00-09:00 PM). sCort was analyzed using chemiluminescence immunoassay and sAA activity by kinetic method at 405 nm, substrate CNPG3. Statistical analysis of the results was performed using Student's t-test and analysis of variance. The sample consisted of 30 women and 30 men, aged between 20 and 35 years (mean age: 24.82 years). Body Mass Index varied between a minimum of 18.0 and a maximum of 38.4, with a range of 20.4 kg/m^2. In all groups, the mean value of sCort in the morning is significantly higher compared to the afternoon (0,29 ± 0,19 vs. 0,09 ± 0,05 µg/dl, $p < 0.0001$) and sAA presented higher levels in the evening than in the morning (160,16 ± 60,42 vs. 32,79 ± 12,98 U/ml, $p < 0.0001$). No significant differences could be detected in the contents of sCort (AM: F= 0,14, p= 0,8677; PM: F= 0,04, p= 0,9573) and sAA (AM: F= 0,04, p= 0,9600; PM: F= 0,07, p= 0,9338) between the three groups. Coffee consumption, in non-stressful conditions, did not alter levels and patterns of sCort and sAA in healthy young adults.

133. (283) **POST-TRANSLATIONAL DOWN-REGULATION OF MRP2 BY OXIDATIVE STRESS IN CACO-2 CELLS: IMPACT ON THEIR BARRIER FUNCTION AND PREVENTION BY ADENOSINE**

Laura Ricardi[1], Felipe Zecchinati [1], Maite Rocío Arana[1], Virgina Gabriela Perdomo[3,4], Aldo Domingo Mottino[1], Fabiana García[2,4], Silvina Stella Maris Villanueva[1]
[1]Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [2]Laboratorio de Fisiología Metabólica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [3]Cátedra de Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [4]CONICET, Rosario, Santa Fe, Argentina.

Oxidative stress (OS) produced by continuous exposure to dietary contaminants, is a key factor in the development of gastrointestinal disorders, in which the intestinal barrier is altered. Its effect on MRP2, an essential component of the intestinal transcellular barrier in the detoxification and disposition of environmental and food toxicants, and therapeutic drugs, has been recently evaluated. Long-term exposure to OS of Caco-2 cells, a model of human intestinal

epithelium, by treatment with tert-butyl hydroperoxide (TBH) for 24 h, showed proteosomal degradation of the MRP2 protein. In turn, adenosine plays an important physiological role in the CNS and in peripheral tissues, and its activity is related to the effect of various drugs. We previously demonstrated a positive acute effect of adenosine on MRP2, consisting of an increase in its apical localization, associated with greater activity barrier. The aim of this work was to evaluate the impact of post-translational down-regulation of MRP2 by TBH 250 μM on the efflux MRP2 activity and its potential prevention by co-treatment of Caco-2 cells with adenosine 50 μM, for 24 h. MRP2 activity was determined by quantifying the efflux of DNP-SG into the incubation medium by HPLC, using CDNB 100 μM as precursor substrate. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test for multiple comparisons and results expressed as a % difference with respect to control (C). We first confirmed that TBH 250 generated OS as indicated by increased lipid peroxidation end products (+140%) and reduced SOD activity (-29%) (p<0.05, n=6). The results showed that the MRP2 activity was decreased significantly in TBH group respect to C (-46%, p<0.05, n=4), while it returned to C levels in cells with adenosine co-treatment. Conclusion: We demonstrated that post-translational down-regulation of the MRP2 expression by OS is of functional significance and that adenosine was able to prevent this alteration

134. (346) **DOWNREGULATION OF INTESTINAL MULTIDRUG RESISTANCE PROTEIN 2 IN OBESE MICE IS REGULATED INDEPENDENTLY OF CYTOKINES PROFILE**

María Manuela Barranco[1,2], Virginia Gabriela Perdomo [2,3], Felipe Zecchinati [4], Romina Manarin [3], Greta Massuh [1], Nicolás Sigal [1], Silvana Vignaduzzo [5], Aldo Domingo Mottino [4], Silvina Stella Maris Villanueva [4], Fabiana García [1,2]

[1]Laboratorio de Fisiología Metabólica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina. [2]CONICET. Rosario, Santa Fe, Argentina. [3]Área Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina. [4]Instituto de Fisiología Experimental-CONICET. Rosario, Santa Fe, Argentina. [5]Área Análisis de Medicamentos, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina.

The inflammatory response generated in obesity is mediated by different cytokines which determined most of the metabolic alterations associated with this disease. The Multidrug resistance protein 2 (Mrp2) and P-glycoprotein (P-gp) efflux pumps involved in the intestinal biochemical barrier, are altered in inflammatory states. Previous studies from our group, demonstrated the involvement of the Tumor Necrosis Factor-alpha receptor 1 (TNFR1) signaling pathway in the downregulation of ileal P-gp from obese mice. Therefore, the aim of this study was to evaluate the mRNA expression and activity of jejunal Mrp2 in wild-type male mice C57BL/6 (C57, 5 weeks old, n: 20) and knockout mice for TNFR1 (R1KO, 5 weeks old, n: 20) subjected to a high-fat diet (HFD, 40% fat) for 16 weeks and to investigate the intestinal cytokines profile developed. The statistical comparisons were done by *t*-student or one-way ANOVA followed by post-test. Results were expressed as % of C57 or R1KO-Control. All mice fed with HFD increased their weight and adipose mass, also showed higher cholesterolemia and triglyceridemia levels respect to controls. Only the C57-HFD group presented increased glycemia levels and insulin resistance. The HFD decreased the mRNA expression of Mrp2, both in C57 (-42%) and R1KO (-66%) mice when comparing to their respective control group. These result correlated well with the 53% and 56% decrease respectively, in the apical transport rate of DNP-SG, in everted intestinal sacs from each HFD group. When evaluating the intestinal cytokines profile from C57-HFD mice, we observed an increase in TNF-α (+52%), IL-6 (+158%) and a decrease in IFN-γ (-88%) levels. However in the R1KO-HFD mice, there were not intestinal expression of TNF-α, with decreased IFN-γ (-77%) and without changes in IL-6 levels. We conclude that the downregulation in the expression and activity of intestinal Mrp2 by the high-fat diet, is regardless of the main intestinal proinflammatory cytokines developed.

135. (371) **BILIARY CHOLESTEROL EXCRETION IS STIMULATED BY HEPATIC HUMAN AQUAPORIN-8 EXPRESSION**

María Celeste Capitani, Alejo M. Capiglioni, Raúl A. Marinelli, Julieta Marrone

Instituto de Fisiología Experimental (IFISE), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Santa Fe, Argentina.

Biliary excretion is a key step in liver cholesterol processing. Cholesterol is excreted into bile directly in its unesterified form or after conversion into bile acids. Our previous studies in cultured hepatocytes suggest that aquaporin-8 (AQP8) is involved in the hepatic metabolism of cholesterol. Here, we begin to study the role of AQP8 in the biliary excretion of cholesterol. Male C57BL/6 mice were transduced with AdhAQP8 adenovector by retrograde intrabiliary infusion to induce the hepatic expression of human AQP8 (hAQP8). Control mice received an empty adenovector. After 72 h, the gallbladder was ligated, the common bile duct was cannulated and bile was collected. hAQP8 expression in hepatocytes (mitochondria and canalicular plasma membranes) was confirmed by immunoblotting with specific antibodies. Gene transfer caused no alteration in serum hepatic enzymes indicating absent of toxic effects. In hAQP8-expressing mice, bile flow increased by about 25% (p<0.05) while the biliary excretions of cholesterol and bile acids were markedly increased over 100%. The biliary cholesterol excretion (nmol/min/100g body weight) was: 0.36±0.04 (controls) vs. 0.96±0.13 (AdhAQP8-treated) (n=5; p< 0.01). The biliary excretion of bile acids (nmol/min/100g body weight) was: 40.7±6.6 (controls) vs. 81.8±8.9 (AdhAQP8-treated) (n=5; p< 0.05). The data suggest that hepatic AQP8 expression plays a regulatory role in the biliary elimination of cholesterol. Studies are being carried out to elucidate the mechanisms involved.

136. (395) **SUSTAINED INTERNALIZATION OF THE HEPATOCANALICULAR TRANSPORTER MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) IN CHOLESTASIS LEADS TO ITS EXACERBATED PROTEOSOMAL DEGRADATION**

Anabela C. Medeot[1], Romina B. Andermatten[1], Gimena Salas[1], Virginia S. Schuck[1], Ismael R. Barosso[1], Fernando A. Crocenzi[1], Marcelo G. Roma[1]

[1]Instituto de Fisiología Experimental. Facultad de Ciencias Bioquímicas y Farmacéutica. Universidad de Rosario.

Exacerbated endocytosis of canalicular carriers, including Mrp2, is a main mechanism involved in cholestasis, as has been shown with the model cholestatic agent, taurolithocholate (TLC) (Crocenzi et al. Gut 52: 1170, 2003). We hypothesized that this exacerbated internalization is followed by accelerated degradation, thus explaining the unchanged carrier protein expression despite increased synthesis observed in chronic cholestatic diseases. We therefore tested here whether sustained Mrp2 internalization leads to accelerated degradation, and if so, which degradation mechanism is involved. Mrp2 protein expression was quantified by Western blot in sandwich-cultured rat hepatocytes (SCRH) incubated with TLC (2.5 μM), or vehicle (DMSO) in controls (C), in the presence of cycloheximide to block “de novo” Mrp2 synthesis. TLC exposure for 12 hs induced no change in Mrp2 expression as compared to C, whereas a significant decrease was observed at 24 h (69±4%; n=7) and 48 h (66±8%; n=3); p<0.05 vs. C. Pretreatment with the proteosomal inhibitor MG132 (10 μM) for 30 min, followed by TLC exposure for 24h, fully prevented this decrease (TLC: 68±4%, p<0.05 vs. C; MG132: 92±7 %; MG132+TLC: 106±6%, p<0.05 vs TLC; n=3). A similar pattern was observed by Mrp2 immunostaining followed by confocal microscopy. This prevention was not observed when SCRH were pretreated with the lysosomal inhibitor pepstain A (50 μM). Mrp2 transport function assays were carried out by preloading SCRHs with chloromethylfluorescein diacetate (CMFDA), and

measuring the initial transport rate (ITR) of its fluorescent metabolite, GSH-S-methylfluorescein (GS-MF), a Mrp2 substrate. ITR, expressed as percent of C, was reduced by TLC (-25%), and this effect was prevented by MG132 (+6%). We conclude that sustained endocytosis of Mrp2 in cholestasis leads to its exacerbated proteosomal degradation. This finding could be relevant to understand the impairment of hepatocanalicular transporters in chronic cholestasis.

137. (524) **ESTRADIOL 17β-D-GLUCURONIDE-INDUCED NADPH OXIDASE IMPAIRS Mrp2 ACTIVITY BY CONTRIBUTING TO ITS CELLULAR INTERNALIZATION IN SANDWICH CULTURED RAT HEPATOCYTES**

Gimena Salas, Alen A. Litta, Virginia S. Schuck, Anabela C. Medeot, Romina B. Andermatten, Cecilia L. Basiglio, Fernando A. Crocenzi[1].
[1]*Instituto de Fisiología Experimental (IFISE)*

Estradiol 17β-D-glucuronide (E17G) alters canalicular secretion via several kinase-mediated signaling pathways which induce endocytosis and intracellular retention of canalicular transporters, the MEK-ERK1/2 pathway contributing to the second process. Previously, we found that NADPH oxidase (NOX)-generated reactive oxygen species (ROS) are partly responsible for the impairment of canalicular Mrp2 transport activity induced by E17G, and that NOX shares the MEK-ERK1/2 signaling pathway, downstream these kinases. We aimed to evidence that E17G-induced NOX-mediated functional impairment of Mrp2 transport is dependent on its internalization. Intracellular distribution of Mrp2 was assessed by immunofluorescence and confocal microscopy analysis in sandwich cultured rat hepatocytes (SCRH). First, SCRH were treated with vehicle (DMSO, control), E17G (200 μM, 20 min) or pre-treated with apocynin (Apo, NOX inhibitor, 100 μM, 30 min) prior to E17G. Then, SCRH were fixed, permeabilized, and incubated with a specific antibody to Mrp2, and then with a secondary antibody and Alexa Fluo 568-conjugated phalloidin for F-actin staining, which is mainly pericanalicular (demarcating the canaliculi) and barely evident in the basolateral membrane. Mrp2 internalization was evaluated in confocal images (n=12 canaliculi, from 3 independent cultures) by calculating the percentage of Mrp2-associated staining within the canaliculi to total cellular staining. Mrp2, mainly confined to the canaliculi in controls, was internalized to intracellular vesicles by E17G. Apo prevented this alteration, showing a control-like distribution pattern. Apo, which inhibits migration of the cytosolic p47phox subunit of NOX, impeding the formation of the active NOX complex in the plasma membrane, prevents internalization of Mrp2 by E17G. This finding supports the location of NOX in the MEK-ERK1/2 signaling pathway, which is involved in E17G-induced internalization of canalicular transporters such as Mrp2.

138. (549) **ANTICANCER EFFECTS OF GLYCOSYLATED 4-METHYLUMBELLIFERONE ON HEPATOCELLULAR CARCINOMA THROUGH REGULATION OF APOPTOSIS AND MIGRATION**

Gisela Weiz, Alina L Gonzalez, Iara Mansilla, Javier D. Breccia, María I. Molejón
Facultad de Ciencias Exactas y Naturales, Instituto de Ciencias de la Tierra y Ambientales de La Pampa (INCITAP), Universidad Nacional de La Pampa – Consejo Nacional de Investigaciones Científicas y Técnicas (UNLPAM-CONICET), Santa Rosa, Argentina

Liver cancer, more specifically hepatocellular carcinoma (HCC), is the second leading cause of cancer-related death and its incidence is increasing globally. We have recently reported that 4-methylumbelliferilrutinose (4MUR), an enzymatically synthetized compound, exhibit antitumoral effect on HCC. 4MUR could be used as HCC targeted therapy, without damaging non-tumoral cells or other organs. In this work we aim to understand the molecular processes involved in 4MUR-related antitumor efficacy. First, we evaluated its effect on cell death using HCC tumoral cell lines. The percentage of apoptotic Huh7 cells exposed to 4MUR significantly increased as compared with 4MU ($p < 0.001$). We next performed wound healing assays to examine the effect of the glycosylated drug on the migration of tumoral cells. 4MUR significantly inhibited the migration of Huh7 cells in a dose-dependent manner compared to the untreated group. Based on the evidence that hyaluronic acid (HA) and its receptors play an important role in tumor migration we quantified by RT-PCR the HA synthases (HAS2 and HAS3) and HA-receptor expression. In Huh7 cells, the expression of HAS2 was significantly reduced upon 4MUR treatment ($p<0.05$), while HAS3 was not significantly affected. On the other hand, 4MU treatment did not affect the expression of HAS2 but up-regulated HAS3 by around 3.5-fold. Whereas, no differences were obtained in CD44 expression. The results of this study revealed that 4MUR effectively induces apoptosis cell death in HCC cells. In addition, 4MUR treatment exerted anti-migration effects on HCC cells. The underlying mechanisms suggested that HCC progression was promoted via HA-dependent pathway, independently of HA-receptos inhibition. Thus, we propose that treatments interfering HA metabolism with 4-MUR may represent effective strategies for HCC treatment in the future.

139. (696) **ANTIOXIDANT ACTIVITY OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE EXOCRINE PANCREAS**

Courreges, Ana P[1], Alvarez Guadalupe I[1], Ochoa Federico[2], Lairion Fabiana[3], Zotta Elsa[2], Repetto Marisa[3], Vatta Marcelo S[1,4], Bianciotti Liliana G[1,4].
[1]*Instituto de Inmunología, Genética y Metabolismo (INIGEM-UBA-CONICET), Hospital de Clínicas "José de San Martín", Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires,* [2]*Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Fisiopatología,* [3]*Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Cátedra de Química General e Inorgánica, Instituto de Bioquímica y Medicina Molecular "Profesor Alberto Boveris" (IBIMOL-UBA-CONICET),* [4]*Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Fisiología.*

We previously reported that ANP is produced by the exocrine pancreas and that it plays a beneficial role in the outcome of acute pancreatitis (AP). It reduces trypsinogen activation and the inflammatory response and restores glutathione depletion. In the present study we assessed different parameters of oxidative stress and antioxidant defenses in Sprague Dawley rats with AP induced by four repetitive cerulein injections (40µg/Kg). Thirty minutes before the first cerulein injection animals were infused with either saline (control) or ANP (1µg/Kg/h) for 60 min. Following euthanasia (60 min after the last cerulein injection) pancreatic samples were harvested. ANOVA followed by a Student Newman Keuls was used for statistical analysis. Results are expressed as the mean±SD and $p<0.05$ or less were considered statistically significant (*). ANP reduced NADPH oxidase activity (U/ug protein) (2180±256 vs. 1410±216**). As ANP increased Nrf2 nuclear translocation (0.158±0.2 vs. 0.398±0.02*) superoxide dismutase (SOD) and catalase expression were assessed by western blot and qRT-PCR. ANP increased SOD protein and mRNA expression (0.9±0.08 vs; 1.20±0.11*; 0.63±0.12 vs. 2.29±0.19*). Catalase showed no protein or mRNA changes as expected. Carbonyl content was decreased by ANP (5.60±0.6 vs. 3.60±0.9*) and TBARS showed no changes in any experimental group. The oxidized form of coenzyme Q9 (isoform in rodents) was also reduced by ANP (477.9±56.9vs. 254.7±86.4*). These results further support previous findings showing that ANP enhances the antioxidant capacity of the pancreas in AP. Current evidence suggests that targeting only oxidative stress would not be sufficient to stop the progression of AP, a pathology characterized by a sudden onset and an unpredictably clinical course. In this sense ANP, which it is produced in the exocrine pancreas, would be beneficial since it not only improves the redox status of the pancreas but also significantly reduces premature trypsinogen activation and the local inflammatory response

140. (744) **LIPOTOXIC EFFECT OF CAFETERIA DIET AND 3-METHYLCHOLANTRENE ON THE LIVER OF RATS**

Marina Labiano[1], Jeremías Pablo Flores-Quiroga [1], Florencia Heinecke [1], María Agustina Meneghini [1], Alicia Graciela Fale-

tti [1], Verónica White [1].
[1]Centro de Estudios farmacológicos y botánicos (CEFYBO-CONICET-UBA), Buenos Aires, Argentina

High caloric diets like cafeteria diet (CD) induce weight gain and metabolic anomalies. In the liver this diet, can drive to the development of fatty liver disease. On the other hand, environmental contaminants like polycyclic aromatic hydrocarbons (PAH) can also cause obesity and metabolic disturbances. Aim: To study whether 3-methylcholantrene (3MC), a PAH member, can potentiate the deleterious effects of CD by enhancing liver metabolic impairments. Methods: Sprague-Dawley male rats were fed with CD (CD rats) or standard diet (SD rats) and were exposed to 3MC three times per week (0.1mg/kg, SD3MC and CD3MC) or vehicle (SD and CD) from weaning for 40 days. The rats were weighed periodically until day 60, at which time they were euthanized by decapitation. Serum and livers were collected and preserved for metabolic analysis. Triglycerides (TG), Cholesterol (Ch), aspartate and oxalacetate transferase (AST and ALT) serum levels were measured by colorimetric assays. TG and Ch Esters (ChE) liver levels were assayed by TLC. Carbonyl groups in proteins were assayed by the diphenylhydrazine method. Results: We found that the CD group was heavier (12%, $p<0.05$) than the SD and CD3MC group.Although we found no changes in TG, Ch, ALT and AST circulating levels but we found an increase in liver levels of TG (100%) and ChE (170%) in CD rats compared to SD $p<0.05$. CD3MC rats also showed an increase in TG (72%) and ChE (157%) compared to the SD rats $p<0.05$. Carbonyl groups were increased in both CD (13%) and CD3MC (16%) compared to the SD rats $p<0.05$. We conclude that the cafeteria diet induces weight gain and liver lipotoxicity without affecting circulating lipid levels. The addition of 3MC does not modify CD-induced liver lipotoxicity.

141. (859) **NOX2 PLASMA MEMBRANE EXPRESSION IS IMPAIRED BY ESTRADIOL 17β-D-GLUCURONIDE IN ISOLATED AND PERFUSED RAT LIVERS**

Alen A. Litta , Gimena Salas , M. Cecilia Larocca , Julieta Marrone , Fernando A. Crocenzi1.
1 Instituto de Fisiología Experimental (IFISE)- CONICET, Rosario, Santa Fe, Argentina

All NADPH oxidases contain a catalytic transmembrane protein known as NOX, which requires its assembly with the membrane subunit p22phox, several cytosolic regulatory subunits and the small GTPase Rac, to get the active NOX oxidase complex. It was seen in different non-hepatic cells that NOX is mostly inactive in cholesterol rich lipid ¨rafts¨, becoming active when present in ¨non-raft¨ microdomains. Previously, we shown that estradiol 17β-D-glucuronide (E17G) impairs the canalicular transporter Mrp2 activity (which depends on raft localization) by inducing its migration to non-rafts and its clathrin-mediated endocytosis. Recently, we got evidence that E17G-induced alteration of Mrp2 activity is partially mediated by NOX2. Thus, we assessed the possible effects of E17G on NOX2 membrane localization and expression. We perfused livers from male Wistar rats, and E17G (2 μmol/liver) or its vehicle (DMSO) were administered as a bolus injection. Twenty minutes later, livers were snap-frozen and purified plasma membranes (PPM) were prepared by ultracentrifugation from liver homogenates; then, by treating PPM with Triton X-100 buffer we obtained purified detergent-soluble (non-raft) and detergent-insoluble (raft) fractions. Western blots from PPM, non-raft and raft samples were made to estimate the total expression of NOX2 in PPM as well as its distribution between microdomains. Caveolin and clathrin expression served as purity markers of raft and non-raft fractions, respectively; actin expression served as load control for PPM. E17G diminished the control PPM expression of NOX2 (1.86±0.08 vs 1.44±0.20; arbitrary values, mean±SEM, n=4; $p<0.05$). Early data suggest that NOX2 is expressed in liver PPM rafts and that E17G leads to a NOX2 enrichment in non-rafts. As we described previously for Mrp2, we hypothesize that E17G could induce migration of NOX2 from rafts to non-rafts, where it is activated and possible endocytosed, thus explaining the decrease in the PPM expression.

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Chairs: Florencia Giliberto - Viviana Dalamon

142. (117) **CHROMOSOME MOSAICISM: METHODOLOGICAL CHALLENGES TO ARRIVE AT DIAGNOSIS**

Claudia Terada[1], Manuel Daroqui[1], Edgardo Baialardo[1], Pablo Ramirez[1], Natalia Perez Garrido[1], Roxana Marino[1], Malena Quarracino[1], Silvia Caino[1], Angélica Moresco[1], María Gabriela Obregon[1], Alicia Belgorosky[1,2], Cristina Alonso[1], Elisa Vaiani[1], Esperanza Berensztein[1,3].
[1]Hospital de Pediatría "Prof. Dr. Juan P Garrahan", [2]Consejo Nacional de Investigaciones Científicas y Técnicas, [3]2a Unidad Académica, Departamento de Histología, Embriología, Biología Celular y Genética, Facultad de Medicina, Universidad de Buenos Aires

Differences of sex development (DSD) with asymmetrical overgrowth is a very rare condition secondary to a chromosomal mosaicism (ChM). ChM is usually a post zygote event and arises when two or more cell lines with different chromosomal constitutions are present in the same individual. Complex methodologies approach is required to reach the diagnosis. Our objective is to describe a patient with asymmetrical overgrowth and DSD and the methodological strategy applied/developed to achieve the patient diagnosis. The patient was born from non-consanguineous parents, at term. Birth weight was 3,250 g. The baby was assigned female sex and was referred at 11 months-old for atypical genitalia and body asymmetries. Physical examination showed dysmorphic features, right hemi-hypertrophy of the face, and left hemi-hypertrophy of upper and lower limbs, cutaneous hypo and hyper- pigmented lesions following Blaschko lines. She presented genital tubercle hypertrophy, labioscrotal asymmetry with urogenital sinus and a palpable right gonad. Pelvic ultrasound showed a lateralized-to-the-left uterus and an intra-abdominal gonad on the left side. Gonadal biopsies showed a right dysgenetic testis and a left ovary. Serum LH/FSH (2.9/3.8 UI/l) and estradiol (44 pg/ml) were according to female reference values. Post hCG-testosterone was 6.3 ng/ml, suggesting the presence of interstitial testicular tissue. The peripheral blood lymphocytes karyotype was 46,XX in 40 metaphases (M), while in fibroblast cell culture of a skin biopsy it was 46,XX (36 M)/69,XXY (6 M) mixoploidy. Molecular diagnosis of SRY resulted negative in peripheral blood lymphocytes, but positive in dysgenetic testis and in skin biopsies. In conclusion, when diagnosis of mixoploidy is suspected and diploid karyotype is found in peripheral blood, chromosome analysis in other tissues should be carried out. In the present case, the described 46,XX/69,XY myxoploidy is the most probable cause of DSD.

143. (228) **REPORT OF A BETHLEM MYOPATHY CASE ASSOCIATED WITH *COL6A3***

Carmen Llames Massini [1,2], Micaela Carcione [1,2], Chiara Mazzanti [1,2], Leonela Luce [1,2], Triana Visconti[1,2], Macarena Bollana[1,2], Florencia Giliberto[1,2].
[1] Laboratorio de Distrofinopatías, Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
[2] Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET - Universidad de Buenos Aires, Buenos Aires, Argentina.

Muscular dystrophies (MD) are a group of rare inherited diseases that cause weakness and progressive degeneration of skeletal muscle. Since the clinical symptoms of MD overlap, it is important to carry out molecular studies to obtain a differential diagnosis and thus establish the standard of care. We describe the case of a mother and two children with symptoms consistent with hereditary myopathy, characterized by waist weakness and keloid scarring. The present work aims to identify the genetic alteration associated with this clinical picture. Whole exome sequencing (WES), variant screening, Sanger sequencing, and intrafamilial segregation of candidate variants were performed. From WES, a variant was found in *COL6A3*, whose alterations are associated with autosomal dominant and recessive inheritance of Limb-girdle MD. A missense variant NM_004369.3:c.6185G>A was identified in *COL6A3*, reported 2

times in LOVD3, classified as pathogenic, and absent in gnomAD. In addition, bioinformatic predictors determined a deleterious effect. Intrafamilial segregation identified the variant in the 3 individuals studied consistent with an autosomal dominant mode of inheritance. In conclusion, the detailed evidence allowed the *COL6A3* variant to be associated with family symptoms. This work highlights the importance of using predictive programs and databases in conjunction with segregation studies to reach an accurate diagnosis.

144. (232) **DIFFERENTIAL DIAGNOSIS OF IDENTICAL TWINS WITH CLINICAL SUSPICION OF LIMB-GIRDLE MUSCULAR DYSTROPHY**

Triana Visconti[1,2], Micaela Carcione[1,2], Chiara Mazzanti[1,2], Macarena Bollana[1,2], Carmen Llames Massini[1,2] ,Leonela Luce[1,2], Florencia Giliberto[1,2].

[1] Laboratorio de Distrofinopatías, Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

[2] Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET - Universidad de Buenos Aires, Buenos Aires, Argentina.

Muscular Dystrophies (MD) are a heterogeneous group of genetic diseases that cause progressive degeneration and weakness of skeletal muscle. They are caused by molecular alterations in genes that encode structural proteins or those necessary for the stability and proper functioning of muscle fibers. Among them is Limb-girdle Muscular Dystrophy (LGMD). This work describes the case of two 7-year-old identical twins who presented muscular weakness, amyotrophy, Gowers sign +, CK of 242IU/L and a clinical suspicion of LGMD. The aim was to identify the genetic alteration associated with the clinical picture of the patients. The diagnostic algorithm was based on a whole exome sequencing (WES) study, gene variant filtering associated with LGMD, Sanger sequencing, and intrafamilial segregation of the candidate variant. From the algorithm used, the variant NM_001848.2:c.868G>A was found in *COL6A1*, in heterozygosis. It is a missense variant that generates the change of glycine for arginine (NP:001839.2:p.Gly290Arg). This substitution is not reported in gnomAD, but it is reported in the "Leiden Open Variation Database" associated with patients with LGMD and classified as pathogenic. Variants in *COL6A1* are associated with LGMD with autosomal dominant and recessive inheritance (Ullrich congenital muscular dystrophy, OMIM:158810 and Bethlem myopathy, OMIM:254090). To corroborate the inheritance mode, an intra-familial segregation study was carried out. Therefore, it was possible to determine that the twins were the only carriers of the variant under study. To conclude, the molecular alteration associated with the patient's clinical picture was identified, resulting in a *de novo* variant concordant with an autosomal dominant mode of inheritance. Finally, this work highlights the effectiveness of the diagnostic algorithm used for the detection of disease-associated variants.

145. (270) **CHARACTERIZATION OF UNIPARENTAL LINEAGES IN A FORENSIC POPULATION OF THE AUTONOMOUS CITY OF BUENOS AIRES (CABA)**

Marcela Azcurra[1], Mariela Caputo[2,3], y Daniel Corach[2,3].

[1] Departamento de Química Legal, Morgue Judicial, Poder Judicial de la Nación, Buenos Aires, Argentina, [2] Facultad de Farmacia y Bioquímica, Departamento de Microbiología, Inmunología y Biotecnología, Centro de Referencia en Identificación Genética Humana, Servicio de Huellas Digitales Genéticas y Cátedra de Genética Forense, Universidad de Buenos Aires, Buenos Aires, Argentina, [3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Objetive: Studying the ethnic diversity of National Judicial Morgue forensic sample through uniparental lineages. Materials and methods: Two hundred cadaveric blood samples (142 male and 58 female) were collected from the Obduction Room of the Judicial Morgue of the Nation from CABA, over a period of 48 months. DNA extraction was performed using modified CTAB and Proteinase K/SDS techniques and quantified by Real Time PCR. The determination of maternal lineage by mitochondrial Native American (NA) haplogroups (A2, B2, C and D1) and of the paternal by Y chromosome (R1b1b2, J2, G2a, Q1a3a, I or E1b1) was carried out by typing SNPs through Real Time PCR followed by high resolution melting. Statistical evaluation was performed using the Chi square test with Yates continuity correction. Results: The presence of NA haplogroups of maternal lineage was evidenced in 63.9% (11.7% A2; 17.3% B2; 20.8% C and 14 .2% D1). A paternal lineage prevalence of 8% of paternal NA (Q1a3a) and 92% of non-Native American (NoNA) origin was revealed (45.7% R1b1b2; 9.4% J2; 3.6% G2a, 9.4% I and 10.9% E1b1b). Conclusions: A percentage of maternal NA component slightly higher was found in the studied group than that reported for the central zone of the country (44.4%-46.6%) and La Plata (45.6%), these differences being statistically significant ($p < 0.001$).
When compared with north western (62.6%-65.6%) and southern regions of Argentina (65.2%-65.6%), no significant differences were observed. Regarding the paternal lineage, 8.0% of the NA haplogroup was evidenced, with no significant differences with respect to what was published for Argentina (4.5%), Buenos Aires (5.1%) and/or urban area of La Plata, Buenos Aires (10.6%). These differences can be explained because the exact proportion of the ancestral component differs according to the sample places and could be related to current migratory flows.

146. (319) **CASES REPORT: CLINICAL, MOLECULAR AND GENETIC CHARACTERIZATION OF ARGENTINEAN CASES DIAGNOSED WITH COLORECTAL CANCER PREDISPOSING SYNDROMES**

Julieta Natalia Soarez [1, 4] , Andrea Constanza Mayordomo [1, 4], Carla Vaccaro [1, 4] , Alisa Olkinuora [2], Josefina Fuhr [1, 4] , Tamara Alejandra Piñero [1, 4], Hernán Garcia Rivello [3], Pablo Germán Kalfayan [4] , Carlos Alberto Vaccaro [1, 4] , Päivi Peltomäki [2] , Walter Hernán Pavicic [1, 2, 4]

1. Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) (HIBA-IUHI-CONICET), CABA, Argentina; 2. Biomedicum Helsinki, University of Helsinki, Helsinki, Finland; 3. Servicio de Patología del Hospital Italiano de Buenos Aires (HIBA), CABA, Argentina; 4. Programa de Cáncer Hereditario (ProCanHe) y Registro Epidemiológico (REM), HIBA, CABA, Argentina.

Lynch syndrome (LS) is characterized by DNA mismatch repair (MMR) deficiency caused by germline pathogenic (GL-*path*) variants. Some patients with suspected LS (displays a similar cancer pattern) have DNA MMR deficiencies but no detectable *path* alteration in those genes: called LS-like (LLS), and others are MMR proficient and does not exhibit microsatellite instability (MSI): called Familial colorectal cancer (CRC) type X (FCCX). Three such cases, classified as LLS (C1) and FCCX (C2-3) based on clinicopathological data, cancer family history and MMR status by immunohistochemistry (IHC), were identified in our local referral register (ProCanHe-REM, HIBA, ARG). However, genetic aetiology has not been elucidated. The aim of our work was to carry out a detailed molecular and genetic characterization to achieve its correct classification. IHC results: while C1 tumor showed no MSH2/6 proteins expression, C2/C3 conserved all four MMRs. MSI analysis (BAT25/26-D2S123-D5S346-D17S250 markers): C1 MSI-high status (3/5) and C2/C3 MSS (stable). MMR-MLPA analysis: no large rearrangement was identified. We performed Target Cancer Panel (578 genes) GL and T seq for C1. No candidate GL variant was identified. At the somatic level, MSH6 showed Loss of Heterozygosity (LOH, strict value 2.59, position 2:48010654-48030838, Hg19), supporting IHC result. The mutational signature analysis identified five SBS signatures, linked to Homologous Recombination or MMR defects. By GL exome sequencing no candidate variant was identified for C2; but C3 showed a variant of unknown significance: BMPR1A gene (c.749T/C, 10:88676964, Hg19; f=0.0000358), *in silico* predicted *path* score 16/23. Segregation analysis (affected/non-affected relatives): variant co-segregate with CRC and high-dysplasia polyps. In sum, for C1 an extended panel, WES or RNA sequencing is required to complete classification; while for C3, combined clinical and molecular data provided evidence to suspect the variant is causative of FCCX syndrome.

147. (347) **GENETIC HETEROGENEITY IN WEST SYNDROME: A SERIES OF CASES**

María Eugenia Martín, Lenin Intriago, Matías Juanes, Gabriel Veneruzzo, Gabriela Zelaya, Giovanna Aschettino, Giovanna Flores, Gabriela Reyes, Mariana Loos, Roberto Caraballo, Cristina Alonso.
Hospital de Pediatría Prof Dr. Juan P Garrahan, Buenos Aires, Argentina.

Purpose: To present the clinical and molecular characteristics of a series of patients with diagnosis of West Syndrome (WS). Methods: We retrospectively analyzed the medical records of 24 patients diagnosed with WS, of non-lesional, metabolic nor chromosomal cause, studied through a Next Generation Sequencing panel of genes associated with epileptic encephalopathy (EE) from 2019 to 2021. Results: We identified pathogenic or likely pathogenic variants in 11 patients. Seven boys and four girls, with a median age of onset of 3 months and present age of 7.8 years. All of them had neurodevelopmental delay prior to the onset of seizures and epileptic spasms with hypsarrhythmia. In 7 of them WS was the initial form of presentation, 2 started with EE with suppression paroxysm and 2 with focal seizures. Eight patients evolved to Lennox-Gastaut syndrome, one to focal epilepsy and two patients discontinued follow-up. Regarding the molecular results, the identified variants corresponded to single nucleotide variants (n=6; 5 missense and 1 splicing) in the genes *KCNQ2* (n=2), *STXBP1* (n=1), *SCN8A* (n=1), CDKL5 (n=1) and *WWOX* (n=1; compound heterozygosity); 5 copy number variants (3 duplications and 2 deletions) involving the genes *UBE3A-GABRB3* (n=3), *SCN2A-SCN1A-SCN9A* (n=1) and *WWOX* (n=1; compound heterozygosity); and a triplet repeat expansion (n=1) in the *ARX* gene. The genetic patterns observed were autosomal dominant, recessive and X-linked. Conclusion: We reached the molecular diagnosis in 46% of the patients. The results obtained demonstrate the wide genetic variability in patients with WS, affecting different genes, with different types of variants that give place to diverse pathophysiological mechanisms that would explain the clinical phenotype of WS. A panel approach showed to be a good strategy to study these cases in our resource-limited settings. In patients without relevant findings, it would be important to complement the study with more comprehensive genomic methods.

148. (620) **PORPHYRIA CUTANEA TARDA IN ARGENTINA. AN UPDATE**

Laura Varela[1], Manuel Méndez[1], Gabriela Cerbino[1], Federico Colombo[1], Viviana Melito[1,2], Ana Buzaleh[1,2], María Rossetti[1], Victoria Parera[1]
1 CIPYP, UBA-CONICET, Buenos Aires, Argentina. 2 Biological Chemistry, FCEN, UBA, Buenos Aires, Argentina.

Porphyria Cutanea Tarda (PCT) is the most common porphyria in Argentina (prevalence 1:20,000). There are two main types: PCT-A (acquired, sporadic or type I) and PCT-H (hereditary or type II). Type I is the most common form of PCT (70-80%) and the deficiency in Uroporphyrinogen decarboxylase (URO-D) is restricted to the liver. In PCT type II blood URO-D activity is reduced by 50%. The manifestation of PCT is associated with triggering factors: alcohol consumption, hormones and iron overload. The association between PCT and other pathologies is remarkable: hereditary haemochromatosis (HH), HCV and HIV. The aim of this study was to analyze the patients diagnosed in CIPYP with PCT to date to evaluate the incidence of the different types of PCT in our population. All patients signed the Informed consent. Among 2230 PCT cases analyzed, 92% were PCT-A; 79% male and 21% female, in a 3.7:1 ratio. In the PCT-H cohort, 179 were symptomatic and 43 were latent in a 1.3:1 male:female ratio in both cases. 16.4% of the PCTs were HIV+ and 34% were HCV+. Only 4.3% were HH, 31.6% carried p.H63D (26.5% heterozygous, 5.1% homozygous). 6.8% carried the p.C282Y mutation (5.1% heterozygous, 1.7% homozygous) and 2.6% were heterozygous for both mutations. In the 84 unrelated PCT-H families, 45 different pathogenic variants were found, of which 16 were reported for the first time in CIPYP. In 23 families, the c.10-12insA variant (27%) was detected, being the most frequent in the country. The second was p.M165R (8%) and in third place p.N304K (7%), which together represent 38% of the allelic variants characterized. We also diagnosed 25 cases of childhood PCT, carrying c.10-12insA (8 cases) and p.M165R (2 cases). Although PCT-H is inherited in an autosomal dominant manner with low penetrance, there are multiple triggering factors. Genetic diagnosis is of utmost importance as it allows counselling about contact with such agents to avoid the clinical expression in latent cases.

149. (632) **EPILEPSY AND NON-EPILEPTIC PAROXYSMAL EPISODES: NOVEL GENETIC VARIANTS IN A COHORT OF ARGENTINIAN PEDIATRIC PATIENTS**

Gabriel Veneruzzo, María Eugenia Martín, Lenin Intriago, Matías Juanes, Mariana Loos, Gabriela Reyes, Eugenia Foncuberta, Giovanna Aschettino, Francisco García, Roberto Caraballo, Cristina Alonso.
Hospital de Pediatría S.A.M.I.C. "Prof. Dr. Juan P. Garrahan", Buenos Aires, Argentina.

Introduction: Monogenic Epilepsies (ME) and Non-Epileptic Paroxysmal Episodes (NEPE) are common neurological disorders in pediatric patients. Molecular studies, mainly NGS and CGH-Array, have contributed to improving the diagnostic process, to understanding underlying neurobiological mechanisms, and to determining precision therapies. Data from our population is underrepresented in genetic databases. Aim: To describe novel and clinically relevant sequence variants detected in a cohort of Argentinian pediatric patients with diagnosis of ME and NEPE. Methods: ME and NEPE patients (N:148) evaluated by the Neurology Department at Garrahan Hospital (period: 2017-2022), with at least one genetic test result were included. Novel sequence variants classified as pathogenic, likely pathogenic or of uncertain significance (ACMG 2015) were identified and reported to ClinVar database. Results: Informative genetic results were obtained in 70/135 patients with ME, and 6/13 with NEPE, identifying a total of 81 relevant variants. Of those found in the ME patients, 44% (33/75) were novel, mainly detected in genes encoding ion channel subunits (*SCN1A*, *SCN1B, SCN2A*, *SCN8A*). The second most frequent genes involved were those related to lysosomal function and endomembrane trafficking (*CLN5, DNM1, MFSD8, PLA2G6*). Additionally, novel variants were also detected in *ALDH7A1, CDKL5, CHD2, DEPDC5, GRIN1, PCDH19, SLC2A1*, *STX1B and WWOX* genes. Within the NEPE patients, one (1/6) novel variant involving *ATP1A3* was identified. Thirty-one novel and clinically relevant variants were reported to ClinVar database. Conclusion: Novel clinically relevant variants were detected in approximately half of the ME patients, in agreement with previously reported data. We emphasize the relevance of reporting novel results to the public databases to extend the genotypic spectrum of neurological disorders in pediatric patients, contributing to improving the interpretation of molecular results in genetic studies.

150. (688) **BIOINFORMATIC PREDICTION OF COPY NUMBER VARIANTS FROM NGS DATA: CLINICAL VALIDATION USING CONFIRMATORY METHODS**

Paula A. Scaglia[1,2], María Esnaola Azcoiti[1,2], Agustín Izquierdo[1,2], Bárbara Casali[1], Lourdes Correa Brito[1], Agustín Bernacchia[3], Gabriela Sansó[1], María Gabriela Ropelato[1,2]
[1]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) CONICET – FEI – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. [2]Unidad de Medicina Traslacional, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. [3]Servicio de Inmunología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

Objective: To confirm the Copy Number Variant (CNV) predictions applying DECoN algorithm to Next Generation Sequencing (NGS) data generated with our own bioinformatic pipeline. Materials and methods: We used NGS to study 215 pediatric patients with presumptive diagnoses of genetic diseases. Different inherited disease targeted capture kits (Illumina or Agilent) or Whole exome sequencing (WES by 3Billion) were performed. NGS results were processed by in house bioinformatic pipeline based on GATK best

practices™ recommendations. CNVs were evaluated using DECoN algorithm, based on coverage. ArrayCGH-SNP 4x180K (aCGH, Agilent), MLPA, or GAP-PCR followed by Sanger sequencing were used to confirm CNVs predictions. Results: DECoN algorithm predicted clinically relevant CNVs in 9 patients, 5 deletions affecting only one or part of a gene (*PAH* exon 3, *ANOS1* exons 4-6, *FBN1* exons 7-16, *HMGA2* exons 1-3, *HPRT1* exon 1, *DOCK8* gene) and 4 deletions involving several genes (67.6 Kb in 16q24.3, 267.1 Kb in Xp22.31, 520Kb in 16p12.1, and 5 Mb in 15q26.2-15q26.3). CNVs were confirmed in 7 cases by: aCGH (n=4), MLPA (n=2) or GAP-PCR followed by Sanger sequencing (n=1). In 3 cases, segregation study was extended to parents and other affected relatives. HMGA2 exons 1-3 deletion was not detected by aCGH as its design has only 1 probe hybridizing in this region, not enough to be called as CNV by the software. However, that probe showed a ratio of 1, compatible with a heterozygous deletion. In a Lesch-Nyhan patient, exon 1 of HPRT1 (Xq26.2-q26.3) was absent by PCR but present in his mother and healthy controls. Although suggestive of a deletion, this finding requires confirmation by appropriate method. Conclusions: We were able to confirm CNVs predicted by DECoN algorithm and validate its use in our NGS data analysis pipeline. This approach allowed us to diagnose 9 additional patients who would have been assumed negative if only sequence variants were considered.

151. (692) **GENETIC HETEROGENEITY IN PEDIATRIC PATIENTS WITH LEIGH SYNDROME**

Maria Paula Rodriguez, Mariana Amina Loos, Gimena Gomez, Gabriel Veneruzzo, Silvina Gomez Montoya, Maria Gabriela Obregon, Giovanna Aschettino, Bárbara Campos, Matías Juanes, Cristina Noemi Alonso, Hilda Verónica Araoz.
Hospital de Pediatría Juan P. Garrahan

Leigh syndrome (LS) is the most frequent mitochondrial disease in children. Molecular diagnosis is a great challenge since it can be caused by pathogenic variants in nuclear (nDNA) or mitochondrial DNA (mtDNA). Aim: To perform molecular characterization of pediatric patients with LS. Methods: We included 34 patients (from 32 families) with LS based on clinical, biochemical and neuroimaging data. Variants in mtDNA were studied by Sanger-Sequencing in all cases. In 15 patients, nDNA variants were analyzed by a customized NGS-panel or by exome sequencing. Parental DNA samples were analyzed as available. Fisher's exact test was used for statistical analysis. Results: Median age at onset disease was 4 months (0-48). Family history of LS or other mitochondrial disorders were present in 9/34. The most frequent initial manifestations were: psychomotor retardation/regression and seizures. During the evolution, neurological involvement was observed in all cases, and extraneurological manifestations in 16/34 patients. Lactic acid was elevated in 22/34. Pathogenic variants were identified in 14: 8/34 in mtDNA genes (4 *MT-ATP6*, 3 *MT-ND6*, 1 *MT-TK*) and 6/15 in nDNA genes (3 *SURF1*, 2 *PDHA1*, 1 *COQ4*). Variants in nDNA were biallelic, except for *PDHA1* (one hemizygous, one mosaic); 4/10 were novel. All mtDNA variants were transmitted by maternal inheritance; nDNA variants (9/10) showed autosomal recessive or X-linked inheritance. No significant differences regarding initial age, presenting manifestations and lactic acid levels were found between mtDNA- and nDNA-patients. After identifying the molecular etiologies, high doses of COQ10 (*COQ4)* and ketogenic diet (*PDHA1, MT-ND6)* were prescribed. Conclusions: Our results highlight the genetic complexity underlying LS, being *MT-ATP6, MT-ND6* and *SURF1* the most frequently involved genes. No clinical differences were observed according to the affected genome. Knowing molecular etiology was relevant for treatment options and genetic counseling.

152. (768) **ACUTE INTERMITTENT PORPHYRIA: INVOLVEMENT OF ABCG2 TRANSPORTER VARIANTS**

Tomas Santillán[1], Sebastián Lucas Yun[1], Johanna Zuccoli[2], Viviana Melito[2,3], Laura Varela[2], Victoria Parera[2], Ana Buzaleh[2,3]
1 Escuelas Técnicas ORT
2 CIPYP, UBA-CONICET
3 Departamento de Química Biológica, FCEN, UBA

Acute Intermittent Porphyria (AIP) is a metabolic disease due to an inherited Porphobilinogen deaminase (PBG-D) deficiency. Enzyme activity reduction is not enough for crisis onset that is precipitated by several factors, including therapeutic drugs. Genetic variants affect ABCG2 transporter expression, altering drugs and heme efflux. NM_004827.3:c.34G>A and NM_004827.3:c.421C>A SNV´s are present at a high frequency. The aim was to evaluate the role of *ABCG2* variants in AIP triggering. Three cohorts were included: Control (non porphyric) (N=40) and AIP patients carrying *PBGD* mutation: symptomatic at diagnosis (S-AIP) (N=20) or without manifestations (latent, L-AIP) (N=20). All subjects have given their informed consent. PCR-RFLP was performed to genotype c.421C>A variant and direct sequencing for c.34G>A. No significant differences of A allele frequency in either SNVs (c.421C>A and c.34G>A) were found and neither for the genotypic frequency of c.421C>A. However, c.34G>A genotypic frequencies differ in control (GG:60%; GA: 40%; AA:0%) respect to S-AIP (GG:72%, GA:22%, AA:6%, $p<0.01$-0.05) and L-AIP (GG: 67%, GA:22%; AA:12%, $p<0.01$-0.05). c.421C>A variant was significant different in control (CC:93%; CA:7%) vs L-AIP (CC:83%; CA:17%, $p<0.05$) and in S-AIP (CC:93%; CA:7%) vs L-AIP (CC:83%, CA:17%, $p<0.05$). AA genotype was only found in c.34G>A. The variation in genotype distribution for c.34G>A between AIP groups and control could imply a difference in the transport of drugs, ABCG2 substrates, that promote the onset of AIP plus other triggering factors. Although, no differences were found between L-AIP and S-AIP, is relevant to consider that latent condition could change to symptomatic, and this fact could be determined by gene haplotypes involved in transport/distribution of xenobiotics. The ABCG2 variants were also found in Porphyria Cutanea Tarda, thus this conclusion could be extended to other types of Porphyria associated with pharmacological triggering factors.

153. (840) **SMALL SEQUENCE VARIANTS IN *RB1* GENE AND THEIR CORRELATION WITH THE FUNCTIONAL DOMAINS OF RETINOBLASTOMA PROTEIN**

Diana Parma[1], Nicohol Tovar Matelo[1], Marcela Ferrer[2], Florencia Giliberto[1], Irene Szijan[1]
1.Cátedra de Genética, INIGEM, UBA-CONICET Buenos Aires, Argentina. 2. Servicio de Genética, Hospital de Clínicas "José de San Martín" UBA Buenos Aires, Argentina.

Retinoblastoma (RB) is the most frequent ocular pediatric tumor, it occurs by biallelic inactivation of the tumor suppressor *RB1*gene. RB may be hereditary or non-hereditary and the predisposition to RB is transmitted as an autosomal dominant trait with a 90% of penetrance. Alterations that inactivate *RB1* may be gross rearrangements or small sequence variants. The small size changes include nucleotide substitutions and indels and they encompass 80% of all *RB1* alterations. Nucleotide substitution, the most common variant, occurs by transient misalignment of one DNA strand. This alteration originates nonsense, missense or splice-site variants. Our aim was to study all the nucleotide substitutions and small indels annotated in the database http://rb1-lovd.d-lohmann.de to uncover the frequency of different variants, their correlation with the clinical presentation and their effect on the retinoblastoma protein (pRb) functional domains. The number of variants analyzed was 1748, 47% of them were nonsense, 14% missense, 31% splice-site and 7% small indels. The small germinal variants mainly gave rise to bilateral RB (72%-92%), while the somatic ones originated unilateral RB. The location of variations in pRb domains showed the highest frequency of them in the Pocket domain, a site of binding of the transcription factor E2F (58% for nonsense, 64% for missense, 50% for splice-site and 45% for small indels). The site more mutated of the consensus sequence for splicing was the first nucleotide of the donor, which is the driver of the splicing process. Moreover, the frequency of variants in the donor site vs acceptor site was 74% vs 26%. Conclusions: The highest percentage of variants was for the nonsense substitutions, followed by splice-site, missense and small indels. All germline variants occurred mostly in bilateral RB. The substitutions and small indels were mainly located in the Pocket domain, which is the major functional site for the regulatory process of pRb.

GENETICS II *Saturday, November 19, 9-10:30 hr*
Chairs: Viviana Melito - Paula Scaglia

154. (176) **DETERMINATION OF 8-OXOGUANINE AND MITOCHONDRIAL DNA MASS IN DIFFERENT OBESITY PHENOTYPES**

Rojo Mailén1,2, Millán Andrea Liliana1,2, Pautasso María Constanza2, Pérez Hernán2, Frechtel Gustavo1,2, Cerrone Gloria Edith1,2
1Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Microbiologia, Inmunologia, Biotecnología y Genética, Cátedra de Genética 2Universidad de Buenos Aires – CONICET. Instituto de Inmunología, Genética y Metabolismo (INIGEM).

Objective: Obesity is characterized by chronic inflammation and increased oxidative stress that can influence mitochondrial DNA (mtDNA) mass. Our objective was to evaluate the oxidation and mtDNA mass and its relationship with metabolic parameters in metabolically healthy obese (MHO) compared with metabolically unhealthy obese (MUO) and normal weight controls (NW). Materials and methods: 151 patients aged between 19 and 65 years were studied, of which 47 were classified as NW, 52 MHO and 52 MUO. The relative determination of the mtDNA mass and its level of oxidation (8-oxoguanine, 8-OG) were performed in peripheral blood leukocytes by real-time PCR, SYBR Green method. We used one-way ANOVA and Tukey test to compare biochemical, clinical, anthropometric characteristics, mtDNA mass, and 8-OG in each group. To evaluate the correlation between mtDNA and the different variables, a linear regression was performed in SPSS 25 with a significance level of 0.05. Results: NW have significantly lower weight, BMI, waist, SBP, TC, LDL ($p<0.05$) than MHO and MUO patients. In addition, NW presents significantly lower values of DBP, glycemia, triglycerides (TG), and higher values of HDL only vs MUO. We can also see that WHO has significantly lower TG and glycemia values and significantly higher HDL values, compared to MUO. A progressive decrease in mtDNA mass was observed between NW, MHO and MUO, (MUO vs NW $p=0.008$, age and sex adjusted). An increase in 8-OG was observed in the MUO compared to the other groups (MUO vs. MHO $p=0.04$). A correlation was observed between the increase in mtDNA mass with an increase in HDL ($p=0.003$), and a decrease in waist circumference ($p=0.001$), weight ($p=0.03$), BMI ($p=0.011$), LDL ($p=0.027$) and TG ($p=0.002$). Conclusion: Changes in mtDNA mass could be explained by damage due to oxidative stress associated with obesity and/or metabolic syndrome. More studies are required to determine the extent of the results obtained.

155. (221) **THE IMPORTANCE OF SCIENTIST´S EXPERTISE IN THE INTERPRETATION OF NGS DATA**

Micaela Carcione[1,2], Chiara Mazzanti[1,2], Macarena Bollana[1,2], Carmen Llames Massini[1,2], Triana Visconti[1,2], Leonela Luce[1,2], Florencia Giliberto[1,2].
[1] Laboratorio de Distrofinopatías, Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [2] Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET - Universidad de Buenos Aires, Buenos Aires, Argentina.

Muscular dystrophies (MD) are a group of rare inherited diseases that cause weakness and progressive degeneration of the muscle. Among them, dystrophinopathies are the most prevalent type of MD and are caused by molecular alterations in the *DMD* gene. Because of the overlap of symptoms between these diseases, genetic or molecular studies are the gold standard for reaching a MD differential diagnosis, for which molecular alterations in MD associated genes can be detected by Whole Exome Sequencing (WES). We aimed to reach a differential diagnosis in patients with MD by a thorough analysis of NGS data. We studied 200 patients with presumptive clinical diagnosis of dystrophinopathy by WES. We applied different predictive programs, conservational and protein modeling tools to establish the pathogenicity of certain variants found. We reached the molecular diagnosis of 151 patients by finding a pathogenic variant in the *DMD* gene, achieving a detection rate of 76%. We found 1 synonymous variant and 2 missense variants that required further analysis to establish their pathogenicity. Also, we detected 2 variant calling errors among the studied individuals, where the VCF results did not resemble the alteration observed in the raw data analysis. These discordances were due to the presence of deletions in the *DMD* gene, which caused problems in the alignment process, so manual annotation was necessary. By deepening the screening to all the MD genes, we were able to identify pathogenic variants in other genes in 30 of the remaining patients, reaching a detection rate of 91%. Finally, this work highlights the importance of the expertise of the scientist in charge of the study in order to determine the pathogenicity of variants and to detect the occurrence of variant calling errors by analyzing NGS raw data.

156. (225) **DMD AND BEYOND: MOLECULAR MODIFIERS THAT ALTER DISEASE PROGRESSION IN PATIENTS WITH DYSTROPHINOPATHY**

Chiara Mazzanti[1,2], Micaela Carcione[1,2], Leonela Luce[1,2], Macarena Bollana[1,2], Carmen Llames Massini[1,2], Triana Visconti[1,2], Florencia Giliberto[1,2].
[1] Laboratorio de Distrofinopatías, Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
[2] Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET - Universidad de Buenos Aires, Buenos Aires, Argentina.

Duchenne muscular dystrophy (DMD) is one of the most frequent and severe presentation of pediatric muscular dystrophies. There is great variability in the progression of the disease between DMD patients, even affected males with the same mutation lose the ability to walk at very different ages. Recent studies point to different SNPs in *SPP1, LTBP4, CD40* and *ACTN3* as the cause of these phenotypic variations. This work focused on characterizing these genetic modifiers in the DMD/DMB argentine cohort and validating their prognostic value. Of a total of 60 DMD patients, two groups of extreme phenotypes were created. The first contained 30 patients with loss of ambulation before the age of 11 (severe phenotype), while the second contained 24 patients who continue walking or who have lost ambulation at ages equal to or greater than 15 (mild phenotype). Previously reported SNPs in the *SPP1, LTBP4, CD40* and *ACTN3* genes were evaluated by PCR-Sanger sequencing. The chi square test was performed to determine that the differences between the severe and mild groups were significant. *ACTN3* SNP showed a significant difference between both groups. The results of this work would indicate that the SNP rs1815739 in *ACTN3* is a modifier for DMD progression, while the SNPs proposed in the other genes couldn´t be associated. The importance of the analysis of these molecular modifiers lies in understanding the origin of the variability of clinical manifestations among individuals affected with these monogenic disorders. This will allow, on the one hand, to improve the design and evaluation of clinical trials, as well as improve patient prognosis and personalization of treatments, opening the way to the development of new molecular targets for gene therapy.

157. (244) **ESTABLISHMENT AND CHARACTERIZATION OF A RSUME KNOCKOUT MODEL UNDER NORMOXIC AND HYPOXIC CONDITIONS**

Nicolas Ciancio Del Giudice[1], David Gonilski Pacin[1], Carol Fagundez[1], Carolina Cárdenas-Figueroa[1], Mariana Fuertes[1], María Belén Elguero[1]* Eduardo Arzt[1, 2,*]
[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society, Buenos Aires, Argentina, [2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
**Equally contributed*

Rwdd3 o RSUME (RWD-containing SUMOylation Enhancer), is a protein whose gene was cloned in our laboratory. In previous works we found a regulatory role for RSUME on the HIF-VHL pathway and

also on its main related process, angiogenesis, in kidney cancer. Recent studies reveal that RSUME is connected with cardiac tissue response to ischemia and stroke. Given the complexity of these pathologies, a whole-organism approach is needed to study the role of RSUME in its target tissues. Taking this into account the aim of this work is to study the role of RSUME in the response to normoxia and hypoxia in a RSUME-knockout (KO)-mouse model. KO mice for RSUME (Tm1d) were generated in our laboratory from the genetically engineered mouse model Rwdd3tm1a. WT and TM1D mice were exposed to normoxia or hypoxia during 3 or 7 days. To study functional alterations induced by hypoxia, cardiac hemodynamic parameters such as Right ventricular pressure, Fulton index and Hematocrit were measured and key proteins for each tissue were analyzed. KO model for Rwdd3 gene was successfully developed and the absence of RSUME expression was validated. We observed a decrease in cardiac connexin43 (CX43) by Western blot (FC=0.714 p= 0.0381) and RT-qPCR in Tm1d mice (FC=0.4928 p=0.008) while the levels of Potassium channel KCNH2 expression measured by RT-qPCR were higher in Tm1d (FC=1.756 p=0.0041). We also found that heart VHL levels were lower by RT-qPCR in Tm1d (FC=0.7397 p=0.0037). Regarding cardiac parameters electrocardiogram (ECG) changes were found in KO mice for Rwdd3. Hypoxia modified hemodynamic parameters showed no differences between WT and Tm1d. These experimental results demonstrate that RSUME modulates key molecules linked with heart electrophysiology and HIF pathways.

158. (480) **UNDERSTANDING THE HUMAN GUT MICROBIOTA IN INFLAMMATORY BOWEL DISEASES**

Ayelén D. Rosso [1,2,4,5], Pablo. N. Aguilera [2,5], Sofía Quesada [1,2,5], Ma. Cecilia Cimolai [2], Ma. Jimena Cerezo [3], Renata A. Spiazzi [3], Ma. Carolina Conlon [3], Claudia Milano [3], Alberto Penas-Steinhardt [1,2,5], Fiorella S. Belforte [1,2,4,5].

[1] Lab. de Genómica Computacional, Dto. Ciencias Básicas, Universidad Nacional de Luján (GeC-UNLu), [2] Programa de Estudios de Comunicación y Señalización Inter-Reino (PECSI)-UNLu, [3] Servicio de Gastroenterología, Hospital Nacional A. Posadas, [4] Instituto de Ecología y Desarrollo Sustentable, CONICET-UNLu, [5] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Background: Inflammatory Bowel Disease (IBD) is the most common form of intestinal inflammation associated with a deregulated immune-system response to commensal microbiota in a genetically susceptible host. IBD includes ulcerative colitis (UC) and Crohn disease (CD). In the present study we aim to describe the unknown gut microbiota of IBD-patients in comparison with healthy individuals of the Argentine population. METHODS: We evaluated 13 non-IBD-controls, 20 UC-patients and 14 CD-patients from Hospital Alejandro Posadas Buenos Aires. Fecal DNA was extracted and hypervariable regions V3-V4 of the bacterial 16SR-gene were sequenced using a MiSeq-Platform and analyzed with the QIIME2 environment. Differential functional pathways were evaluated using PICRUSt. Core microbiota was defined as the set of amplicon sequence variants detected in 50--100% of the samples with a relative abundance threshold value above 0.01% (calculated with Core microbiome from R package). RESULTS: Beta diversity was significantly different between groups (UniFrac distances PERMANOVA p-value <0.05). In UC-patients we found no significant differences in alpha diversity compared to CD and non-IBD-control. However, differences in alpha diversity were found in CD compared to controls (Shannon q=0.04). The genus *Bifidobacterium* was found to be overrepresented in UC compared to controls. Our analyzes also indicate that multiple genera had a higher representation in the CD group. Finally, core microbiota and functional pathways were analyzed, finding that UC and CD have different core microorganisms with different metabolic capabilities compared to the control group. CONCLUSIONS: Overall, our study provides new knowledge on the gut microbiota composition of our population, allowing the association of local changes in gut microbial diversity in UC and CD. These novel findings would enable personalized therapies development through the use of metagenomic profiles of the Argentine population.

159. (532) **ANALYSIS OF NOVEL THYROID PEROXIDASE GENE VARIANTS FROM THE GNOMAD DATABASE USING IN SILICO BIOINFORMATICS ALGORITHMS AND LITERATURE REVIEW**

Maricel F. Molina[1,2], Mauricio Gomes Pio[1,2], Karen G. Scheps[1,2], Ezequiela Adrover[1,2], Miguel M. Abelleyro[3], Héctor M. Targovnik[1,2], Carina M. Rivolta[1,2].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Microbiología, Inmunología, Biotecnología y Genética/Cátedra de Genética. Buenos Aires, Argentina.

[2]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Buenos Aires, Argentina.

[3]CONICET-Academia Nacional de Medicina. Instituto de Medicina Experimental (IMEX). Buenos Aires, Argentina.

Thyroid peroxidase (TPO) is a thyroid-specific enzyme that plays a key role in thyroid hormones biosynthesis and is the major autoantigen in Hashimoto's disease, the most common organ-specific autoimmune disease. TPO catalyzes both iodination and coupling of iodotyrosine residues within the thyroglobulin molecule. Variants in TPO gene cause congenital hypothyroidism (CH) by iodide organification defect and are commonly inherited in an autosomal recessive manner. In the present study, we report a detailed analysis and bioinformatic prediction of the TPO variants reported in the Genome Aggregation Database (gnomAD) v2.1.1. 456 variants from unrelated individuals were analized using prediction tools such us PROVEAN, SIFT, PolyPhen-2, Fsplice, among others. The proportion of missense cysteine, nonsense, frameshift, and splice acceptor/donor variants were analyzed in each ethnic group included in the gnomAD v2.1.1 dataset. The results showed a clear predominance of frameshift variants in the East Asian (82%) and European (Finnish) (75%) population, whereas the splice site variants predominate in African/African Americans (99.46%), Other (96%), Latino/Admixed American (94%), South Asian (86%), European (Non-Finnish) (56%) and Ashkenazi Jewish (56%) populations with a significant p value <0.0001***. The analysis of the distribution of the variants revealed that most missense variants identified in the An peroxidase domain map in exon 8, followed by exons 11, 7 and 9. In total, 183 novel TPO variants were described (13 missense cysteine's variants, 158 missense variants involving the An peroxidase domain and 12 splicing variants) which were not reported in the literature and that would have deleterious effects on prediction programs. The estimated prevalence of heterozygous carriers of the potentially damaging variants was 1:77. In conclusion, we provide an updated and curated reference source of new TPO variants for application in clinical diagnosis and genetic counseling.

160. (638) **TDP1 PROMOTE DNA END RESECTION AT STALLED AND COLLAPSED REPLICATION FORKS AFTER GENOTOXIC INSULTS**

Néstor Aznar, Camila Gosso, Irene Larripa, Marcela González-Cid, Marcelo de Campos Nebel.

Instituto de Medicina Experimental (CONICET-ANM), Argentina.

The Topoisomerase II (Top2) poison etoposide (ETO) induces the accumulation of Top2 cleavage complexes (ccTop2), resulting in DNA end blocking adducts. Protein adducts interfere with replication. Several nucleases and hydrolases, including MRE11 and TDP1, can remove these adduct to avoid forks stalling. The relative contribution of each enzyme in this process is not clear. We evaluated the roles of MRE11 and TDP1 in the removal of ccTop2α during replication. We utilized two HeLa cell lines stably expressing a shRNA against TDP1 (TDP1kd) or a non-silencing control (NS), in the presence of a chemical inhibitor (MRE11i) of MRE11. Flow cytometry analysis of ETO-induced ccTop2α showed an increase during the S and G2-phases in TDP1kd cells (p<0.05). The MRE11i on NS cells led to similar results (p<0.05). However, there was no additive or synergic increase on MRE11i–pretreated TDP1kd cells. The induction of stalled replication forks was analyzed by the DNA fiber as-

say. ETO induced stalled replication forks in both TDP1kd cells and MRE11i-pretreated NS cells (p<0.05). However, MRE11i-pretreated TDP1kd cells and treated with ETO showed similar levels to those found in NS pretreated with MRE11i and in TDP1kd cells without the MRE11i. Lastly, as MRE11 has a primary role for the initiation of DNA end resection; we analyzed the formation of single-stranded DNA (ssDNA) by BrdU and RPA foci by immunofluorescence. ETO induced an increase of ssDNA by both markers in NS cells ($p<10^{-4}$), which were not shown in MRE11i pretreated NS cells and treated with ETO ($p<10^{-4}$). MRE11i pretreated TDP1kd cells showed basal levels of ssDNA. However, ETO-treated TDP1kd cells displayed a middle amount of ssDNA compared with NS cells treated with ETO, and NS or TDP1kd pretreated with the MRE11i ($p<10^{-4}$) before ETO. Overall, our results suggest TDP1 acts together MRE11 but later, allowing a complete long-range DNA end resection following replication fork stalls and collapses by Top2α-blocked DNA ends.

161. (668) **IMPACT OF BARIATRIC SURGERY ON ABSOLUTE TELOMERE LENGTH AND MITOCHONDRIAL DNA MASS IN OBESE PATIENTS**

Millán Andrea Liliana[1], Rojo Mailén[1], Pautasso Constanza[1], Beskow Axel[4], Gutt Susana[3], Cerrone Gloria Edith[1,2], Frechtel Gustavo Daniel[1,2]

1. Universidad de Buenos Aires. CONICET Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina.

2. Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Microbiología, Inmunología y Biotecnología. Cátedra de Genética. Ciudad Autónoma de Buenos Aires, Argentina

3. Hospital Italiano. Servicio Clínica Médica. Sección Nutrición. Ciudad Autónoma de Buenos Aires, Argentina.

4. Hospital Italiano. Servicio Cirugía General. Sección Esofagogástrica. Ciudad Autónoma de Buenos Aires, Argentina

Objectives: The main objective of the present study was to evaluate the change in the mass of mitochondrial DNA (mtDNA) and the absolute leukocyte telomere length (LTLa), in patients with obesity before and after 6 months of the bariatric surgery. The secondary objective was to evaluate the probable association of the change in these markers, with the expression of key molecules of inflammation such as TLR4 and IL1-β. Methods: 37 individuals with an indication for bariatric surgery were included. LTLa was determined in leukocytes using qPCR through standard curves by the T/S ratio. mtDNA mass, TLR4 and IL1b expression were determined by the $2^{-\Delta\Delta Ct}$ method. We evaluated the change of variables by analysis of paired samples and correlations and linear regressions between variables by SPSS V20. Results: After surgery patients showed a significant weight loss and an improvement in the metabolic lipid profile, accompanied by a decrease in inflammation determined by hs-CRP. The change in visceral fat correlated significantly with changes in blood glucose (r=0.391; p=0.020), HbA1c (r=0.448; p=0.013) and HOMA index (r=0.548; p=0.022). We did not observe changes in the expression of TLR4 and IL1-β, although the reduction in blood glucose levels and HbA1c associated with the lower expression of IL1-β (p=0,042 y p=0,048 respectively). Finally, the patients showed an increase of LTLa (p=0,004) and higher mtDNA mass (p=0.015) after surgery. The increase in LTLa mass was correlated to the decrease in hs-CRP (r=-0.71; p<0.001). On the other hand, we observed a trend between the increase in mtDNA and the decrease in hs-CRP (r=0,431; p=0,074) using the type of surgery as a covariate. Conclusions: Bariatric surgery not only leads to a decrease in weight, improvement of the lipid-metabolic profile and the inflammatory state of the obese patient in the short term, also have an impact at the cellular level, demonstrated by the increase in the size of the telomeres and mtDNA mass.

162. (750) **CLINICAL, MOLECULAR GENETIC AND BIOINFORMATIC STUDIES IN PATIENTS WITH A FAMILY HISTORY OF SUDDEN DEATH**

Romina Soledad Navarro1, Juan Ignacio Fernandez Lopez1, Monica Paola Bellazzi1, Patricia Liliana Perucca1, Marisol Delea2, Liliana Francipane3, Sebastian Menazzi3, Sabrina Valeria Lopez3, Viviana Cosentino4, Maria Amalia Elizar5, Hugo Javier Altube6, Gustavo Ontiveros7, Soledad Andersen1, Guillermo Corró1, Carlos David Bruque1.

1. Unidad de Conocimiento Traslacional Hospitalaria Patagónica, Hospital de Alta Complejidad El Calafate - SAMIC, El Calafate, Santa Cruz, Argentina.

2. Centro Nacional de Genética Médica "Dr. Eduardo E. Castilla", Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Ciudad Autónoma de Buenos Aires, Argentina.

3. División Genética, Hospital de Clínicas "José de San Martín", Ciudad Autónoma de Buenos Aires, Argentina.

4. Hospital Gandulfo, Provincia de Buenos Aires, Argentina.

5. Instituto Cardiovascular de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

6. Servicio de Cardiología, Hospital de Alta Complejidad El Calafate - SAMIC, El Calafate, Santa Cruz, Argentina.

7. Fundación para la Prevención de la Muerte Súbita FUPREMUS, Ciudad Autónoma de Buenos Aires, Argentina.

Introduction: Sudden death in patients over 40 years is commonly a result of atherosclerotic occlusion of coronary arteries. On the other hand, these events in younger patients (<35 years) are usually caused by hereditary genetic diseases (cardiac channelopathy, cardiomyopathies or aortopathy). Material and Methods: Patients (Index cases n=15) and relatives (n= 46) with cardiac familial/hereditary genetic diseases were tested using Sanger, Exome or targeted gene sequencing. The identified variants were classified according to ACMG criteria or the experts panel for each specific pathology (MYH7, FBN1, etc). The observed variants were evaluated in crystallography structure, molecular homology models generated with the Modeller 9.22 software or AlhpaFold2 structure. Stability programs were used to predict ΔΔG caused by residue changes. Results: 15 families were studied in this work. We found variants on several genes related to cardiac channelopathy, cardiomyopathies or aortopathy: ACTA2, TNNT2, MYBPC3, SCN5A, TRPM4, TTN, FBN1 and others. Bioinformatics analysis of protein structure, family history, phenotype and literature revision suggested that these mutations may be directly associated to the clinical outcomes described for each patient. Conclusion: The found variants were analyzed by means of a global study of genetic variants considering protein structure, protein stability, family history, clinical manifestations and several databases in order to determine their possible effects and their correlation with patients' phenotypes. The joint analysis of the families and the bioinformatic approach was strong enough to associate phenotypic effects to each genetic variant found.

163. (799) **NEW ALGORYTHM FOR THE CHARACTERIZATION OF NOVEL VARIANTS IN THE THYROGLOBULIN GENE: INTEGRATION OF *IN SILICO* TOOLS AND EXPRESSION ESSAYS**

Mauricio Gomes Pio, Maricel F. Molina, Ezequiela Adrover, Carina M. Rivolta, Héctor M. Targovnik.

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Microbiología, Inmunología, Biotecnología y Genética/Cátedra de Genética. Buenos Aires, Argentina.

[2]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Buenos Aires, Argentina.

Thyroglobulin (TG) is a homodimeric glycoprotein synthesized by the thyroid gland. To date, two hundred twenty-seven variations of the TG gene had been identified in humans. Thyroid dyshormonogenesis due to TG gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. The clinical spectrum ranges from euthyroid to mild or severe hypothyroidism. Missense variants represent a large number of spontaneous variations that cause human disease. Such variants can behave with heterogeneous patterns of pathogenicity, depending of the amino acids and

structures involved and the impact of the variant to create folding rearrangements. Therefore, the pathogenicity of missense mutations can be more challenging to predict. In the present work we show pathogenicity predictions of two novel variants in TG identified by our group, p.Pro2232Leu and p.Cys1282Tyr, where we combine the performance between pathogenicity prediction programs, protein modeling using ChimeraX and the gold standard protein expression system in order to accurate our knowledge in the interpretation of results using *In Silico* tools. The results show that of 20 programs, Pro2232Leu and p.Cys1282Tyr variants were defined as pathogenic by 17 and 15 programs respectively. QuimeraX analysis showed important structural changes as rupture of hydrogen's bonds and the arisement of Clashes that could affect the correct folding for both variants. To corroborate the results identified *In Silico*, we proceeded to perform directed mutagenesis on recombinant plasmids (pcDNA6-TG) and transfection of the same into HEK93T cells. The Western Blot to compare the cell lysate and supernatant showed that both p.Pro2232Leu and p.Cys1282Tyr variants produced intracellular retention. Our results show that the combination of *In Silico* prediction programs with protein modeling analysis improves and makes the identification and characterization of pathogenic variants more effective.

164. (853) **ANALYSIS OF GENETIC VARIANTS ACCORDING TO THE GIPSS PROGNOSTIC MODEL IN PATIENTS WITH MYELOFIBROSIS**

Camacho María Fernanda[1], Heller Paula[2], Enrico Alicia[3], Moiraghi Beatriz[4], Castro Rios Miguel[5], Sackmann Federico[6], Bendek Georgina[7], Vallejo Veronica[8], Varela Ana[4], Romina Montivero[9], De Luca Geraldine[2], Gutiérrez Marina[10], Flores Daiana[10], Patricio Pereyra[11], Belli Carolina[1], Larripa Irene[1]

[1]Laboratorio de Genética Hematológica, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina (ANM), [2]Instituto Lanari, Universidad de Buenos Aires-CONICET, Instituto de Investigaciones Médicas (IDIM), [3]Hospital Italiano de la Plata, [4]Hospital Ramos Mejía, [5]Centro Hematológico San Isidro, [6]Fundaleu/Centro de Hematología Pavlovsky (CHP), [7]Hospital Italiano de Buenos Aires, [8]Instituto Cardiovascular de Buenos Aires, [9]Hospital Privado de Córdoba, [10]Laboratorio Stamboulian, [11]Hospital Profesor Alejandro Posadas

Myelofibrosis (MF) is characterized by stem cell-derived clonal myeloproliferation, associated with bone marrow fibrosis. Most patients present one driver mutation in *JAK2*, *CALR* or *MPL* genes, which are mutually exclusive. The majority acquire others high molecular risk (HMR) in genes affecting epigenetic regulation (*ASXL1* and *IDH1/2*) and splicing machinery (*SRSF2* and *U2AF1*), that may be combined. The Genetically-Inspired Prognostic Scoring System (GIPSS) includes three genetic risk factors: very high risk (VHR) or unfavorable karyotype, HMR mutations and the absence of type 1/like *CALR* mutations. Our aim was to analyze the karyotypes, driver and HMR genetic variants according to the GIPPS criteria without other clinical parameters. The cohort included 84 patients (56% females) with MF diagnosed according to the 2016 WHO criteria. Driver mutations were 45% *JAK2* (p.V617F), 20% Type 1 *CALR* (p.L367fs*46), 5% Type 2 *CALR* (p.K385fs*47), 8% *MPL* (p.W515L/K) and 21% triple-negatives. Genomic DNA samples were analyzed using allele-specific-primers for *IDH1/2* (exon 4), Sanger sequencing for ASXL1 (exon 12-13) and high-resolution melting confirmed by Sanger sequencing for *SRSF2* (exon 1) and *U2AF1* (exon 2). HMR variants were detected in 32 patients (38%), 7 (8%) of them with ≥2 variants. *ASXL1* was characterized by frameshift or nonsense variants (n23) while the remaining by missense changes in *IDH1* (n1, p.R132H), *IDH2* (n5, p.R140Q), *SRSF2* (n7, p.P95H/L) and *U2AF1* (n4, p.Q157P/R). Abnormal karyotypes were identified in 10 (12%) patients: 6 VHR and 4 unfavorables. The overall survival was 86 months with a median follow-up of 25 months (1-182). The GIPSS score differentiated: High, Intermediate and Low risk (median survival: non reached vs 86 vs 27 months, respectively, p=0.029). No differences were observed between intermediate findings (Int-1 vs Int-2, p=0,866). Our results support the GIPSS model as a prognostic tool to risk-adapt therapy in our MF population.

HEMATOLOGY *Thursday, November 17, 14-15:30 hr*
Chairs: Romina Maltaneri - Rosana Marta

165. (159) **RED BLOOD CELL SENESCENCE IN CHAGAS DISEASE**

Rocío Stampone[1], Antonella Pacini[1], Alejandra Ensinck[2], Brenda Dinatale[1], Ana Rosa Pérez[1], Carlos Cotorruelo[1], Silvina Villar[1]

[1]Instituto de Inmunología Clínica y Experimental Rosario (IDICER-CONICET-UNR), Rosario, Argentina
[2]Área de Inmunología. Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina
E-mail: rociostampone@gmail.com

Red blood cells (RBC) have a life expectancy of 120 days; however, there is evidence supporting that chronic inflammatory processes can alter RBC lifespan. Chagas disease (CD) is an infectious and inflammatory condition with the possibility of modifying the half-life of RBC. This study aims to identify possible aging mechanisms of RBC in patients with CD. For this, the relationship between RBC aging and membrane marker changes which lead to the clearance of senescent erythrocytes was investigated. Our study was designed to evaluate young RBC (YRBC) and senescent RBC (SRBC) in individuals without Chronic Chagas Cardiomyopathy (Indeterminate form IND, n=4) and with Chronic Chagas Cardiomyopathy (CCC, n=4). Healthy volunteers were matched according to sex and age (Co, n=5). Flow cytometry has been applied for detection of RBC bound Immunoglobulin G (IgG), CD47 and Annexin V. RBC were gated on the basis of their forward and side scatter characteristics (YRBC: FSC high; SRBC: FSC low). The percentage of YRBC decreased in both IND and CCC vs Co. Also, the percentage of SRBC increased in both IND and CCC vs Co (*p<0.05). CD47 shows a decrease in the Median Fluorescence Intensity in YRBC and SRBC in CCC vs Co (*p<0.05). There was no significant difference in the percentage of Annexin V between patient and control groups. However, there was an increase of phosphatidylserine exposure on the cell surface in SRBC vs YRBC in both IND and CCC (*p<0.05). Although the detection of all RBC bound IgG did not show significant difference between the groups, YRBC had a lower amount of membrane bound IgG in IND and CCC vs SRC (*p<0.05). Considering the three aging-associated mechanisms studied, in SRBC, the predominant process of erythrocyte clearance in CD could be by CD47 acting as a molecular switch for controlling erythrocyte phagocytosis. In IND and CCC, comparing YRBC and SRBC, an increased in bound IgG and Annexin V was known, as promoters of the selective clearance of SRBC.

166. (175) **CONTRIBUTION OF THE AIM2 INFLAMMASOME TO CHRONIC INFLAMMATION IN PATIENTS WITH MYELOFIBROSIS**

De Luca G[1], Goette NP[1], Lev PR[1], Camacho MF[2], Castro Ríos MA[3], Sackmann F[4], Moiraghi B[5], Cortes V[6], Caula V[6], Verri V[6], Bendek G[7], Carricondo E[8], Vicente A[9], Varela A[5], Vallejos V[10], Caruso V[11], Gutierrez M[12], Larripa IB[2], Marta RF[1], Glembotsky AC[1], Heller PG[1].

1 Instituto de Investigaciones Médicas "Alfredo Lanari", Departamento de Hematología Investigación, IDIM UBA-CONICET, Buenos Aires, Argentina. 2 Academia Nacional de Medicina, IMEX CONICET, Buenos Aires, Argentina. 3 Consultorios Hematológicos, Buenos Aires, Argentina. 4 Fundaleu, Buenos Aires, Argentina. 5 Hospital Ramos Mejía, Buenos Aires, Argentina. 6 Instituto de Investigaciones Médicas "Alfredo Lanari", Departamento de Hematología Clínica, Buenos Aires, Argentina. 7 Hospital Italiano, Buenos Aires, Argentina. 8 Hospital Austral, Buenos Aires, Argentina. 9 Hospital Alemán, Buenos Aires, Argentina. 10 Instituto Cardiovascular de Buenos Aires, Buenos Aires, Argentina. 11 Hospital Posadas, Buenos Aires, Argentina.12 Laboratorio Stamboulian, Buenos Aires, Argentina.

Myelofibrosis (MF) is a myeloproliferative neoplasm associated with poor outcome. It is caused by driver (JAK2/CALR/MPL) and high-

risk mutations (HRM) coupled to a chronic inflammatory process that accentuates the disease. We previously described high levels of cell-free DNA (cfDNA) in MF patients that increase as disease progresses. cfDNA has pro-inflammatory effects being one of its targets the AIM2 inflammasome, responsible for maturation and release of IL-1β and IL-18 and involved in pyroptotic cell death. The aim of this work was to assess the activation of the inflammasome in MF patients and the role of AIM2 in monocytes. IL-18, an inflammasome product, was elevated in plasma from MF (n=66) vs. controls (P<0.0001, ELISA), reflecting in vivo inflammasome activation. Levels were higher in JAK2+ patients (P<0.01) and those with high vs low-risk (MIPSS70) categories (P<0.001). Patients with high (upper two quartiles) IL-18 had increased frequency of HRM (P<0.05), clinical complications and shorter survival (P<0.05), suggesting that IL-18 contributes to disease progression. Correlation was found between IL-18, cfDNA levels (P<0.0001), C-reactive protein (P<0.0001), and tumour burden parameter, LDH (P<0.05). Considering that monocytes are effectors of inflammation in MF, that patients monocyte counts were increased and correlated with IL-18 (p<0.05), we assessed their contribution to systemic IL-18. Patient monocytes had increased AIM2 expression (P<0.01, qPCR) and tended to release higher amounts of IL-18 after stimulation with AIM2 agonist polydA/dT (synthetic DNA) (n=10), (P=NS). Altogether, these results indicate that monocytes are a relevant source of IL-18 in MF. The novel finding of elevated IL-18 reveals its involvement in MF cytokine network. The association between cfDNA and systemic IL-18, together with monocyte AIM2 overexpression and preserved response to stimuli suggests cfDNA triggers inflammation, at least in part, through AIM2 inflammasome activation.

167. (183) **EFFECT OF ANAGRELIDE ON MEGAKARYOCYTIC CYTOSKELETON DURING PLATELET PRODUCTION. POSSIBLE GENES INVOLVED**

Cellucci AS[1,2], Lev PR[1,2], Yañuk DB[1,2], Goette NP[1], Glembotsky AC[1,2], De Luca G[1,2], Lira MC[1,3], Baroni Pietto MC[1,2], Heller PG[1,2], Marta RF[1,2].

[1] Universidad de Buenos Aires, Facultad de Medicina, Instituto de investigaciones Médicas A Lanari, Departamento de Hematología Investigación, Buenos Aires, Argentina.

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad de Buenos Aires, Instituto de investigaciones Médicas (IDIM), Departamento de Hematología Investigación, Buenos Aires, Argentina.

[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad de Buenos Aires, Instituto de investigaciones Médicas (IDIM), Departamento de Biología Molecular y Apoptosis, Buenos Aires, Argentina.

Previously, we reported increased maximum platelet diameter (MPD) in patients with Phi-negative chronic myeloproliferative neoplasms treated with Anagrelide (ANA), alpha Interferon (IFN) and ruxolitinib (Ruxo), while patients under hydroxyurea (HU) had decreased MPD. Evaluation of proplatelet (PP) morphology in normal megakaryocyte (MK) culture showed that ANA and IFN increased tips and swellings size (structures that give rise to platelets), while Ruxo and HU did not. To study possible causes of these changes during ANA treatment, two approaches were carried out A) Morphological analysis of MK cytoskeleton by immunofluorescence: ANA induced a trend toward decreased actin polarization (phalloidin-FITC), suggesting that demarcation membrane development necessary for thrombopoiesis is abnormal. Besides, microtubular disorganization was observed (anti-alpha tubulin-FITC) (Wilcoxon test p<0.05), which would interfere with PP elongation. B) To identify molecular targets responsible for ANA-induced cytoskeleton abnormalities, gene expression data from Ahluwalia (GEO database:GSE60621) was analyzed. 81 genes were found down-regulated (Log-fold change ≤3). This group was further analyzed with Cytoscape v3.8.1 to build a visualization network of associated biological processes. From three groups detected, the one corresponding to Positive regulation of phospholipase activity showed topological features that suggest high number of processes with potential relevance in MK. Within this group, CCL5, an inflammatory cytokine that stimulates PP formation, is of particular interest. We confirmed decreased CCL5 expression by qPCR in our culture conditions (Wilcoxon test, p<0.05). Our results suggest that ANA alters actin and tubulin cytoskeleton, which could be responsible for the increased tips and swelling size and could lead to increased MPD observed in patients. In addition, decreased CCL5 expression could be related to cytoskeletal PP alterations under ANA treatment.

168. (265) **EVALUATION OF THE IMPLEMENTATION OF WHOLE EXOME SEQUENCING (WES) FOLLOWED BY A VIRTUAL GENE PANEL FOR DIAGNOSIS OF INHERITED THROMBOCYTOPENIAS (IT)**

Kamiya LJ,[1] Ganiewich D,[2] Marta RF,[1] Goette NP,[3] De Luca G,[1] Basak N,[4] Veber E,[5] Donato H,[6,7] Negro F,[8] Altuna D,[9] Marti A,[10] Arrieta ME,[11] Lagrotta P,[12] Llera A,[2] Heller PG,[1] Glembotsky AC,[1]

[1]Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Médicas A Lanari, IDIM UBA-CONICET, Departamento de Hematología Investigación, Buenos Aires.

[2]Laboratorio de Terapia molecular y celular, Fundación Instituto Leloir, FIL, Buenos Aires.

[3]Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Médicas A Lanari, Departamento de Hematología Investigación, Buenos Aires.

[4] Hospital de Niños "Ricardo Gutierrez", Hematología, Buenos Aires.

[5] Hospital Pedro de Elizalde, Buenos Aires.

[6]Hospital del Niño de San Justo, Pcia. de Buenos Aires

[7]Consultorios de Hematología Infantil, Buenos Aires

[8]Sanatorio Sagrado Corazón, Buenos Aires

[9]Hospital Italiano de Buenos Aires.

[10] Hospital "El Cruce", Florencio Varela, Pcia. de Buenos Aires.

[11]Hospital Guillermo Rawson, San Juan

[12]Hospital Nacional "Profesor Alejandro Posadas", Pcia. de Buenos Aires

IT are a heterogeneous group of rare disorders caused by defects in genes involved in platelet production and function. Although 50 genes have been described, the defect is unknown in a large proportion of cases. Development of extrahematological manifestations or leukemia in certain disorders highlights the importance of accurate diagnosis allowing personalized follow-up. Introduction of NGS has revolutionized IT diagnosis. We describe our experience on IT diagnosis after implementation of WES. Patients (n=31) from 20 IT families were included after ethical approval. Clinical and platelet phenotype were obtained and WES was performed in an Illumina sequencer and analyzed following good bioinformatic practices. Variants from a virtual panel of IT-related genes were selected for curation and confirmed by Sanger sequencing. Twenty variants were found in 10 genes:*MYH9*,*NBEAL2*,*WAS*,*GP1BA*,*GP1BB*, *ACTN1*,*RUNX1*,*IKZF5*,*ITGB3*,*ITGA2B*, 6 of them were novel. According to ACMG guidelines, 6 variants were classified as pathogenic and 9 as likely pathogenic, yielding a diagnostic rate of 65% (13/20 families): MYH9-related disorder, 5 families; Gray Platelet Syndrome, 3; monoallelic Bernard-Soulier, 3; Wiskott-Aldrich, 1; ACTN1 related-thrombocytopenia, 1. The phenotype matched the genetic diagnosis in 77% of these families, while in 23% WES was essential for diagnosis, as there was no previous clinical suspicion. Variants of uncertain significance (n=5) were found in 4 other families. Analysis of the rest of the exome in cases without causative variants failed to identify candidate variants in genes currently unrelated to IT. In conclusion, combination of clinical and platelet phenotypic characterization with genomic techniques proved to be fundamental for IT diagnosis. Our diagnostic rate compared favorably with other centers, indicating the feasibility of this approach in our setting. Considering there are no similar local initiatives, this would cover unmet medical need in our region.

169. (286) **REGULATION OF ERYTHROFERRONE EXPRESSION BY ERYTHROPOIETIN IN ERYTHROID CELLS**

Julieta Villarosa Outes, María Eugenia Chamorro, Juan Ignacio Maciel Paccini, Alcira Nesse, Daniela Vittori, Romina

Maltaneri.
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto del Departamento de Química Biológica (IQUIBICEN) de la Facultad de Ciencias Exactas y Naturales. Buenos Aires, Argentina.

The recently characterized protein Erythroferrone (ERFE), produced by erythroid precursors, is known to reduce Hepcidin (Hep) levels in hepatocytes, allowing the release of intracellular Fe into the bloodstream. Previous research proposed ERFE as an indirect mediator of Erythropoietin (Epo) in hepatocytes. This mechanism plays a key role in the regulation of Fe availability for erythropoiesis. With the aim of studying the mechanism through which Epo induces ERFE expression, erythroleukemic K562 cells were treated with Epo (80 ng/mL). ERFE expression was significantly induced by Epo after 1 h, and returned to baseline levels after 24 h (RT-qPCR, a.u.: t_0=1, *t_{1h}=3.68±1.2, t_{24h}=0.98±0.24, *p<0.01 vs t_0, n=4). Inhibition of the JAK2 kinase, associated with the Epo receptor, with AG490 25 μM, impaired this effect (a.u.: C=1, *E=4.16±0.82, AG+E=1.23±0.28, *p<0.01 vs C, n=5). Similar results were obtained with PI3K inhibitor LY294004 10 μM (a.u.: C=1, *E=3.20±0.53, LY+E=1.11±0.38, *p<0.05 vs C, n=5) and mTOR inhibitor rapamycin 50 μM (a.u.: C=1, *E=2.27±0.35, Rap+E=1.38±0.27, *p<0.01 vs C, n=5). In order to evaluate the indirect effect of Epo on hepatocytes, K562 cells were cultured with Epo (80 ng/mL, 24 h), thoroughly washed and cultured another 24 h without Epo. The conditioned media were collected and added to hepatocarcinoma HepG2 cells (6 h). Hep mRNA levels appeared significantly reduced in HepG2 cells cultured with media from Epo-treated K562 cells (CM-E), compared with those obtained from untreated cells (CM-C), indicating that upon stimulation with Epo K562 cells produce a factor, consistent with ERFE expression, capable of suppressing Hep (RT-qPCR, a.u.: CM-C=1, CM-Epo=0.42±0.08, p<0.05, n=5). These findings reveal that the action of Epo on erythroid cells affects Fe metabolism. Moreover, the inductive effect of Epo decreased considerably in the presence of signaling pathway inhibitors, suggesting the participation of JAK2/PI3K/mTOR in ERFE expression.

170. (298) **MECHANISM OF ACTION OF ERYTHROPOIETIN ON HEPCIDIN REGULATION IN MACROPHAGES**

Joaquín Iolster, Romina Maltaneri, Julieta Villarosa Outes, Alcira Nesse, Daniela Vittori, María Eugenia Chamorro.
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto del Departamento de Química Biológica (IQUIBICEN) de la Facultad de Ciencias Exactas y Naturales. Buenos Aires, Argentina.

Iron (Fe) homeostasis, required for essential cellular processes, is systemically regulated by hepcidin (Hep). Fe deficiency, stress erythropoiesis or erythropoietin (Epo) administration inhibit Hep expression, increasing systemic Fe availability. We have recently described a direct effect of Epo and that of the erythropoietic intermediary erythroferrone (ERFE) on Hep regulation in hepatocytes. Since this Epo action has not been evaluated in macrophages –fundamental cells in Fe homeostasis– the aim of this work was to investigate mechanisms by which Epo regulates Hep in these cells. We previously observed the presence of the Epo receptor (EpoR) and a inhibitory effect of Epo (160 ng/mL, 6 h) on Hep mRNA levels in the THP-1 monocytic cell line differentiated to macrophages with PMA. To determine if this action occurs through the homodimeric receptor, EpoR was silenced using a specific siRNA, which prevented the suppressive effect of Epo on Hep expression (RT-PCR, a.u.: Control=0.36±0.05; *Epo=0.19±0.03; siEpoR+Epo=0.53±0.04; *siGFP+Epo=0.21±0.05, *P<0.05 vs. C and siEpoR+Epo, n=5). We later assessed the phosphorylation of Jak2 and AKT by Western blotting. An increase in Jak2 and AKT phosphorylation was determined in the presence of Epo in differentiated THP-1 cells. The indirect action of Epo was evaluated using conditioned media (MC) obtained from K562 erythroid cells treated with Epo (80 ng/mL, 24 h, MC-E), washed and incubated again in the absence of Epo (24 h). In differentiated THP-1 cells cultured with MC-E, Hep mRNA was decreased (RT-PCR, a.u.: MC=1; *MC-Epo=0.76±0.09, *P<0.05, n=3). ERFE induction was confirmed in Epo-treated K562 cells (RT-PCR; a.u.: Control=1; *Epo=3.6±0.6, *P<0.05, n=4). These results show that the decrease in Hep expression caused directly by Epo in macrophages occurs through its EpoR and the classical mediators of the Epo signaling pathway, Jak2/AKT. In addition, the indirect pathway by ERFE also decreased Hep levels in macrophages.

171. (530) **INCREASED MEGAKARYOPOIESIS AND THROMBOPOIESIS INDUCED BY PLASMA FROM MYELOFIBROSIS (MF) AND ESSENTIAL THROMBOCYTHEMIA (ET) PATIENTS**

Danila Belén Yañuk[1], Rosana Fernanda Marta[1], Adela Soledad Cellucci[1], Geraldine De Luca[1], Ailen Dalma Discianni Lupi[2], Paula Graciela Heller[1], Nora Paula Goette[2], Paola Roxana Lev[1].
[1]Instituto de Investigaciones Médicas "Alfredo Lanari", Departamento de Hematología Investigación, IDIM UBA-CONICET, [2]Instituto de Investigaciones Médicas "Alfredo Lanari", Departamento de Hematología Investigación, UBA.

MF and ET are characterized by abnormal proliferation of hematopoietic progenitors and chronic inflammation. MF presents medullary fibrosis, which confers a worse prognosis. Our objective was to evaluate the effect of plasma from MF and ET patients on megakaryo/thrombopoiesis. Megakaryopoiesis was evaluated in CD34+ hematopoietic progenitors (umbilical cord blood) cultured with TPO and 10% recalcified plasma (patients n=51, 29 MF and 22 ET; controls n=45) for 12 days. Total cell number (proliferation) and percentage of CD61 (differentiation) and CD42b (maturation) cells were quantified by optical microscopic and by flow cytometry, respectively. Thrombopoiesis was evaluated in mature megakaryocytes (MKs) cultured for 72 hours with TPO and 10% recalcified plasma. Proplatelets (PP) were counted using an inverted microscope and PP morphology was analyzed by immunofluorescence. Statistical analysis was performed using Mann Whitney and Spearman's correlation tests. An increase in MKs number and maturation was observed in the presence of plasma from MF compared to ET and control (p<0.001, p<0.0001, respectively). CD61 and CD42b percentages were higher in both pathologies compared to controls (MF: p<0.0001, ET: p<0.05). In contrast, PP count was increased in ET (p<0.001) while PP morphology was normal. According to their mutational status (JAK2V617F vs CALR), MF JAK2V617F patients showed an increase in total and mature MKs (p<0.05). A direct correlation was observed between PP count and peripheral platelet count in both pathologies (p<0.05). Exacerbated proliferation, differentiation and MKs maturation induced by MF patient plasma suggests an increase in soluble mediators that could be related to the chronic inflammatory state present in this pathology. In contrast, increased PP formation was specifically observed in ET, in accordance with elevated peripheral platelet count. These results suggest a differential profile of soluble inflammatory mediators between MF and ET.

172. (624) **REGULATION OF MEGAKARYO/THROMBOPOIESIS BY ENDOSOMAL TLR7/TLR8 ACTIVATION OF CD34$^+$ CELLS IN A VIRAL INFECTION MODEL**

Camila Sofía Rodríguez[1], Nancy Lorena Charó[1], Silvio Tatti[2], Ricardo Martín Gómez[3], Lina Paola D'Atri[1] y Mirta Schattner[1].
[1]Laboratory of Experimental Thrombosis and Immunobiology of Inflammation, IMEX-CONICET-National Academy of Medicine, Buenos Aires, Argentina. [2]Clinical Hospital, Buenos Aires, Argentina. [3]Laboratory of Animal Viruses, Institute of Biotechnology and Molecular Biology, UNLP-CONICET, La Plata, Argentina.

Background: Although they are not immune cells, hematopoietic progenitor cells (CD34$^+$ cells), megakaryocytes (MKs), and platelets (PLT) express Toll-like receptors (TLRs) that enable these cells to amplify the host innate immune response during infectious or inflammatory processes. However, the role of TLR7/TLR8, which recognizes single-stranded RNA (ssRNA) viruses, in megakaryopoiesis has not yet been investigated. Objectives: We evaluated the effect of the positive sense ssRNA Coxsackievirus B3 (CVB3) and synthetic TLR7/TLR8 agonists on human MK development and PLT pro-

duction. Methods: CD34+ cells from the human umbilical cord were exposed to CVB3 or UV-irradiated CVB3 virus or stimulated with TLR7 (imiquimod or loxoribine) or TLR8 (TL8-506 or ssPolyU) agonists and cultured in the presence of thrombopoietin. MK viability, caspase-3 activation (flow cytometry) and IL-1beta release from mature MKs was measured 12 days later (ELISA). Inflammatory markers of MKs were evaluated on day 12 of culture (flow cytometry). Results: Cell expansion, differentiation into MKs, MK maturation, and PLT biogenesis, were significantly reduced in CD34+ infected cultures (n=5, p<0.05). The reduction in MK growth was not due to an alteration in cell proliferation but was accompanied by an increase in cell apoptosis and pyroptosis (n=3-5, p<0.05). Impairment of MK generation and maturation of viable cells was completely abrogated by TLR7 but not TLR8 antagonists and mimicked by TLR7 but not TLR8 agonists (n=4, p<0.05). CVB3 infection of CD34+ cells increased immunophenotype of MKs characterized as CD148+/CD48+ or CD41+/53+ cells (n=4, p<0.05). Conclusions: These data suggest a new role for TLR7 in megakaryo/thrombopoiesis during viral infections. Our data also contribute to the new concept of MKs as cells with immune and inflammatory functions by describing a population of immune MKs resulting from TLR7 activation.

173. (811) **EXPRESSION OF *NCR2* AND THE IMMUNE CHECKPOINT *TIM3/LGALS9* IN CHRONIC MYELOID LEUKEMIA PATIENTS DURING THE FIRST YEAR OF TREATMENT WITH IMATINIB**

María Jazmín Toloza[1], Yesica Bestach[2], Marco Lincango-Yupanki[1], María Fernanda Camacho[1], Javier Bordone3, Romina Mariano[4], Melissa Tarqui[5], Mariel Pérez[6], Pedro Negri Aranguren[7], Alicia Enrico[5], Irene Larripa[1], Carolina Belli[1]

[1]Instituto de Medicina Experimental, IMEX-CONICET/ Academia Nacional de Medicina, Buenos Aires, Argentina, [2]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina, [3]Hospital de Alta Complejidad en red "El Cruce", [4]Hospital "San Martín de Paraná", [5]Hospital Italiano de La Plata, Argentina, [6]Hospital Interzonal General de Agudos "Prof. Dr. Rodolfo Rossi", La Plata, Argentina, [7]Instituto Privado de Hematología y Hemoterapia, Paraná, Argentina.

Our previous results evaluating T-cell responses showed a basal suppression in Chronic Myeloid Leukemia (CML). A slow restoration after imatinib initiation was observed with some mediators requiring more than one year to normalize. Interestingly, responses were associated with some components differentially expressed (i.e. *ARG1, TNF or IL6)*. The aim was to include *TIM3/LGALS9*, important member of the immune checkpoints in myeloid leukemia, and *NCR2,* a cytotoxicity-activating receptor that contributes to the efficiency of activated natural killer cells. Total RNA from peripheral blood samples was collected from 89 CML patients at diagnosis (n:28) or under treatment at 3 (n:50), 6 (n:50) or 12 (n:44) months classified according to the molecular response at each time. Gene expression was evaluated by quantitative real-time PCR applying the comparative method $2^{-\Delta CT}$ relative to *GAPDH* gene. Statistical analyses were performed using the InfoStat v2019 and p-values <0.05 were considered statistically significant. Baseline expression of the mediators evaluated was significantly decreased when compared with healthy donors (n:50) (Mann-Whitney test p<0.0001). On therapy, *NCR2* and *LGALS9* reached normal levels after 3 months (Kruskal Wallis test-KWt: p=0.1251 and p=0.6638, respectively) while *TIM3* continued downregulated (kWt: p=0.0172). At 6 months, *NCR2* and *LGALS9* sustained normalized levels and *TIM3* remained downregulated (kWt: p=0.0014). No differences were observed at both times among responders or not. However, at 12 months in non-responders *LGALS9* was significantly elevated (KWt: p=0.0225) and *TIM3* increased reaching control levels (KWt: p<0.0001). The obtained results complement our previous data on a suppressed immune system in CML at diagnosis and a re-activation once imatinib is initiated. Besides *ARG1* as marker of early poor responders, *LGALS9* may impair the immune system providing an ideal environment for the proliferation of CML cells in non-responder patients.

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Chairs: Rossana Ramhorst - Carolina Veaute - Luciana Balboa

174. (36) **ANGIOTENSIN II TREATMENT OF ENDOMETRIAL STROMAL CELLS MODULATES FIRST TRIMESTER TROPHOBLAST CELL FUNCTION**

Rosario Macchi[1,2], Lucía Zavattieri[1,2], Luciana Salaverry[1,2], Cecilia Parrado[1,2], Marisa Castro[1,2], Estela Rey-Roldán[1,2], Andrea Canellada[1,2]

Affiliations: 1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología, Buenos Aires, Argentina. 2. Universidad de Buenos Aires-CONICET, Instituto de Estudios de la Inmunidad Humoral Dr. Ricardo Margni (IDEHU), Buenos Aires, Argentina.

Prenatal exposure to Angiotensin Converting Enzyme inhibitors or Angiotensin II (Ang II) AT1 receptor blockers has been associated with adverse pregnancy outcomes. Given Ang-II treated T-HESC conditioned media increased trophoblast cell migration, we aim to further explore their effect on early pregnancy trophoblast function and related cytokine secretion. T-HESC were pretreated or not with either AT1R antagonist losartan (Los) (5μM) or NFAT inhibitor INCA-6 (10μM) for 30 min and/or Ang II (1.25-5x10^{-7}M) for 48 h; culture medium was replaced and after 3 days conditioned media (CM) were obtained. HTR-8/Sv neo (H8) cell invasion was assessed through transwell invasion assay; tube formation was assessed through endothelial-like tube formation assay. H8 were pretreated or not with either JNK inhibitor SP600125 (SP) (10μM) or mTOR inhibitor Rapamycin (R) (50nM). TGFβ production was assessed by Western Blot; IL-6 and VEGF production was assessed by ELISA. Differences were considered statistically significant with p<0.05. CM from Ang II-treated T-HESC increased invasion of H8 cells (p<0.0001 *vs.* control CM), effect that was inhibited by pretreatment of T-HESC with Los (p<0.0001) and by pretreatment of H8 with SP (p=0.9979 *vs.* control CM) but not with R (p<0.05 *vs.* control CM). Ang II-treated T-HESC CM was quemotractant to H8 (p<0.0001) in an AT1R- and NFAT-dependent manner. Ang II-treated T-HESC CM increased number of meshes (p<0.001), master segments (p<0.01) and master junctions (p<0.05) on tube-like H8; effect that was inhibited by pretreatment of T-HESC with Los and INCA-6. Ang II-treated T-HESC CM increased TGFβ (p<0.05) and IL-6 (p=0.0512) production in an AT1R- and NFAT-dependent manner but did not affect VEGF secretion. Ang II/AT1R signaling in endometrial stromal cells could play a role in early pregnancy, promoting migration and invasion of trophoblast cells and enhancing their capacity for spiral artery remodeling.

175. (53) **MONONUCLEAR IMMUNE CELLS DISTRIBUTION IN THE ENDOMETRIUM DURING POSTPARTUM OF DAIRY COWS**

Sofía Cainelli[1], María Belén Peralta[2], Emmanuel Angeli[1], Antonela F. Stassi[1], Hugo H. Ortega[1], Melisa ML. Velázquez[2]

[1]Laboratorio de Biología Celular y Molecular Aplicada. Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), (3080) Esperanza, Santa Fe, Argentina

[2]Instituto de Ecología Humana y Desarrollo Sustentable, Universidad Católica de Santa Fe, (3000) Santa Fe, Argentina

The processes involved in the resolution of uterine inflammation during the postpartum period are closely related to an enhanced fertility during the subsequent lactational period. However, little is known about the role of immune cell populations in the endometrial remodeling, restoration and clearance during bovine postpartum. The aim of this study was to analyze the endometrial profile of mononuclear immune populations in absence of disease during bovine postpartum, and their possible relation with delayed conception. Endometrial biopsies were taken from twenty multiparous Holstein dairy cows (second to four lactation, at 45 and 60 days in milk (DIM). All dairy cows returned to their normal cycles before 45 DIM.

Their voluntary waiting period was 70 DIM and after one they received the first insemination. Histological sections from endometrial biopsies were processed to analyze the cell immunolocation of total T lymphocytes (LT), Tγδ lymphocytes (LTγδ), B lymphocytes (LB) and macrophages (MØ) by indirect immunohistochemistry (IHC).
Dairy cows were grouped according to conception parturition interval (CPI) for comparison: CPI fewer than or equal 90 DIM ($CPI_{\leq 90}$), CPI between 90 and 120 DIM ($CPI_{90\text{-}120}$) and CPI greater than 170 DIM ($CPI_{\geq 170}$). A Generalized Linear Model (GLM) was used to analyze the mononuclear immune cells distribution in the endometrium. Immunostained cells were mainly located in the endometrial stroma. The number of MØ and LB per mm^2 at 45 DIM between the groups showed significant differences ($P<0.05$). Specifically, cows of the $CPI_{90\text{-}120}$ group showed the highest number of MØ. In contrast, cows of the $CPI_{90\text{-}120}$ group showed a lower number of LB in relation to the group $CPI_{\leq 90}$. These results suggest that MØ and LB may be part of the appropriate environment and maternal immune response mechanism necessary for there to be early post-partum conception.

176. (235) **CHRONIC INFLAMMATION OF THE MALE GENITAL TRACT IMPAIRS FERTILITY**

María Sol Martínez, Fernando Nicolás Ferreyra, Daniela Andrea Paira, Carolina Olivera, Virginia Elena Rivero, Rubén Darío Motrich.
CIBICI-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Argentina.

Urogenital inflammation has been proposed as a cause of male infertility as epidemiological studies revealed that it underlies at least 15% of male infertility cases. However, supporting evidence from animal models is scarce. Herein, we analyzed the development of Experimental Autoimmune Prostatitis (EAP) and its impact on fertility. C57BL/6 male mice were immunized with prostate antigens (PA) or saline on days 0 and 15. At day 24, males were mated with BALB/c female mice and different fertility parameters and uterine immune changes that occur after insemination were analyzed. Male mice were euthanized on day 26 and the specific immune response and prostate histopathology were assessed. Chronic pelvic pain development was evidenced by increased allodynia responses in PA-immunized male mice. Furthermore, significantly increased PA-specific lymphoproliferative responses with IFNg and IL17 secretion ($p<0,0001$) together with marked prostate periglandular macrophage and CD4+ T cell infiltration and tissue inflammatory lesions were observed. None of these changes were present in control mice. Interestingly, mating experiments revealed significantly decreased fertility indexes and augmented rates of pre- and post-implantation embryo loss in female mice mated with PA-immunized C57BL/6 males with respect to controls ($p<0,05$). Remarkably, these females showed alterations in the immune cell changes that physiologically occur in uterine mucosa after insemination such as significantly increased infiltration of macrophages, dendritic cells, NK cells and CD4+ T cells ($p<0,05$). Our results indicate that PA/specific Th1/Th17 immune responses underlie EAP associated chronic pelvic pain and prostate inflammation development. Of clinical interest, chronic inflammation of the prostate significantly impairs fertility by reducing the fertilizing ability of sperm, altering the uterine immune response triggered after insemination, and increasing embryo loss.

177. (274) ***Ureaplasma urealyticum* AND *Mycoplasma hominis* UROGENITAL INFECTIONS ASSOCIATED WITH SEMEN INFLAMMATION AND DECREASED SPERM QUALITY**

Daniela Andrea Paira[1], Carolina Olivera[1], María Sol Martinez[1], Fernando Nicolas Ferreyra[1], Andrea Tissera[2], Rosa Molina[2], José Olmedo[3], Virginia Elena Rivero[1], Héctor Alex Saka[1], Rubén Darío Motrich[1].
[1] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. [2] Laboratorio de Andrología y Reproducción (LAR), Córdoba, Argentina. [3] Fundación Urológica Córdoba para la Docencia e Investigación Médica (FUCDIM), Córdoba, Argentina.

Ureaplasma urealyticum (UU) and *Mycoplasma hominis* (MH) are among the most prevalent sexually transmitted infections proposed to induce urogenital inflammation and impair sperm quality. However, the topic remains controversial since contradictory findings have been reported. Herein, we performed a comprehensive analysis of UU and MH urogenital infections and their association with semen inflammation and sperm quality parameters in a cohort of men with couple's primary infertility undergoing initial infertility evaluation or with lower urinary tract symptoms. A total of 212 patients were included. Semen samples were collected by masturbation and the detection of common uropathogens was performed by PCR. Semen analysis was assessed according to the WHO manual. Levels of IL-8, TNFα, IL-1β, IL-6, IFNγ, IL-10 and IL-17A and leukocyte cell subsets were analyzed by ELISA and flow cytometry, respectively. Data were analyzed using the non-parametric Mann-Whitney or Kruskal-Wallis tests and the chi-square test as appropriate. The prevalence of UU and MH infection was respectively 17.0% and 23.6%. In addition, a similar prevalence of both infections was found when patients were divided by the presence or absence of clinical symptoms. Moreover, infections associated with elevated semen levels of TNFα, IL-1β, IL6 and/or increased counts of total leukocytes and their subsets, including CD4 and CD8 T lymphocytes and neutrophils. In addition, MH infection and the co-infection with UU associated with impairments in sperm quality variables. Our results indicate that UU and MH urogenital infections induce urogenital inflammation and decrease sperm quality thus impairing male fertility potential. Screening for these infections and performing a comprehensive analysis of leukocyte subsets and cytokines in semen would be clinically helpful in the diagnosis and follow up of male urogenital infection.

178. (335) **RECURRENT IMPLANTATION FAILURE PATIENTS DISPLAY AN IMBALANCE IN DECIDUAL AND SENESCENT CELLS MARKERS**

Laura Fernández[1], Lara Castagnola[1], María Soledad Gori[1], Elizabeth Soczewski[1], Esteban Grasso[1], Guillermina Calo[1], Marcela Irigoyen[2], Gustavo Martínez[2], Claudia Pérez Leirós[1], Rosanna Ramhorst[1].
[1]Laboratorio de Inmunofarmacología, IQUIBICEN-CONICET, FCEN-UBA. Buenos Aires, Argentina. [2]Fertilis Medicina Reproductiva. Buenos Aires, Argentina.

Objectives: During the decidualization process, a subset of endometrial stromal cells (EnSCs) undergoes cellular senescence. We hypothesize that recurrent implantation failure (RIF) patients display an imbalance between both subpopulations. Since these patients have been reported to show alterations in endoplasmic reticulum stress (ERS), we studied the link between both senescence and ERS using an *in vitro* model. Materials and methods: Deiodinase 2 (DIO2), Lumican (LUM) and Clusterin (CLU) were evaluated as markers for the senescent subset, while Ferritin (FTL) was used to evaluate the decidual one. Forkhead box protein O1 (FOXO1) was tested as a key transcription factor involved in the divergence of the subpopulations. Gene expression was tested in endometrial biopsies from fertile women, RIF patients and Human Endometrial Stromal Cell line (HESC) by RT-qPCR. To evaluate how ERS affects senescence in EnSCs, HESC cells were ERS induced using 1µg/ml of thapsigargin (Tg) for 4h. Then, stimulus was washed, and supernatants and RNA were recovered after 48 h *$p<0.05$ was considered significant. Results: RIF biopsies showed a downregulation of the transcription factor FOXO1* (vs fertile controls). This was associated to alterations in senescence markers DIO2* and LUM as well as decidual maker FTL*. An *in silico* analysis of EnSCs primary culture showed that HESCs *in vitro* model displayed similar expression pattern of the evaluated genes. This model also showed that ERS induction downregulated senescence markers DIO2* and LUM*, indicating that high ERS levels might pre-condition the endometrium to an initial imbalance of senescence levels and highlighting the link between both processes. Conclusion: Endometrial samples from RIF patients showed an imbalance between both senescent and decidual subpopulations, which might be related to endometrial receptivity failure. Additionally, ERS alterations modulated senescence

markers expression in HESC.

179. (552) **FUNCTIONAL ALTERATIONS IN DECIDUAL CELLS AND TROPHOBLASTS INDUCED BY *BRUCELLA* INFECTION**

Lucía Zavattieri[1,2], Rosario Macchi[1,2], M. Victoria Aramburu [1,2], Andrea M. Canellada[1,2], Mariana C. Ferrero[1,2], Pablo C. Baldi[1,2]

[1]IDEHU (CONICET-UBA), [2]Facultad de Farmacia y Bioquímica UBA, Buenos Aires, Argentina

Brucella infections are associated with reproductive complications in humans and animals. We evaluated if *Brucella* can impair functions of decidual cells and trophoblasts relevant for a successful pregnancy. Human endometrial stromal cells (THESC) were infected with different *Brucella* species (*B. abortus, B. suis, B. melitensis*) before or after decidualization with medroxiprogesterone and cAMP. Prolactin (PRL), used as a marker of decidualization, was reduced in THESC infected with *B. abortus* (500 CFU/cell) before decidualization (94±10 pg/ml vs 260±21 pg/ml), but not in cells infected after decidualization. Morphological analysis agreed with PRL observations. Similar results were obtained with other *Brucella* strains. LDH activity, a marker of cytotoxicity, was not increased at any multiplicity of infection (MOI). The effect of *Brucella* infection on trophoblasts migration was evaluated with the scratch test using the Swan71 cell line. Wound closure was significantly reduced in trophoblasts infected with *B. abortus* (MOI 50: 45%; MOI 250: 38%; MOI 500: 36% vs. 83% in uninfected controls), and with other *Brucella* strains. Factors produced by decidualized or not decidualized THESC are known to promote migration and proinflammatory mediators in trophoblasts. Wound closure was reduced in Swan71 cells treated with conditioned media (CM) from *Brucella*-infected decidualized THESC as compared to uninfected CM (20% vs. 86%). Similarly, wound closure was inhibited by CM from THESC cells infected before decidualization (8.5% vs. 72.8%). While inflammatory signals are known to promote trophoblast migration, we did not find a reduction of IL-8 (2933±125 vs 820±308 pg/ml) or IL-6 (371±2.3 vs 57±1.3 pg/ml) in CM from infected decidualized THESC used to stimulate Swan71 cells (versus uninfected CM). Anti-inflammatory cytokines are under investigation. Overall, the normal function of trophoblasts and decidual cells, and cross-talk between them, can be affected by *Brucella* infection.

180. (594) **ENDOPLASMIC RETICULUM STRESS INDUCES AN INFLAMMATORY RESPONSE DURING DECIDUALIZATION: POTENTIAL REGULATION BY miR-17-5p, miR-21-5p AND miR-193b-3p**

Elizabeth Soczewski[1], José Martín Murrieta-Coxca[2], Paulina Fuentes-Zacarias[2], Ruby Gutiérrez-Samudio[2], Lucas Miranda[3], Esteban Grasso[1], Marcelo Martí[3], Claudia Pérez Leirós[1], Diana Morales-Prieto[2], Udo Markert[2], Rosanna Ramhorst[1]

1. Laboratorio de Inmunofarmacología, IQUIBICEN-CONICET, UBA. Buenos Aires, Argentina.

2. Placenta-Labor, Jena University Hospital, Jena, Germany.

3. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

Considering that differentiation of endometrial stromal cells involves the induction of the endoplasmic Reticulum Stress (RS) and Unfolded Protein Response (UPR), here we identified and explored the contribution of miRNAs in the regulation of these processes. We used an *in vitro* model of decidualization based on the endometrial stromal cell line St-T1b, treated with 8-Br-cAMP during 5 days, or Thapsigargin (Tg, a RS-inducer). After decidualization St-T1b, increased the RS-sensors ATF6α, PERK and IRE1α, and the UPR marker CHOP, in comparison with non-decidualized cells ($p<0.05$, t-test). Moreover, thioredoxin-interacting protein (TXNIP), a link between the RS-pathway and inflammation increased in decidualized cells, and also the inflammasome NLRP3 and IL-1β expression ($p<0.05$, t-test). Then, by performing an *in silico* analysis using miRNet 2.0 we identified three miRNAs able to regulate the RS/UPR pathways and the inflammation associated to embryo implantation: miR-17-5p, miR-21-5p and miR-193b-3p. The three miRNAs significantly decreased in non-decidualized cells in the presence of Tg. Finally, to validate the present results we used endometrial biopsies from patients with recurrent pregnancy loss (RPL) and recurrent implantation failures (RIF). Using *in situ* hybridization technique in endometrial samples we confirmed the localized of the three miRNAs in stromal and epithelial glandular cells in both group of patients. In fact, stromal cells from RPL patients displayed lower intensity in comparison with those from RIF patients indicating an alteration in the regulation of the RS/UPR processes. The key message is that decidualization is accompanied by the induction RS/UPR processes associated with a sterile inflammatory response to sustain embryo implantation. We identified novels miRNAs, miR-17-5p, miR-21-5p, and miR-193b-3p that might be involved in the regulation of these processes and are differentially expressed in endometrium from RPL and RIF patients.

181. (619) **INDUCTION OF THE TOLEROGENIC DENDRITIC CELLS DC-10 INTO THE DECIDUA: THE CONTRIBUTION OF TROPHOBLAST CELLS**

Ana Schafir[1], Vanesa Hauk[1], Brenda Lara[1], Laura Fernández[1], Jorgelina Blejer[2], Alejandra Grassi Bassino[2], Esteban Grasso[1], Claudia Pérez Leirós[1], Rosanna Ramhorst[1] and Soledad Gori[1]

[1]Laboratorio de Inmunofarmacología, IQUIBICEN-CONICET, Departamento de Química Biológica, FCEN-UBA, Buenos Aires, Argentina, [2]Fundación Hemocentro de Buenos Aires, Argentina.

Immunomodulation and tolerance induction are strictly required during pregnancy. A novel and distinctive subset of HLA-G$^+$ tolerogenic dendritic cell, DC-10, was found increased in first-trimester decidua. Recently, we showed that decidualized cells can condition maternal monocytes to DC-10 profile during the pre-implantation period. Here we aimed to explore the potential contribution of DC-10 in first-trimester decidua and the impact of trophoblast cells in its induction. First, we performed an integrated bioinformatic analysis since public array-based data. We found 212 up-regulated genes in DC-10 relative to mature and immature DC ($p<0.05$) and a significant enrichment of this specific genes in first-trimester decidua CD14$^+$ cells (FDR<0.25). Interestingly, at least 10 genes are associated with characteristic processes related to pregnancy such as decidualization, implantation, and placentation, suggesting a potential role of DC-10 in these processes. Then, we evaluated the contribution of first-trimester trophoblast cells to the induction of decidual DC-10. Thus, isolated monocytes from blood of healthy women were cultured with rhGM-CSF+rhIL-4 for 5 days in the absence/presence of conditioned media (CM) of human first-trimester extravillous trophoblast cell line (HTR8). We observed that HTR8-CM inhibited the differentiation to the CD1a$^+$ immature DC ($p<0.05$, Wilcoxon test) and induced the maintenance of CD14 on monocyte-derived cells, a marker of tolerogenic DC. Also, a significant increase of CD83$^+$CD86^{low} subset was observed in HTR8-CM culture, accompanied by a higher production of IL-1β, a key implantation mediator ($p<0.05$). Notably, after HTR8-CM cultures, DC displayed a higher expression of tolerogenic markers associated to DC-10 profile, IL10 and HLA-G ($p<0.05$). The present results suggest that trophoblast cells contribute to the induction of DC-10, whose may play a key role in different processes related to embryo implantation and pregnancy maintenance.

182. (647) **THE ABSENCE OF FETAL ALLOANTIGEN INDUCES MODIFICATIONS IN PLACENTAL DEVELOPMENT, PREGNANCY OUTCOME, AND OFFSPRING SURVIVAL IN MICE**

Natalin Valeff[1], Marcos Dibo[1], María Silvia Ventimiglia[1], Federico Jensen[1]

1 Center for Pharmacological and Botanical Studies (CEFYBO-UBA-CONICET), Medical Faculty, Buenos Aires University, Buenos Aires, Argentina.

Reproductive immunology is a fascinating area of immunology that studies the cellular and molecular mechanisms occurring in the maternal immune system during pregnancy that allows the gestation of the semi-allogeneic fetus. Though recognition of paternally inherited antigens present in the fetus by maternal immune cells seems to be fundamental for proper embryo implantation as well as placentation and fetal development, syngeneic matings, where semi-allogenic antigens are absents, as animal models of pregnancy are widely used and accepted. The aim of this work was to compare syngeneic versus allogeneic pregnancies in mice in terms of fetal development as well as offspring survival. C57BL/6j females were mated with BALB/c (allogeneic matings) or C57BL/6j (syngeneic matings) males. The day of vaginal plug detection was considered gestational day (gd) 0. Pregnant females were either sacrificed on gd 14 and placental and fetal weight were assayed, or they were left to deliver, and pregnancy outcome and offspring survival were analyzed. Along with a tendency for higher fetal resorption rate, we observed diminished fetal (P*= 0.0002) and placental weight (P*<0.0001), in syngeneic (P*<0.0001) compared to allogenic pregnancies (2way ANOVA). Moreover, we also observed a significantly higher rate of stillbirth (P*= 0.0094), as well as a diminished fetal survival rate (P*=0.0011) in syngeneic compared to allogenic pregnancies (χ^2, Fisher´s exact test). This was mirrored with a tendency of lower litter size in syngeneic pregnancies. Overall, we demonstrated in this study that syngeneic pregnancies do not behave as allogeneic, naturally occurring pregnancies, highlighting the importance of choosing allogeneic matings when studying reproductive processes.

183. (697) **ALTERATIONS IN ENDOMETRIAL PROGRAMMING LEADS TO RECURRENT IN VITRO FERTILIZATION FAILURES**

Lara Castagnola[1], María Soledad Gori[1], Elizabeth Soczewski[1], Esteban Grasso[1], Laura Fernández[1], Ana Schafir[1], Marcela Irigoyen[2], Gustavo Martínez[2], Lucila Gallino[1], Claudia Pérez Leirós[1] y Rosanna Ramhorst[1]

[1]Laboratorio de Inmunofarmacología, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN) CONICET e Universidad de Buenos Aires, Argentina.

[2] FERTILIS Medicina Reproductiva, Buenos Aires, Argentina.

Embryo implantation efficiency in humans is very low because it requires a competent blastocyst during the endometrium's window of implantation (WOI). Patients with repeated implantation failure (RIF) don't reach implantation even if a competent blastocyst is transferred during WOI. Our hypothesis is that RIF patients present alterations on the decidualization program, affecting decidual cells' functions, and on the associated inflammatory response. First, we performed bioinformatic analysis in endometrial biopsies from RIF patients vs fertile, based on standardized pathways in public data bases. We focused on genes that connected and were modulated in processes involved in implantation, angiogenesis, placentation, decidualization, inflammation and immune regulation. Then, we validated expression of 15 genes with highest score in biopsies from RIF or fertile women taken on LH+7 by RT-qPCR. We found decreased IGFBP1 expression, early decidualization marker, and the progesterone receptor (p<0,0001), suggesting alterations in the decidualization program. Since inflammation is associated with decidualization, we evaluated IL1b pathway. RIF biopsies had similar IL1b expression while NRLP3 was reduced (associated with inflammasome). So, we evaluated genes involved in processes regulated by inflammation. We observed increased MUC1, avoiding blastocyst adhesion, and decreased ITGA8 preventing its attachment (p<0,0028); accompanied by decreased MMP9 levels, inhibiting trophoblastic invasion. Finally, we isolated and cultivated stromal cells from RIF samples to analyze migration ability (process that mediates blastocyst inclusion into the decidua) by wound healing assay. These primary cultures showed a differential migration pattern compared with decidualized stromal cells (with MPA and cAMP). Our results demonstrate that RIF patients display alterations in the decidualization program that might condition inflammation, attachment and migration preventing embryo implantation.

184. (778) **EFFECT OF TROPHOBLAST CELL EXOSOMES ON HUMAN HOFBAUER CELLS PHENOTYPE AND FUNCTION**

Daniel Paparini[1], Esteban Grasso[1], Brenda Lara[1], Ana Schaffir[1], Vanesa Hauk[1], Daiana Vota[1], Soledad Gori[1], Fátima Merech[1], Guillermina Calo[1], M. Agustina Arslanian[2], J. Ignacio Abasolo[2], Gustavo Izbizky[2], Rosanna Ramhorst[1] & Claudia Pérez Leirós[1]

[1] Department of Biological Chemistry, School of Sciences, University of Buenos Aires. IQUIBICEN-CONICET. Ciudad Universitaria, Pab. 2, (1428) Buenos Aires, Argentina.

[2] Obstetric Service, Hospital Italiano, Buenos Aires, Argentina

Hofbauer cells (HBC) are the only foetal immune cell population within the stroma of healthy placenta. However, the functional properties of these cells are poorly described. HBCs are transcriptionally similar to yolk sac macrophages. Trophoblast cells release exosomes (Ex) that regulate target cell function during pregnancy. Here we studied the profile of HBC modulated by trophoblast exosomes, physiological or pathological stimuli. Methodology: Trophoblast cell line (Swan 71-Tb) Ex were obtained by differential centrifugation and characterized. HBC were isolated from human term placentas (N= 25) by enzymatic digestion. They were cultured with 75-100 ng/ml Tb-Ex for 18 h to assess phenotypic profile, glucose uptake and lipid droplets formation by flow cytometry. 100 nM vasoactive intestinal peptide (VIP) or 100 ng/ml E. coli lipopolysaccharide (LPS) were used as anti or proinflammatory stimuli. HBC supernatant was used to study endothelial cell (EC) migration by wound healing assay. Results: Tb-Ex increased antiinflammatory marker CD39 (*P<0.05 *vs.* basal), without changing IL-1β production in CD14+ HBC, like the effect of VIP. HBC stimulated with LPS increased CD11c, IL-1β and CD39 expression (*P<0.05 vs. basal). Only when HBC were cultured with Tb-Ex but not with VIP, EC migration was enhanced (45±3.6% *vs.* 35±4.5%, results are expressed as Mean ± SEM, *P<0.05). Interestingly, phenotypic differences were found between HBC from female (F-HBC) and male (M-HBC) new-born placentas as a higher % of CD14/CD163+ cells in F-HBC (*P<0.05) and lower secretion of IL-1β (*P<0.05) compared to M-HBC. Also, F-HBC displayed higher CD36 expression (*P<0.05) and lipid droplets accumulation than M-HBC without changes in glucose uptake suggesting different metabolic profiles. Conclusions: Trophoblast cell exosomes promote an M2-like phenotype on HBC and endothelial cell migration, consistent with a favouring role at placentation.

185. (817) **GESTATIONAL ADMINISTRATION OF PROBIOTIC *LENTILACTOBACILLUS KEFIRI* ALTERS CYTOKINE RESPONSE AND STRUCTURAL PLACENTA ALTERATIONS IN A MOUSE MODEL OF PRETERM BIRTH**

Marcos Dibo[1], Natalin Valeff[1], Marlon Pozo-Albán[1], María de los Ángeles Serradell[2], María Silvia Ventimiglia[1*], Federico Jensen[1*]

[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO-UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

[2]Cátedra de Microbiología, Departamento de Ciencias Biológicas, UNLP, La Plata, Argentina.

** Shared senior authorship*

Fetal well-being relays on an adequately functioning placenta. It has been proven that placenta dysfunction is associated with severe pregnancy outcomes, increasing stillbirth rates as well as health risk for mother and neonate/child. We have previously reported that prophylactic treatment with probiotic *Lentilactobacillus kefiri* (Lk48) was highly effective in preventing lipopolysaccharide (LPS)-induced preterm birth and stillbirth in mice, favoring the good health and development of progeny. Moreover, we showed that Lk48 treatment ameliorated changes in placental labyrinths circulation by reducing the thickening of interhaemal membranes and neutrophil infiltration. The aim of the present study was to assess the effects *L. kefiri* on secretion profiles of cytokines from the placenta as well as vascu-

lar space quantification as a measure of the biological function in a mice model of LPS-perturbed pregnancies. C57BL/6 females were treated every 48h by oral gavage during a week with Lk48. Next, females were mated with BALB/c males. On gestational day (gd) 16, females were challenged with LPS. Tissue samples from placenta were harvest 18h after LPS injection. Levels of cytokines were evaluated on the supernatant from placental explants using ELISA kits. Histological examination and vascular space quantification of placental labyrinth were assessed by image analysis using ImageJ (www.imagej.nih.gov/ij/). Treatment with Lk48 altered cytokine production when compared to Control (t-test $p<0.05$). Placentas from LPS-treated animals showed a reduction in vascular area 18h compared to untreated animals (t-test $p<0.05$). Lk48 administration showed a tendency to prevent vascular space reduction induced by LPS (one-way ANOVA) 18h after challenge. Our results suggest that probiotic Lk48 administration modulates the immune response to LPS by placental tissues and promotes placental vascular homeostasis, suggesting its potential role in preventing adverse effects on the mother and neonate.

IMMUNITY OF TRANSPLANT

Thursday, November 17, 9-10:30 hr
Chairs: Ruben Motrich - Silvina Villar

186. (457) **CHARACTERIZATION OF AN EXPERIMENTAL MODEL OF ALLOGENEIC HETEROTOPIC INTESTINAL TRANSPLANTATION IN RATS**

I. Ivanoff Marinoff[1], R. Papa-Gobbi[1], L. Vecchio[2], N. Lausada[2], M. Machuca[3], P. Stringa[1,2], M. Rumbo[1]
[1] Instituto de estudios Inmunológicos y Fisiopatológicos (IIFP – UNLP-CONICET-CIC)
[2] Laboratorio de Trasplante de Órganos y Tejidos (LTO – UNLP)
[3] Laboratorio de Patología Especial (UNLP)

Graft rejection is one of the most common complications associated with intestinal transplantation (ITx). High doses of immunosuppressants are needed to prevent rejection but have a negative impact in the long term. Regulatory T cells (T regs) play an important role in the induction of graft tolerance. Our aim is to evaluate the frequency of T regs in the graft and blood with the kinetics of rejection in an experimental intestinal transplant rejection model using tacrolimus, one of the most used immunosuppressive agents in the clinics. Allogeneic heterotopic ITxs were performed in rats (Sprague Dawley as donor, Wistar as recipient); tacrolimus 0.6 mg/kg/day was administrated subcutaneously for 7 days as immunosuppressive therapy. Graft and blood samples were taken at 0, 4, 7, 14, 21 and 28 postoperative days (POD). Wu' score was used for histopathological diagnosis of acute cellular rejection (ACR). The frequency of T regs (defined as CD4+CD25hiFOXP3+) was determined by flow cytometry. Histopathological analysis demonstrated that tacrolimus-treated group developed moderate-severe ACR between 21 and 28 POD ($p<0.0001$). The frequency of T regs in PBMCs increased during the first 4 POD and then declined to baseline values, while in the graft an increase in T regs was observed at 14 POD and then decreased to baseline levels (or below???). Difference in the rejection kinetics and severity were observed in this group compared to previously reported models of allogenic heterotopic ITx without immunosuppressant. Although tacrolimus blocks T cell activation and inhibits the production of cytokines such IL-2, our results indicate that T regs generation in the graft and blood is not impaired by immunosuppressive treatment. Rejection progression decrease the relative proportion of Tregs in the graft.

187. (855) **EFFECTS OF AGING IN AN EXPERIMENTAL MODEL OF INTESTINAL TRANSPLANTATION. PRELIMINARY RESULTS**

María Virginia Gentilini[1], Pablo Stringa[2*], Constanza Arriola Benítez[1], Natalia Lausada[3], Jeremías Morerira[1], Ivana Ivanoff Marinoff[2], Rodrigo Papa-Gobbi[2], Martín Rumbo[2], Stefan Tullius[4], Gabriel Gondolesi[1].
*Equal contributions
[1] Instituto de Medicina Traslacional, Trasplante y Bioingeniería, IMETTyB- CONICET- Universidad Favaloro, Argentina, [2] Instituto de Estudios Inmunológicos y Fisiopatalógicos, IIFP-CONICET- Universidad Nacional de La Plata, Argentina, [3] Cátedra Trasplante de Órganos y Tejidos- Facultad de Ciencias Médicas-Universidad Nacional de La Plata, Argentina, [4] Division of Transplant Surgery, Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

Older transplant recipients experience increased incidence of infection, malignancy, and death compared with younger patients, likely because of aging-associated immune senescence. Youthful allografts could theoretically drive rejuvenation, but the data are less clear. We aimed to evaluate the effects of aging in an experimental model of intestinal transplantation (ITx) Materials and Methods. Intestinal allografts from young Sprague-Dawley rats were transplanted in Wistar rats. Based on age of the recipient, two experimental groups were performed: 1-Old Recipient (OR, >9 months, n=4) and 2-Young Recipient (YR, 2 months, n=4). Intestinal graft and blood samples were collected at 30 minutes, 5, 7, 9 and 18 postoperative days (POD) after ITx. Graft and recipient survival, histopathological analysis in search of acute cellular rejection (ACR) signs (Wu Score), intestinal absorptive capacity; Kinetics of CD4 and CD8 frequency (flow cytometry) were compared between groups. Also, donor specific allorecognition were evaluated by Mixed Lymphocyte Reaction (MLR) assay (flow cytometry). Results: Despite the kinetic of histological ACR process was similar in both groups (mild to moderate at 7 POD, severe ACR at end-point), OR survived longer (OR: 14 vs YR: 8 POD) and showed lower percentage of body reduction than YR at the clinical endpoint (O: 20% vs Y: 25%). Glycemia curves were similar in both groups, demonstrating comparable graft absorptive capacity. During the ACR, a tendency to a reduction of CD4 (OR: 12% vs YR: 35%) and an increment in CD8 frequency (OR: 65% vs YR: 180%) in the gut was observed in both groups. Finally, a donor specific CD4 and CD8 allorecognition was demonstrated by MLR assays. Conclusion: Our preliminary results suggest that there is a difference in the ACR process depending on the recipient age. This study is currently being extended with a large number of animals.

188. (861) **INTESTINAL TRANSPLANT REJECTION IS DRIVEN BY A DISBALANCE BETWEEN REGULATORY AND INFLAMMATORY IMMUNE STATUS IN THE ALLOGRAFT**

Constanza Arriola Benítez[1], Andrés Machicote[2], Luis Perez Illidge[1], Araceli Castro[3], Claudia Fuxman[3], Diego Ramisch[3], Martín Rumbo[4], Leonardo Fainboim[5], Gabriel Gondolesi[1,3], María Virginia Gentilini[1]
[1] Instituto de Medicina Traslacional, Trasplante y Bioingeniería, IMETTyB- CONICET- Universidad Favaloro, Argentina,[2] Hamburg Center for Translational Immunology, University Medical Center Hamburg, Germany, [3] Hospital Universitario Hospital Favaloro, Argentina, [4] Instituto de Estudios Inmunológicos y Fisiopatalógicos, IIFP- CONICET- Universidad Nacional de La Plata, Argentina, [5] Instituto de Inmunología, Genética y Metabolismo, INIGEM- CONICET, Argentina

Background: Acute cellular rejection (ACR) is leading cause of graft loss and morbitidy in intestinal transplant (ITx) patients. Reduction of rejection episodes and induction of immune tolerance are critical to reverse the situation. Regulatory T (T regs) cells have been shown to play a pivotal role in the prevention of rejection in other solid organ transplants. Aim: to evaluate the immunological status during the ACR focusing on the study of CD4 and CD8 Tregs. Material and methods: lamina propria cells of ileum biopsies and explants were isolated from ITx [Non-rejection (NR)=16; Rejection (Rx)= 3] and non-transplant patients [NITx=12]. CD4+ CD25+ Foxp3+ and CD8+HLD-DR+ cells were determined by flow cytometry. Total levels of functional markers of Tregs (Foxp3, TGF-β, IL-10), Th22/17 (RORγ, IL-22, IL-17A), and inflammatory response (IL-1, IL-6) in biopsies [NR=6; Rejection (Rx)=4; NITx=8] were measured by qPCR. CD4+, CD8+, Foxp3+ cells were detected by immunohistochemical

staining [NR=40; Rx= 16]. Results: Total percentage of Tregs CD4+ CD25+ Foxp3+ were decreased in ITx patients during the ACR (Rx, p=0.039) in comparison with NITx patients. Although, no changes in Foxp3+ cells were observed (p=0,30), the expression of Foxp3+ mRNA was significantly diminished (p=0,04) accompanied by a tendency to the reduction of the ratio IL-10/IL-17 (p=0,06). RORγ/ Foxp3 ratio did not show a different expression between groups (p=0,9). As was expected, CD8+ cells were increased during the ACR (p=0,026), but a reduction of regulatory CD8+HLD-DR+ cells frequency was observed. Significant relative expression of IL-1 (p=0,04) and IL-6 (p=0,01) mRNA demonstrated a proinflammatory immune status during the rejection. Conclusion: our preliminary results indicate that ITx rejection is driven by a disbalance between regulatory and inflammatory immune status in the allograft. Therapies directed to expand Tregs population can potentially be used as an approach to prevent ACR.

IMMUNOMETABOLISM

Wednesday, November 16, 13:30-15 hr
Chairs: Claudia Perez Leiros - Cinthia Stempin

189. (166) **REWIRING OF MACROPHAGE METABOLISM BY LIPID MEDIATORS DERIVED FROM OMEGA-3 FATTY ACIDS PRESENT IN TUBERCULOUS PLEURAL EFFUSIONS: ITS IMPACT ON THE CONTROL OF MYCOBACTERIUM TUBERCULOSIS INFECTION**

Joaquina Barros[1], Mariano Maio[1], José Luis Marín Franco[1], Marine Joly[1,2], Domingo Palmero[3], Xavier Aragone[3], Rafael J Argüello[4], Emilie Layre[2], Geanncarlo Lugo-Villarino[2], Olivier Neyrolles[2], Christel Vérollet[2], María del Carmen Sasiain[1], and Luciana Balboa[1].

[1] Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.
[2] Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, Toulouse, France.
[3] Instituto Prof. Dr. Raúl Vaccarezza, Hospital de Infecciosas Dr. F.J. Muñiz, Buenos Aires, Argentina.
[4] Aix Marseille Univ, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, France.

The success of *M. tuberculosis* (Mtb) as a pathogen derives from its efficient adaptation to the intracellular milieu of macrophages, entailing the regulation of their metabolic pathways. Previously, we found that M1 macrophages exposed to the acellular fraction of pleural effusions from TB patients (TB-PE) displayed a reduced glycolytic activity and an increased mitochondrial respiration by targeting HIF-1α expression, and ultimately impairing the resistance to infection. Such properties were driven by polyunsaturated fatty acids (PUFA) metabolites, and herein, we aim to identify them. For this purpose, were determined PUFA metabolites within TB-PE by LC-MS/MS. The abundances of 18-HEPE, 7(R)-Maresin 1, Protectin Dx and Resolvin D5 correlated with the inhibition of M1 macrophages' glycolysis ($p<0.05$). These lipids are omega-3-derived pro-resolving mediators. To explore their implication in the regulation of the M1 metabolism, monocyte-derived macrophages were stimulated with LPS/IFN-γ for 24h (M1 profile) in the presence or not of those lipids, and the metabolic profile was assessed by the SCENITH method. Alternatively, macrophages were infected with Mtb at the BSL-3 facility (ANLIS-MALBRAN). Unlike 7MaR1 and PDx, RvD5 and 18-HEPE reduced the glycolytic activity by M1 macrophages, leading to an increased mitochondrial respiration as well as high intracellular bacillary loads, features that could be reverted after the chemical stabilization of HIF-1α ($p<0.05$). Finally, we determined the *ex vivo* metabolism of CD14+ cells from paired TB pleural effusions and blood samples provided by the Muñiz Hospital and found that pleural CD14+ cells showed a lower glycolytic capacity and a higher mitochondrial dependency than their blood counterpart ($p<0.05$). Unraveling the mechanisms by which lipids found in a TB microenvironment can drive metabolic alterations of macrophages leading to poor local protection will significantly advance knowledge on TB immunity.

190. (295) **WNT SIGNALING PATHWAY IMPACTS ON MACROPHAGE (Mo) METABOLIC PROGRAMMING DURING *TRYPANOSOMA CRUZI* INFECTION**

Juan Nahuel Quiroz[1,2], María Belén Brugo[1,2], Ximena Volpini[1,2], Camila Fontanari[1], Cinthia Carolina Stempin[1,2], Claudia Cristina Motran[1,2].

1. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Argentina; 2. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Argentina.

The metabolic programming of immune cells is strongly associated with effector functions. Thus, while M1 Mo increase glycolysis (GLY) and reduce oxidative phosphorylation (OXPHOS); M2 Mo relies on OXPHOS and lower GLY rates. Several intracellular pathogens, like *T. cruzi* (Tc), exploits host metabolic pathways for their own benefits. Still, targeting host cell metabolism to improve Mo response against Tc represents a new paradigm for parasite control. We reported that the activation of Wnt signaling pathways is important for Tc replication inside Mo, since when the secretion of Wnts is blocked with IWPL6, the intracellular replication was inhibited. Whether Wnt signaling has the ability of modulating Tc-infected Mo metabolism is something that has not been reported. Then, we are focusing in the metabolic characterization of IWPL6- treated Mo during Tc infection. For that, bone marrow derived Mo were treated with IWP-L6 or Vehicle (Mock) for 24 h and then infected with Tc trypomastigotes. Non-infected Mo was set as control. At 72 hpi, Seahorse analyzer and fluorescent probes were used to assess the bioenergetic and mitochondrial status, respectively. Glucose uptake was estimated with glucose analogue 2-NBDG. The frequency of Mo with functional mitochondria were identify as MytoGreen^{hi} MytoOrange^{hi} F4/80+ cells. Compared to control, both mock and IWPL6-treated Tc-infected Mo displayed an increased OCR-linked basal and maximum respiration at 72 hpi ($p<0.05$) and, higher frequencies of MytoGreen^{hi} MytoOrange^{hi} F4/80+ cells were observed ($p<0.05$). Moreover, ECAR-related GLY and glycolytic capacity were reduced for Tc-infected Mo compared to non-infected controls ($p<0.05$), although they showed increased 2-NBDG uptake($p<0.05$). Nevertheless, when Wnt signaling was arrest, Tc-infected Mo showed higher glycolytic capacity than the mock-treated counterpart ($p<0.05$). In summary, Wnt signaling inhibition could switch macrophage metabolic programming during Tc infection.

191. (360) **TROPHOBLAST-MONOCYTE/MACROPHAGE INTERACTION ELICITS BIDIRECTIONAL METABOLIC REPROGRAMMING**

Fatima Merech[1], Soledad Gori[1], Graciela Reyscher[1], Daniel Paparini[1], Vanesa Hauk[1], Mariela Videla[2], Mariela Garcia[2], Rosanna Ramhorst[1], Maria Eugenia Monge[2], Daiana Vota[1] and Claudia Pérez Leirós[1]

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inmunofarmacología. Buenos Aires, Argentina. CONICET, Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.
[2]Centro de Investigaciones en Bionanociencias (CIBION), CONICET.

Bidirectional interaction of trophoblast cells (Tb) and maternal leukocytes support homeostasis maintenance at placentation. Tb modulate decidual macrophages to an anti-inflammatory profile through cytokines and other factors released. Both Tb and macrophages display highly active metabolism to adapt to variable nutrient and oxygen placental microenvironments. However, a role for metabolites as mediators in the trophoblast-macrophage interaction is unclear, entailing a new research area within Immunometabolism sustained on novel metabolomics approaches. We have shown that the conditioned media of Tb (Tb-CM) prevent LPS-induced glucose uptake in monocytes/macrophages (CD14+) and promote anti-inflammatory markers. Here we studied the trophoblast-CD14+ cell metabolic rewiring upon in vitro interaction.

Monocytes were isolated from peripheral blood of female donors by Percoll. M1 macrophages were obtained after differentiation 5+2 days with GM-CSF and E. coli LPS; M1 conditioned media (M1-CM) collected in RPMI-FBS 2% 24h later. Tb metabolism was studied in the human trophoblast cell line Swan-71. Tb-CM was collected after 18h. Glucose, long chain polyunsaturated fatty acids (LCPUFAs) uptake and lipid droplets were evaluated using D-glucose analog (2-NBDG), Bodipy FL C12 or 493/503 respectively by flow cytometry, and gene expression by RT-qPCR. Proinflammatory M1-CM induced rapid (4h) glucose uptake in Tb and increased cell migration. However, it decreased TNFa and IL1b ($p<0.05$) as well as GLUT1 expression and glucose uptake at 24h. In parallel, Tb-CM promoted anti-inflammatory CD14+ and metabolic reprogramming with higher lipid droplet accumulation and LCPUFAs uptake ($p<0.05$ ANOVA). Conditioning CD14+ with Tb-CM prevented TNF-a expression and lactate release induced by LPS (100 ng/ml) while further promoted LPUFAs uptake ($p<0.05$). Results show a bias toward lipid metabolism in Tb and monocytes displaying lower levels of proinflammatory cytokines upon interaction.

192. (539) **MITOCHONDRIAL CHARACTERIZATION OF CD8 T CELL DURING *TRYPANOSOMA CRUZI* INFECTION**

María Florencia Hellriegel, Yamile Ana, Ruth Eliana Baigorri, Matías Ezequiel Vazquez Vignale, Fabio Marcelo Cerbán, Cinthia Carolina Stempin.
CIBICI-CONICET. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba, Argentina.

We have previously demonstrated that acute *T. cruzi* infection triggers mitochondrial ROS (mROS) production and mitochondrial alterations in effector (E) CD4 T cells leading to functional changes and apoptosis. Moreover, it has been shown that mitochondrial membrane potential (MMP) and mROS levels are important for E and memory CD8 T cell function. The aim of this work was to evaluate metabolic and mitochondrial parameters in CD8 T cells during acute phase (AP) of *T. cruzi* infection. Then, spleen cells were isolated from non-infected (NI) or AP infected BALB/c mice (with 500 trypomastigotes, at 19 days post infection). Mitochondrial parameters were measured by FACS in total (Tot) and E (CD44+) CD8 T cells combining MMP-dependent (MTst) and MMP-independent mitochondrial dyes (MTgr) while mROS was tested using MitoSOX. We gated on $MTgr^+MTst^-$ cells to identified T cells with depolarized mitochondria or $MTgr^+MTst^+$ as cells with normal mitochondria. We found an increase in the frequency of CD8mROS and CD8CD44mROS producing cells in spleen of AP compared to NI mice ($p<0.005$;$p<0.05$) compatible with cell activation. However, we observed higher levels of mROS being produced by Tot and E CD8 T cells with depolarized mitochondria ($p<0.005$;$p<0.001$) and the frequency of these cells is increased in AP mice ($p<0.005$). Next, we test if PD1 expression is related to accumulation of depolarized mitochondria. In the AP of infection, a higher proportion of E CD8 T cells express PD1 and showed loss of MMP ($MTst^-PD1^+$) compared to cells with an activated phenotype that had conserved MMP ($MTst^+PD1^+$)($p<0.0001$). Besides, Tot and E CD8 T cells with loss of MMP and ROSm production exhibited reduced functional activity measured by lower levels of 2NBDG uptake, a fluorescent glucose analog, compared to cells with conserved MMP ($p<0.0005$;$p<0.005$). These results showed that study of mitochondrial parameters in CD8 cells could also be relevant to understand the immune response in *T. cruzi* infection.

193. (598) **PREGNANCY SHAPES IMMUNOMETABOLIC REPROGRAMMING OF MATERNAL CIRCULATING MONOCYTES**

Fátima Merech[1], Graciela Reyscher[1], Vanesa Hauk[1], Guillermina Calo[1], Sofía Novoa[2], Luciana Doga[3], Luciana D´Eramo[3], Pablo Fabbiano[2], Aldo Squassi[3], Rosanna Ramhorst[1], Rafael Argüello[4], Claudia Pérez Leiros[1], Daiana Vota[1].
1. Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inmunofarmacología. Buenos Aires, Argentina. CONICET, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.
2. Servicio de Obstetricia, Hospital General de Agúdos Dr. Cosme Argerich, Ciudad Autónoma de Buenos Aires, Argentina.
3. Cátedra de Odontología Preventiva y Comunitaria, Facultad de Odontología, Universidad de Buenos Aires, Buenos Aires Argentina.
4. Aix Marseille University, CNRS, INSERM, CIML, Centre d´Immunologie de Marseille-Luminy, Marseille, France.

Metabolic reprogramming of macrophages is associated to functional polarization upon environmental stimuli. Decidual macrophages display high plasticity to face opposing demands such as tolerance to embryonic antigens and efficient control of pathogens. In normal pregnancy, trophoblast cells drive macrophages to alternative M2 phenotypes. So far, the immunometabolic profile of maternal monocytes during pregnancy and the effect of infectious stimuli were not characterized. Objective: To investigate the metabolic profile of monocytes at early pregnancy and the effect of inflammatory bacterial stimuli. Methods and Results: Peripheral blood mononuclear cells (PBMC) were isolated from fertile non-pregnant and 16-20 week pregnant women by Ficoll-Paque and stimulated with 100 ng/ml LPS from E. coli or periodontal pathogens. CD14+ cell metabolism was analyzed by flow cytometry after SCENITH protocol. Briefly, metabolic inhibitors (2-Deoxy-D-glucose, Oligomycin or their combination) were added to PBMC for 15 min. Then puromicyn was added 30 min and CD14 antibody staining was followed by intracellular antipuromycin staining. Glucose and long chain polyunsaturated fatty acids (LCPUFA) uptake was assessed by flow cytometry with D-glucose fluorescent analog (2-NBDG) or Bodipy FL-C12, respectively. CD14+cells from pregnant vs. non-pregnant women showed higher glucose dependency and glycolytic capacity along with decreased mitochondrial dependency and fatty acid & amino acid oxidation capacity ($p<0.05$). CD14+cells from pregnant women displayed higher glucose uptake. LPS strongly reduced mitochondrial dependency and increased glycolytic capacity in CD14+cells from non-pregnant women ($p<0.05$). Conversely, a trend increase of mitochondrial dependency with higher LCPUFA uptake and decreased glycolytic capacity were elicited by LPS in the pregnant group. **Conclusion:** Pregnancy shapes monocyte immunometabolic profile and alter their metabolic rewiring in response to LPS.

194. (699) **METABOLIC PROFILING OF MACROPHAGE VS DENDRITIC CELL DIFFERENTIATION**

Fernando Erra Díaz[1] , Lucía Bleichmar[1], Ignacio Mazzitelli[1], Claudia Melucci [1], Jorge Geffner[1]
[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA , INBIRS, Facultad de Medicina ,UBA-CONICET

Monocytes are highly plastic cells and can differentiate into macrophages (Mo-Macs) or dendritic cells (Mo-DCs). However, the environmental signals and the mechanisms that skew monocyte fate are poorly understood. While the cytokine GM-CSF induces monocyte differentiation into MoMacs, the combination of GM-CSF + IL-4 is the canonical way to generate MoDCs. Based on our previous findings indicating that mTORC1 inhibition in human monocytes promotes Mo-DC differentiation, we hypothesized that monocyte differentiation into either Mo-Macs or Mo-DCs could be associated with differential nutrient utilization and biased metabolic activity. To gain insight into the metabolic changes associated with these processes, we performed untargeted metabolomics (HPLC/MS) of cell culture supernatants (after 6 days of culture) of human monocytes undergoing Mo-Mac or Mo-DC differentiation *in vitro* (n=6). Differentiation of Mo-Macs was induced by GM-CSF (50ng/ml) (GM-MoMacs) while Mo-DC differentiation was induced by GM-CSF and IL-4 (30ng/ml) (GMIL4-MoDCs) or GM-CSF and Temsirolimus (50nM) (GMT-MoDCs). We detected 385 metabolites, 97 of which were differentially abundant among comparisons ($p<0.05$). Unsupervised clustering of the samples clearly showed three different groups, corresponding to each of the cell populations analyzed. However, Mo-DCs metabolic profiles showed closer similarity. Looking at the shared metabolic changes between GMIL4-MoDCs and GMT-MoDCs vs GM-MoMacs, we found that monocytes undergoing

macrophage differentiation showed a higher production of the TCA cycle metabolites fumaric and aconitic acid, acetylcarnitine, 3-hydroxybutyric acid, and several lipids, while showing an increased uptake of different aminoacids, when compared to Mo-DCs. This suggests that MoDC vs MoMac differentiation is associated with a lower metabolic activity, an observation that is consistent with the regulatory role of mTORC1 on this process.

195. (882) **IMMUNOMETABOLIC REPROGRAMING OF NEUTROPHILS BY TROPHOBLAST CELLS IN AN *IN VITRO* MODEL OF EARLY MATERNAL-PLACENTAL INTERFACE**

Guillermina Calo[1], Daiana Vota[1], Florencia Sabbione[2], Fátima Merech[1], Vanesa Hauk[1], Rosanna Ramhorst[1], Analía Trevani[2] And Claudia Pérez Leiros[1]

[1] Laboratorio de inmunofarmacología, IQUIBICEN-CONICET, FCEN, UBA.

[2] Laboratorio de inmunidad innata, IMEX-CONICET, Academia Nacional de Medicina.

Trophoblast cells (Tb) interact with maternal immune cells at placentation favoring an anti-inflammatory microenvironment required for fetal growth. We have previously shown that conditioned media from Tb cells (CM) induced a proangiogenic functional profile on neutrophils (PMN) but their immunometabolic reprogramming in the context of pregnancy was not yet studied. The aim of this work was to evaluate the effect of trophoblast cell-derived factors in neutrophils' metabolic, phenotypic, and functional profile. PMN from healthy donors were cultured with human first trimester trophoblast cell line Swan71 or HTR8 CM. PMN profiles were assessed by qPCR and flow cytometry. Glucose uptake, lipid accumulation, long chain polyunsaturated fatty acids uptake reactive oxygen species (ROS) production and apoptosis were determined by flow cytometry using 2-NBDG, BODIPY 493/503, FLC12, DCFH-DA or Annexin V– Propidium Iodide respectively. PMN cultured with CM enhanced glucose uptake and lipid droplet (LD) accumulation although to a lower extent than PMA. Also, an increase in the glucose specific transporter GLUT1 and lactate dehydrogenase A was observed. Treatment with lactate -a metabolite greatly released by Tb- slightly increased ROS production (X±SE Basal 12152±1018 20mM lactate 16405±991, $P<0.05$, n=7) and LD accumulation similar to the CM, with no changes in glucose uptake. Furthermore, spontaneous apoptosis of PMN in CM was increased by the glucose metabolism inhibitor 2-DG (X±SE CM 21.4±4.7 CM+2DG 44.1±4.8, $P<0.05$, n=4) with no effect of the mitochondrial respiratory chain complex I inhibitor rotenone. Migration of PMA-stimulated PMN to CM was prevented by both inhibitors. They also inhibited ROS release by CM (X±SE CM 7516±775 CM+2DG 4549±645, $P<0.05$, n=4). PMA stimulated PMN showed similar results with the inhibitors. Our results support that immunometabolic reprogramming of neutrophils shapes the proangiogenic and deactivating effect of factors derived from Tb cells.

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Chairs: Carolina Domaica - Gabriela Lombardi - Romina Gamberale - Mariela Paz

196. (11) **IMMUNOGENICITY ASSESSMENT AND CYTOKINE PROFILE IN COVID-19 PATIENTS TREATED WITH HORSE ANTI SARS-COV-2**

Silvia Miranda[a], Javier Coria[a], Luciana Madeo[b], Adolfo de Roodt[c], Claudio Bonel[c], José Christian. Dokmetjian[c], Guillermo Di Girolamo[a] and Guillermo A. Keller[b,d].

a-Universidad de Buenos Aires- Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET). Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional (IATIMET).

b-Hospital General de Agudos Donación Francisco J. Santojanni, Departamento de Urgencias.

c- Administración Nacional de Laboratorios e Institutos de Salud (ANLIS-Malbrán), Instituto Nacional de Producción de Biológicos (INPB).

d-Universidad de Buenos Aires, Facultad de Medicina, Centro de Vigilancia y Seguridad de Medicamentos.

This study was approved in 2021 by ANMAT for the evaluation of a product ("P") -horse Fab´2 anti SARS-Cov-2-, developed by INPB (ANLIS-Malbrán). Patients with COVID diagnosis (n=20) were recruited in Hospital Santojanni to perform a blinded-randomized controlled study comparing iv administration of "P" (T group) at t=0 and t=48hs versus control (C group). Methods: 1)Plasma concentration of "P" was monitored during 28 days with a double antibody ELISA, employing unconjugated and HRP conjugated IgG anti horse and "P" as standard. Plasma "P" concentration was expressed as mg/L. 2)Antibodies anti "P" were evaluated at t=0, 14 and 28 days by an indirect ELISA where a horse IgG pepsin digest was used as antigen coating and anti human IgG-HRP as developer. Samples were loaded at dilutions 1/150 until 1/2400. Titer of anti-equine Fab'2 antibodies was expressed as the average value of the area under the curve OD/log dil. 3)Plasma levels of IL-1β, IL-6, TNFα and IFNα were assessed by a multiplex magnetic bead assay at 96hs and 14 days. Standards were plotted and concentrations determined using xPONENT version 4.2 software and expressed as pg/ml. Results: 1)Samples from the C group were identified due to the lack of circulating horse protein, while "P" was quantified in the T group from t=3hs (<36 mg/L) until t=21 days (<4.6 mg/L). 2)The presence of heterophile anti-horse immunoglobulin antibodies was detected in all samples. Titers did not show significant differences along study. 3) Plasma levels of IL-6, TNFα and IL-1β did not show significant differences with respect to baseline conditions, either in T and C groups. T group showed lower plasma IFNα levels at 14 days than baseline values (B=31,36±2,8, T=15,38±4,8, C= 25,18±5,6; T vs B: $P<0.05$). Conclusions: The administration of "P" demonstrated to be safe and beneficial for COVID+ patients as it lacked of immunogenicity capacity nor inflammatory response improving clinical outcome and recovery from infection.

197. (24) **IMMUNOMODULATORY OLIGONUCLEOTIDE IMT504 TRIGGERS DOWN-REGULATION OF T CELLS IN ADULT NON-OBESE DIABETIC MICE**

Sofía Gómez Bustillo[1], Silvia Bianchi[2], Victoria Lux-Lantos[2], Hannah Hipp[3], Maria Kral[3], Isabelle Ser[3], Carolin Daniel[3] and Alejandro D. Montaner[1].

1. Institute of Science y Technology "Dr. Cesar Milstein" (CONICET –Pablo Cassará Fundation), Buenos Aires, Argentina. 2. Institute of Biology and Experimental Medicine (IBY-ME-CONICET) 3. Institute for Diabetes Research, Helmholtz Diabetes Center at Helmholtz Zentrum München.

Type 1 diabetes (T1D) is a multifactorial autoimmune disease in which insulin-producing pancreatic β-cells are destroyed by autoreactive T cells. Currently, there are no effective clinical interventions for T1D, and patients are treated with insulin for life. There is a consensus that new innovative approaches to predict, treat, and prevent T1D are urgently needed. Several strategies to modulate the immunological response and restore β-cell mass have been performed but are limited by the availability of grafts and the need for chronic immunosuppression. IMT504 is the prototype of the PyNTTTTGT family of immunomodulatory oligonucleotides (ODNs), which are known for their regenerative properties and have been shown to be effective in lowering glycemia in nonobese diabetic (NOD /LtJ or simply NOD) mice. Here, we investigate the effect of IMT504 on T cells from NOD mice. Infiltrating T cells were isolated from the pancreas and lymph nodes of pre-diabetic and diabetic NOD mice. Cells were then sorted and treated in vitro with 0.5 μg/ml IMT504 or control ODN. After treatment, lymphocyte activity and functionality were characterized (differentiated T cell subsets, gene expression (cytometry), activated signaling pathways (qPCR), and induction of regulatory T cells (cytometry)). IMT504 induced TGF-β ($*p < 0.05$ vs control), a cytokine that stimulates regenerative properties in stem cells and higher β-cell number. We analyzed the molecular signaling pathways involved in the deregulation of T1D. We measured lower levels of PI3K ($*p < 0.01$ vs control), mTOR ($*p < 0.05$ vs control), and STAT ($*p < 0.01$ vs control). Downregulation of these genes is associated with higher levels of glucose transporter 1 (GLUT1),

normalized glycemia, and cellular reprogramming into β-like cells, respectively. MAP3K7 expression which is associated with β cell destruction was also downregulated (*p < 0.05 vs control). In conclusion, our results indicate that IMT504 exerts an effect on T cells that may prevent the onset of diabetic autoimmunity.

198. (35) **AN UPDATE OF CUTANEOUS MELANOMA PATIENTS TREATED IN ADJUVANCY WITH THE ALLOGENEIC MELANOMA VACCINE VACCIMEL AND PRESENTATION OF A SELECTED CASE REPORT WITH IN-TRANSIT METASTASES**

Ana Mordoh* 1, Mariana Aris* 2, Ibel Carri 3, Alicia Inés Bravo 4, Enrique Podaza 5, Juan Carlos Triviño Pardo 6, Gerardo Rubén Cueto 7, María Marcela Barrio 2, José Mordoh 2 4 8
**Equal contribution*
1División Dermatología, Hospital de Clínicas, Buenos Aires, Argentina.
2Centro de Investigaciones Oncológicas Fundación Cáncer, Buenos Aires, Argentina.
3Laboratorio de Inmunoinformática y Machine Learning, Instituto de Investigaciones Biotecnológicas-CONICET, Universidad Nacional de San Martín, Buenos Aires, Argentina.
4Laboratorio de Cancerología, Instituto de Investigaciones Bioquímicas de Buenos Aires-CONICET-Fundación Instituto Leloir, Buenos Aires, Argentina.
5Englander Institute for Precision Medicine, Weill Cornell Medicine, New York, NY, United States.
6Unidad de Bioinformática, Sistemas Genómicos Grupo Biomédico ASCIRES, Valencia, Spain.
7Grupo de Bioestadística Aplicada, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
8Departamento de Bioterapia, Instituto Alexander Fleming, Buenos Aires, Argentina.

The CSF-470 vaccine (VACCIMEL) plus BCG and GM-CSF as adjuvants has been assayed in cutaneous melanoma patients. In the adjuvant randomized Phase II study CASVAC-0401, vaccinated patients had longer distant metastasis-free survival (DMFS) than those treated with IFNα2b. Five years after locking the data, an actualization was performed. The benefit in DMFS was maintained in the vaccinated group versus the IFNα2b-treated group (p = 0.035), with a median DMFS of 96 months for VACCIMEL and 13 months for IFNα2b. The favorable risk-benefit ratio was maintained. DMFS was also analyzed as a single cohort in all the IIB, IIC, and III patients (n = 30) who had been treated with VACCIMEL. The median DMFS was 169 months, and at 48 months follow-up, it was 71.4%, which was not statistically different from DMFS of previously published results obtained in adjuvancy with ipilimumab, pembrolizumab, nivolumab, or dabrafenib/trametinib. The possible toxicity of combining VACCIMEL with anti-immune checkpoint inhibitors (ICKi) was analyzed, especially since VACCIMEL was co-adjuvated with BCG in every vaccination. A patient with in-transit metastases was studied to produce a proof of concept. During treatment with VACCIMEL, the patient developed T-cell clones reactive towards tumor-associated antigens. Three years after ending the VACCIMEL study, the patient progressed and was treated with ICKi. During ICKi treatment, the patient did not reveal any toxicity due to previous BCG treatment. When she recurred after a 4-year treatment with nivolumab, a biopsy was obtained and immunohistochemistry and RNA-seq were performed. The tumor maintained expression of tumor-associated antigens and HLA-I and immune infiltration, with immunoreactive and immunosuppressive features. VACCIMEL plus BCG and GM-CSF is an effective treatment in adjuvancy for stages IIB, IIC, and III cutaneous melanoma patients, and it is compatible with subsequent treatments with ICKi.

199. (267) ***LACTOCOCCUS LACTIS* AS A DELIVERY SYSTEM OF STAPHYLOCOCCAL PROTEIN A IN THE SKIN**

Victoria Y. Lee[1], Camila Ledo[2], Ana-Katharina E. Gehrke[2,3], Edith Illescas[2], Marisa I. Gómez[2,3,4] and Cintia D. Gonzalez[2,3].
[1]Facultad de Ciencias de la Salud, Universidad Maimónides, Buenos Aires, Argentina. [2]Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD), Departamento de Investigaciones Biomédicas y Biotecnológicas, Universidad Maimónides, Buenos Aires, Argentina. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [4]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Staphylococcus aureus is the primary cause of skin and soft tissue infections (SSTI). We have previously shown that staphylococcal protein A (SpA) induces the early recruitment of neutrophils, modulate their lifespan in the skin and contribute to proper abscess formation, bacterial eradication and wound healing. Therefore, SpA promotes beneficial immune responses in the skin and could be considerate as a candidate for the development of co-adjuvant immunomodulatory therapies for *S. aureus* SSTI. The aim of this study was to determine the feasibility of using *Lactococcus lactis* as a delivery system for SpA administration in the skin. This system has been previously used for the expression of cytokines and antigens in treatments that were proven safe in clinical trials. We determined the safety of the inoculation of heat-killed *L. lactis* SpA by intradermal route and its immunostimulatory potential. Mice were inoculated with 10^8 CFU of heat-killed *L. lactis* SpA (25 *µ*l in two inoculation points 1 cm apart). Histopathological analysis of the skin at 6 days post-inoculation (dpi) showed that mice inoculated with *L. lactis* SpA presented conserved epidermis and dermis as well as no evidence of skin damage due to the presence of the bacterium. Interestingly, in mice inoculated with *L. lactis* SpA a significant increase in the number of mature mast cells present in the dermis was observed compared with non-inoculated skin ($p<0.05$, Student *t* test) as determined by Toluidine Blue staining. We then characterized the cytokine and chemokine profile induced in the skin by *L. lactis* SpA at 2 and 6 dpi. The levels of IL-1β, IL-6, TNF-α and KC in skin homogenates from mice inoculated with *L. lactis* SpA at 6 dpi were higher than those in mice inoculated with *L. lactis* that does not express SpA. Taken together these results suggest the potential of using *L. lactis* SpA as a locally delivered immune modulator in the skin.

200. (282) **STUDY OF FOXP3 EXPRESSION IN TUMOR SAMPLES AND PERIPHERAL BLOOD MONONUCLEAR CELLS FROM BREAST CANCER PATIENTS**

Canzoneri R[1], Lacunza E[1], Berman C[1], Melchiori P[1], Creton A[2], Barbera LA[2], Croce MV[1], Isla Larrain M[1].
[1]Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA),
[2]Fundación Breast
e-mail: marinaislalarrain@hotmail.com

Breast cancer constitutes a heterogeneous group of malignant neoplasms with high incidence and mortality rates. The study of tumor evasion mechanisms is relevant to identify new prognostic biomarkers and therapeutic targets. The combination of immunotherapy agents with conventional therapies shows promising results in cancer treatment. Several clinical trials combining immune checkpoints inhibitors are in course both in breast cancer (BC) and in other tumors. Foxp3, known as a Treg marker, has been detected in tumor cells, being a controversial finding. The aim of this study was to evaluate Foxp3 expression in the tumor microenvironment and in peripheral blood mononuclear cells (PBMC) from BC patients. Tumor and peripheral blood samples from 127 breast cancer (BC) patients without treatment were obtained during surgery at the Hospital Italiano de La Plata with informed consent. The expression of ER, PR and HER2-neu receptors, Foxp3, CD45RO and CD8 was studied. The expression of FOXP3 mRNA in peripheral blood mononuclear cells (PMBC) was evaluated by PCR. Statistical analysis was performed in SPSS program. A bioinformatic analysis of the expression of FOXP3 and co-expressed genes was carried out, on RNA-Seq data (PANCANCER_TCGA) and RNA microarrays (GSE21653), considering the intrinsic subtypes of breast cancer. Foxp3 was found in 61% of BC samples, showing a positive correlation with CD8+ cells and a negative association with tumor stage. In 73.5% PBMC samples, FOXP3 expression was found showing a positive association with advanced tumor stages and PR (p<0,05). In silico anal-

ysis showed that FOXP3 and coexpressed genes are associated to immune pathways and FOXP3 RNA levels were higher in Basal and Her2 subtypes. The presence of Foxp3 in breast cancer cells and *FOXP3* expression in PBMC associated with advanced stages, makes this transcription factor a potential target for immunotherapy.

201. (342) **GENERATION OF BISPECIFIC SINGLE-DOMAIN ANTIBODIES WITH POTENTIAL APPLICATION IN CANCER THERAPY**

Diego Duarte Gareta, María Moreno Jauge, Gualberto González Sapienza
Universidad de la República, Uruguay.

Objectives: Generate bispecific single-domain antibodies (bispecific nanobodies (b-Nbs)) from the combination of individual Nbs against immunity targets and evaluate their anti-tumor activity in murine models. Specifically, it seeks to combine different Nbs with an anti-PD-L1 Nb in order to: 1-Promote a connection between effector immune cells and tumor cells. 2-Increase the specificity of binding towards dendritic cells. Materials and methods: Production of b-Nbs by recombinant DNA techniques in *E. coli* or HEK293T. Evaluation of its functionality by ELISA and flow cytometry. Evaluation of the antitumor activity in B16F10 model (metastatic melanoma) in C57Bl/6 mice and CT26 model (colon carcinoma) in Balb/c mice where tumor volume and survival curves are constructed, using ANOVA test followed by Dunnett test, and Logrank test for statistical analysis respectively. Results: In B16F10 model, the combination of an anti-PD-L1 Nb with a Nb against a dendritic cell receptor showed the best effect, although this was moderate, on the tumor volume curve P=0.01 and on the survival curve P=0.0078 compared to the control group. In the CT26 model, the same combination, to which the Fc region of mouse IgG2a without effector functions was added only in order to extend the half-life, showed a powerful antitumor effect, on the tumor volume curve P=0.0001 and on the survival curve P=0.0002 compared to the control group. Conclusion: Using a b-Nb generated by the combination of a Nb against a dendritic cell receptor and a Nb against PD-L1, a potent antitumor effect was observed in the CT26 model. This may be due to dendritic-tumor cell connection and/or increased binding specificity by dendritic cells acting as a checkpoint inhibitor.

202. (413) **ADAPTIVE NK CELLS IN BREAST CANCER PATIENTS DISPLAY SUPERIOR CYTOKINE RESPONSES AFTER STIMULATION WITH THERAPEUTIC ANTIBODIES AND SHOW INCREASED RESILIENCE TO NEOADJUVANT CHEMOTHERAPY**

María B. Bordignon[1,Φ], Ayelén I. Pesce Viglietti[1,Φ], Estefanía P. Juliá[1], Sánchez María Belén[1], Alexander Rölle[2,3], Pablo Mandó[4], Luciana Sabatini [5], Alexis Ostinelli[5], Manglio M. Rizzo [6], José Mordoh[1,7], Leonardo Fainboim[8] and Estrella M. Levy [1]. [Φ]These authors contributed equally.
1 Centro de Investigaciones Oncológicas CIO-FUCA, Ciudad Autónoma de Buenos Aires, Argentina; 2 Clinical Cooperation Unit "Applied Tumor Immunity", German Cancer Research Center, Heidelberg, Germany; 3 Department of Medical Oncology, National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany; 4 Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC), Ciudad Autónoma de Buenos Aires, Argentina; 5 Instituto Alexander Fleming, Ciudad Autónoma de Buenos Aires, Argentina; 6 Cancer Immunobiology, Instituto de Investigaciones en Medicina Traslacional, Facultad de Ciencias Biomédicas, CONICET, Universidad Austral, Derqui, Pilar, Argentina 7 Instituto de Investigaciones Bioquímicas. Fundación Instituto Leloir, Ciudad Autónoma de Buenos Aires, Argentina; 8 Laboratorio de Inmunogenética, INIGEM, CONICET-UBA, Hospital de Clínicas, Universidad de Buenos Aires, Argentina;

Adaptive NK (aNK) cells are a subpopulation of $CD3^{-}CD56^{dim}$ cells that proliferate after human cytomegalovirus (HCMV) infection. This subpopulation has more robust production of cytokines via CD16 stimulation (ADCR), longer lifespan and persistence compared to conventional NK cells (cNK) and is therefore interesting for cancer immunotherapy. Because there is limited information on aNK cells in cancer patients, we studied this population in peripheral blood samples from 140 Breast cancer (BC) patients using flow cytometry in the context of HER2+ BC-directed therapy. We characterized aNK cells by the lack of FcεRIγ (γ-) protein expression or the presence of the NKG2C receptor. Phenotypically, we analyzed the expression of various markers within the γ+ and γ- cell subsets. Functionally, we examined IFN-γ production and CD107a expression in both cell subsets by CD16 stimulation with anti-HER2 antibodies (abs). The seroprevalence of HCMV in BC patients was high (86%) and comparable with the age-matched healthy donors (85%). Nearly 60% of HCMV+ BC patients presented aNK cell subpopulation. Compared with γ+, γ- cells had higher levels of CD85j but lower levels of NKp30 and CD161 receptors ($p<0.0001$, Wilcoxon test), and lower expression of CD16 ($p<0.05$, Wilcoxon test). In response to abs-opsonized HER2+ cells, aNK cells produced more IFN-γ ($p<0.001$, Wilcoxon test), but presented lower degranulation capacity ($p<0.05$; Wilcoxon test) than cNK cells. Moreover, in 21 HCMV+ HER2+ BC patients treated with neoadjuvant regime based on chemotherapy, trastuzumab (TRZ) and pertuzumab (PER), the proportion of NKG2C+ cells augmented after therapy ($p<0.05$, Wilcoxon test) and aNK cells retained greater IFN-γ production than cNK cells ($p<0.01$, Wilcoxon test). Our results suggest that aNK cells are important mediators of ADCR in HER2+ BC and support efforts to determine the role of these cells and explore their possible relevance for clinical decisions in TRZ/PER treatment outcome in patients.

203. (418) **A PROSPECTIVE STUDY OF PREDICTIVE BIOMARKERS OF RESPONSE TO TREATMENT WITH BACILLUS CALMETTE-GUERIN (BCG) IN PATIENTS WITH NON MUSCLE INVASICE BLADDER CANCER. PRELIMINAR RESULTS**

José León Mellado[1], Joaquín Chemi[2], María Teresa Pombo[3], Mariana Aris[1], Roberto Villalba Bachur[2], Juan Camean[2], Jorge Jaunarena[2], Ximena Garcia[4], Gisela Coliva[4], Mora Amat[4], José Mordoh[1], Gustavo Villoldo[2], María Marcela Barrio[1]
[1] Centro de Investigaciones Oncológicas, Fundación Cáncer FUCA; [2] Servicio de Urología, Instituto Alexander Fleming; [3] Servicio de Immunohistoquímica, Instituto Alexander Fleming; [4] Servicio de Patología, Instituto Alexander Fleming, Ciudad de Buenos Aires, Argentina.

Intravesical administration of BCG is the main therapy for non-muscle invasive bladder cancer (NMIBC) pts. However, the response rate is ~60%. BCG is a local immunomodulatory that induces a strong inflammatory response that ultimately eliminates the tumor. In a retrospective study we defined a Th2-score evaluated in pre-BCG biopsies significantly associated with response to BCG. We have launched this prospective study to validate the Th2-score in biopsies and to evaluate immune parameters in urine and blood samples obtained before and throughout BCG treatment. Pts with NMIBC receiving BCG (6+3 scheme) were included. In pre-BCG biopsies, TILs Th2-score was calculated combining T-bet+(Th1) and GATA-3+(Th2) ratio and the density/activation of EPX+ eosinophils by immunohistochemistry. In urine, lymphocytes (UL) were analyzed by FACS and IP-10 chemokine was evaluated by ELISA. The first pts (n=10, 8 T1 and 2 Ta) were included and are still completing BCG treatment. 4/10 pts had a Th2-score predictive of favorable response to BCG; 1 pt with low Th2-score progressed early to metastatic disease. Urinary IP-10 chemokine increased in all pts during treatment, probably facilitating the recruitment of immune cells. UL increased along treatment, reflecting progressive infiltration of bladder tissue. Although mainly innate immune cells (PMN, monocytes) were present in the urine, CD3+T cells could be recovered after 3-4 BCG instillations in all pts. Most UL were CD4+ and CD8+, with few were NK/NKT cells. As treatment advanced, PD-1+TIM-3+CD8+ UL reached >15% In pts with a low Th2-score, but this population constituted <5% in pts with high Th2-score. Also, exhausted CD103+CD39+CD8+ UL showed different frequencies in low and high Th2-score pts. This is the first prospective study of biomarkers of response to BCG in NMIBC pts performed in Argentina. These initial results highlight the feasibility of analyzing UL as a dynamic

liquid biopsy to search for biomarkers of response to BCG.

204. (483) **ROLE OF SIALYLTRANSFERASES AND SIALIDASES ON GALECTIN-1-DRIVEN RESISTANCE TO IMMUNOTHERAPY: A BIOINFORMATIC APPROACH**

Marco A. Scheidegger[1,*], Joaquín P. Merlo[1,2,*], Yamil D. Mahmoud[1], Karina V. Mariño[2], Tomas Dalotto-Moreno[1], Gabriel A. Rabinovich.[1]

[1] Glycomedicine Lab, Instituto de Biología y Medicina Experimental (IByME) – CONICET, Buenos Aires, Argentina. [2]Functional and Molecular Glycomics Lab, Instituto de Biología y Medicina Experimental (IByME) – CONICET, Buenos Aires, Argentina.

**These authors contributed equally.*

Objectives. Here we aimed to explore, using a bioinformatic approach, the role of sialyltransferases ST3GAL1 and ST6GAL1 as well as sialidases NEU1-4 in resistance to immunotherapy. Materials and Methods. Publicly available single-cell transcriptomics of tumour samples from melanoma patients treated with immunotherapy was analysed using Seurat package v4.0.6 and R software v4.2.0. Background and Results. Galectin-1 (Gal1) has emerged as a critical mediator of resistance to different anti-cancer therapies, including immunotherapy and anti-angiogenic therapies. Gal1 induces apoptosis of fully activated Th1 and Th17 cells, confers tolerogenic potential to dendritic cells and favours M2 polarization of macrophages. Gal1 also confers resistance to anti-VEGF therapies through VEGFR2 binding on endothelial cells and inducing angiogenesis. Since Gal1 binding is inhibited by terminal α(2,6)-sialylation of glycoproteins, we focused on sialyltransferases which add terminal sialic acid, and on neuraminidases, able to hydrolyse this monosaccharide. Using publicly available transcriptomics data from melanoma patients, we found that cytotoxic and exhausted $CD8^+$ T lymphocytes of non-responding patients showed a decreased expression of *ST6GAL1* mRNA compared to responding patients after treatment ($p<0.01$), and increased expression of *LGALS1* mRNA ($p<0.05$). We also found a tendency toward an increase of *NEU1* mRNA on dendritic cells and macrophages from non-responding patients. To further explore these processes, we developed exosialidase and sialylation scores and analysed their impact on immune cells. Exosialidase score was higher in macrophages from non-responding patients before their treatment (0.5 log2 fold change). These effects were further confirmed in *in vitro* cell cultures. Conclusions. Our preliminary bioinformatic analyses shed light on the contribution of the sialylation machinery in regulating resistance to immunotherapy by modulating sensitivity to endogenous galectins.

205. (570) ***T. CRUZI*-DENDRITIC CELLS INTERACTION: EXTRACELLULAR VESICLES AS MEDIATORS FOR IMMUNE MODULATION**

Brenda Gutierrez[1], Maria E Ancarola[1], Izadora Volpato-Rossi[2], Antonio Marcilla[3], Mara Rosenzvit[1], Marcel Ramirez[2], Marcela Cucher[1], Carolina Poncini[1]

1IMPAM, UBA-CONICET, Dpto Microbiología, Facultad de Medicina, UBA, 2Fiocruz Paraná-Instituto Carlos Chagas, Curitiba, Brazil, 3Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Universitat de Valencia, Burjassot, Valencia, España.

Extracellular vesicles (EVs) include a heterogeneous group of particles. Microvesicles, exosomes and apoptotic bodies are the most characterised vesicles. EVs reach considerable interest in the scientific community due to their role in cell-to-cell communication, including antigen-presentation, stimulation of anti-tumoral immune responses, tolerogenic or inflammatory effects. In pathogens, EVs shedding is well described in fungus, bacteria, protozoan parasites and helminths. In *Trypanosoma cruzi* not only EVs liberation, but also protein composition was described. Dendritic cells (DCs) are important antigen presenting cells, key players promoting immunity against pathogens and maintaining self-tolerance. Previously, we demonstrated that *T. cruzi* down regulates DCs immunogenicity in vitro and in vivo. Here we analyze EVs from the in vitro interaction between blood-circulating-trypomastigotes (Tp) and bone marrow-derived DCs. EVs were enriched by ultracentrifugation and characterized by TEM. We found that Tp incremented the number, but not the size of EVs in DC culture supernatants evaluated by nanoparticle tracking analysis and flow cytometry. Referring composition, EVs displayed some exosome markers and intracellular RNA. By proteomics, we found that the parasite changes the protein cargo in EVs from DCs. In addition, EVs from Tp-DCs interaction (EVs DCs-Tp) were easily captured by unstimulated DCs in comparison to EVs from DCs cultured without the parasite and also that EVs DCs-Tp modified the activation status of LPS-stimulated DCs. Immunization assays with EVs DCs-Tp versus EVs DCs, showed that EVs DCs-Tp partially protects animals from the challenged with *T. cruzi* lethal infection. Animals displayed low parasitemia and 60% of survival in comparison to EVs DCs-treated that showed 100% of lethality. Our goal is to go deep into the molecular characterization of EVs DCs-Tp, in order to identify mediators with therapeutic purpose.

206. (589) ***TOXOPLASMA GONDII* SERINE PROTEASE INHIBITOR-1 (TgPI-1) DIFFERENTIALLY MODULATES DENDRITIC CELL SUBSETS**

Joel Katan Piñeiro[1], Julieta Santos[1], Valentina Martín[1], Paula Berguer[2], Ignacio Martín Fenoy[1], Ariadna Soto[1], Alejandra Goldman[1].

[1]Centro de Estudios en Salud y Medio Ambiente, ITECA, ECyT, Universidad Nacional de San Martín. Consejo Nacional de Investigaciones Científicas y Técnicas

[2] Laboratorio de Inmunología y Microbiología Molecular, Fundación Instituto Leloir, Instituto de Investigaciones Bioquimicas De Buenos Aires.

Background**:** We previously showed that treatment with *T. gondii* serine protease inhibitor-1 (TgPI-1) significantly reduced experimental asthma. BMDC differentiated in the presence of TgPI-1 (DC_{TgPI-1}) significantly lowered IL-12 secretion, CD80 and CD86 expression and increased PDL-1 and CD45Rb. AIM: Further characterize TgPI-1 effect on dendritic cells (DCs) *in vitro* by analyzing the T response profile promoted by DC_{TgPI-1} and *in vivo* after treating asthmatic mice with TgPI-1. Methods: BMDCs were obtained from BALB/c mice bone-marrow cultured with GM-CSF in presence or not of TgPI-1 and stimulated with LPS. Then a mixed lymphocyte reaction (MLR) was done co-culturing CD11c+ cells with *naive* C57BL/6 mice splenocytes. For *in vivo* study, BALB/c mice were ip. sensitized with OVA/Alum and aerosol challenged. Later, were intranasally treated with TgPI-1+OVA (OPI). Controls included *naive*, and sensitized mice treated with OVA (OO). Eight h later, cDC1 ($MHCII^+CD11c^+CD103^+$), cDC2 ($MHCII^+CD11c^+CD11b^+CD64^-$) and moDCs ($MHCII^+CD11c^+CD11b^+CD64^+$) were assessed by flow cytometry in lung and lymph nodes. RESULTS: MLR supernatants from $DC_{rTgPI-1}$, showed significantly less IL-4 and IL-17 ($p<0.05$). No differences in IFN-γ levels were detected. Lung dendritic cells obtained from OPI mice showed a significantly lower percentage and total number of cDC2 ($p<0.05$) compared to OO group. While moDCs percentage showed no significant differences between OO and OPI mice, a tendency to decreased total number was observed in mice treated with TgPI-1. cDC1 subset didn´t show significant differences between OO and OPI groups. The same trend in cDC2 and cDC1 percentage was observed in draining lymph nodes ($p<0.01$). Conclusions: DC_{TgPI-1} showed a lower capacity to induce type 2 and type 3 immune responses. Interestingly, these results correlate with the diminished cDC2 subset detected in asthmatic mice treated with TgPI-1. TgPI-1 would differentially modulate different T response profiles

207. (702) **AN ONCOGENIC VIRUS FOR THE TREATMENT OF LEUKEMIA AND LYMPHOMA**

Mercedes Pastorini[1,2], Gebhard Leopoldo[2], Iara L. Scialfa[2], Mercedes Alemán[1] and Sandra E.Goñi[2].

[1]Laboratorio de Inmunología Experimental, IMEX-Academia Nacional de Medicina, CABA,Argentina [2]Laboratorio de Virus Emergentes, Instituto de Microbiología Básica y Aplicada, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina.

Superantigens (SAgs) are proteins that activate T or B lymphocytes

through non-conventional interactions. Mouse Mammary Tumor Virus (MMTV) is a type B retrovirus that encodes a SAg with the ability of stimulating and inducing apoptosis of T cells, but only those that have a determined Vβ chain on the T cell receptor. *Virus-like particles (*VLPs) are multiprotein nanostructures that mimic the organization and conformation of viral particles. Given their lack of genome, they cannot infect or replicate but they maintain their immunogenic capacity. The aim of this work is to design and develop a specific treatment for leukemia and lymphoma, using MMTV SAgs vehiculized in JUNV VLPs. Our hypothesis is that VLPs carrying different viral SAgs will be able to induce apoptosis specifically to T cells that bear the respective Vβ. We generated the plasmids pZ-SAgs, carrying the ORF sequences of BALB2, BALB14, LA and mtv-7 SAgs fused to JUNV Z protein, allowing their vehiculization into VLPs. These constructions were employed to transfect HEK 293T cells and the fusion proteins were detected by Western Blotting using a rabbit anti Z serum. We are currently concluding the batch production of Z-mtv7 VLPs to start the *in vitro* assays with healthy donor samples, patient samples and related cell lines.
Besides, it was necessary to use the soluble SAgs as control for immunological assays, but their full sequences were unknown. Thus, we generated the plasmidic constructions pET-28a-SAgs, carrying the ORF of MMTV SAgs, we expressed them in *E. coli* and detected the presence of the recombinant proteins through Western Blotting. We are currently optimizing the expression and purification.
Current therapies for leukemia and lymphoma affect neoplastic cells and also a high proportion of normal cells, impacting on the patient's health during and after the treatment. The use of SAgs may reduce the undesired effects of those therapies offering a therapy that affects a limited repertoire of cells.

INFECTIOUS DISEASE - *(18/11 9-10:30 hs)*

Chairs: Virginia Rivero - Karina Gomez - Andrés Alloatti Luciano D´attilio - Silvana Spinelli Ariana Díaz - Carolina Poncini - Romina Fernandez-Brando - Samanta Funes

208. (16) **HYDATID FLUID FROM *ECHINOCOCCUS GRANULOSUS* IS RECOGNIZED BY CLEC9A IN BMDCs, INDUCES AUTOPHAGY AND POLARIZES THE T CELL RESPONSE TOWARDS TH-1/TH-17 PROFILES *IN VITRO*.**

Maia Chop[12], Camila Ledo[13] María Celeste Nicolao[13], Julia Loos[13], Andrea Cumino[13], Christian Rodriguez Rodrigues[12].
1 CONICET, 2 Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. 3 IIPROSAM, Universidad Nacional de Mar del Plata.

Background: Cystic echinococcosis is caused by Echinococcus *granulosus.* This parasite develops cysts in the host that are filled with hydatid fluid (HF). Autophagy plays a key role in antigen presentation promoting activation of T cells. The aim of this work is to analyze if HF could polarize the T cell response in vitro. Methods: HF was collected from infected cattle. Expression of Clec9a was evaluated by FACS in FLT3L-BMDCs. Autophagy induction was evaluated by FACS, qPCR, confocal and TEM. Rapamycin (20 nM) and chloroquine (100μM) were used to analyze autophagic flux. T cell cytokine profiles were analyzed by qPCR. Results: HF induces a downmodulation of Clec9a in BMDCs (n=3 **p<0.01). Enhanced number of autophagosomes were observed by TEM in HF-stimulated BMDCs compared to control cells. Also, HF-stimulated BMDCs significantly enhanced the MFI of $LC3^+$ structures in comparison with unstimulated cells (**p<0.01). The autophagic flux was analyzed by measuring LC3II attached to autophagosome membranes by FACS. HF induced an increased in the percentage of $LC3^+$ cells compared to untreated cells (30.2% vs 23.1%) and this phenomenon was enhanced by the inhibition of lysosomal acidification (28.4% vs 40.6%). Then, we evaluated the expression of *beclin-1, lc3, tfeb and atg16l-1* genes. We observed that HF induces a significant increase in the transcriptional expression of *lc3* and *beclin-1* (n=3, **p <0.01 vs control). Finally, we measured the expression of *il-4, il-5, il-6, il-10, il-12, il-13, il-17, tnf-α, tgf-β and inf-γ* in splenocytes by qPCR, finding that HF-stimulated BMDCs increases significantly the levels of *il-6, il-10, il-12,* t*nf-α, tgf-β and inf-γ* (n = 3, *p<0,05, **p<0,01, vs control). Conclusions: These results suggest that HF could be recognized by Clec9a in BMDCs, inducing autophagy and promoting antigen presentation. HF-stimulated BMDCs modulate gene expression of cytokines related to the Th1, Th17 and Treg profiles, and strongly inhibit Th2 response.

209. (23) **TUBERCULOSIS-ASSOCIATED MICROENVIRONMENT MODULATES THE METABOLISM OF MACROPHAGES RENDERING THEM MORE SUSCEPTIBLE TO HIV-1 INFECTION**

Zoï Vahlas[1,2], Sarah Monard[1,2], Alexandre Lucas[3], Thien-Phong Vu Manh[4], Rémi Mascarau[1,2] ; Myriam Ben Neji[1], Renaud Poincloux[1,2] , Brigitte Raynaud-Messina[1,2], Olivier Neyrolles[1,2], Geanncarlo Lugo- Villarino[1,2], Luciana Balboa[5,2] and Christel Vérollet[1,2]
[1] Institut de Pharmacologie et Biologie Structurale, Université de Toulouse, CNRS, Toulouse, France ; [2]International Research Project CNRS " MAC-TB/HIV ", Toulouse and Buenos Aires ; [3] Institut des maladies métaboliques et cardiovasculaires, Inserm, Toulouse, France ; [4]Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France ; [5]Academia de Medicina, CONICET, Buenos aires, Argentina.

Tuberculosis (TB) is the most frequent co-infection in $HIV\text{-}1^+$ patients, making it a well-known risk factor to exacerbate HIV-1 morbidity and mortality. We recently identified macrophages as main actor in the exacerbation of HIV-1 infection in this context, mainly through high HIV-1 cell-to-cell transmission *via* tunneling nanotubes (TNT). *Mycobacterium tuberculosis* (Mtb), the etiological agent for TB, is known to regulate macrophage metabolic state to thrive in the host, but nothing is known about the role of macrophage metabolism in HIV/Mtb co-infection. Our transcriptomic data highlight an upregulation of glycolytic pathway in human macrophages when they are differentiated in Mtb-derived microenvironments, using in vitro and ex vivo models, compared to controls. After a deep characterization of their metabolism, it appears that these macrophages have increased glucose uptake, lactate release and maximal respiration without induction of ROS production. These changes in metabolic activity are also associated with a stronger ATP production, mainly coming from aerobic glycolysis. Finally, we show that specific alteration of aerobic glycolysis in macrophages in Mtb- associated environments impact HIV-1 infection of macrophages, virus production and formation of TNT. Thus, those results show that the glycolysis metabolic pathway is determinant for the worthening of HIV-1 pathogenesis in co-infection with Mtb. Our results will open up, through the modulation of the metabolism, innovative therapeutic perspectives making it possible to control HIV infection in patients infected with Mtb.

210. (101) **EVALUATION OF THE ROLE OF TISSUE REPAIR REGULATORY T CELLS DURING ACUTE *Trypanosoma cruzi* INFECTION**

Santiago Boccardo, Cintia L Araujo Furlán, Constanza Rodriguez, Carolina P Abrate, Laura Almada, Adriana Gruppi, Carolina L Montes, Eva V Acosta Rodríguez
CIBICI - CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba

T. cruzi (Tc) and the type I response required to control it promote damage in target tissues. Tissue repair regulatory $Foxp3^+$ $CD4^+$ T cells (trTreg) are defined as $ST2^+$ $KLRG\text{-}1^+$ and specialized in maintaining tissue homeostasis and favoring repair upon injury in addition to their immunoregulatory properties. We aimed to study trTregs role in damage control during acute Tc infection. To this end, Foxp3-GFP C57BL/6 mice were infected (INF) with 5000 Tc parasites (Tulahuen). Skeletal muscle, liver and spleen (Sp) infiltrate was evaluated by flow cytometry in a kinetic study. trTregs were reduced in all tissues at 21 days post infection (dpi) in correlation with maximum tissue damage and parasitism, and decreased systemic levels of their growth factor IL33 (p<0.05). Recombinant IL33 allowed the *in-vitro* expansion of trTregs among sorted Tregs from INF Sp, but

we could barely increase them in vivo after intraperitoneal injections at 12, 15 and 18 dpi in mice. To identify signals that could counteract IL33 effect *in-vivo*, we cultured Tregs from non-INF Sp with IL33 plus IFNg/TNF/IL18, parasites or INF Sp conditioned media. In all cases, trTregs expanded normally. As IL33 could expand trTregs in non-INF mice, we treated INF mice at early dpi (0, 3 and 6) and evaluated disease evolution. This treatment resulted in increased trTreg in all tissues ($p<0.05$) lower levels of plasma LDH, CPK, GOT and Glucose and reduced body weight loss and tissue parasitism at 21 dpi. These was accompanied by an increase of Tc-specific CD8+ T cells and ILC2. We conclude that trTreg cell response is compromised during acute Tc infection likely because the inflammatory milieu is unfavorable for trTregs survival, preventing IL-33-mediated rescue. Yet, early IL-33 treatment improves the course of the acute infection. As trTregs and effector immune cells are increased by IL-33, further studies are required to establish the underlying mechanisms in the IL-33 protective effects.

211. (143) **INTERACTION OF HELJA LECTIN ISOLATED FROM *HELIANTHUS ANNUUS* WITH *CANDIDA ALBICANS* INHIBITS PHAGOCYTOSIS AND PROMOTES PHENOTYPIC MATURATION IN DENDRITIC CELLS.**

Maia Chop[12#], Melisa Radicioni[13#], Marianela Del Rio[13#], Christian Rodriguez Rodrigues[12&], Mariana Regente[13&].
1 CONICET, 2 Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. 3, Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata. #& equal contribution.

Background: Helja is a sunflower lectin that recognizes mannose motifs. Candidiasis is a fungal infection caused by *Candida spp*. that affects immunocompromised individuals. This opportunistic yeast can be recognized by dendritic cells (DCs). The aim of this work is to analyze if the interaction between Helja and *Candida albicans* (*Ca*) cell wall could inhibit recognition and phagocytosis by DCs and modulate cell maturation. Methods: Bone marrow dendritic cells (BMDCs) were cultured in RPMI 1640 medium supplemented with FLT3-L. Helja was purified from sunflower seeds with a D-mannose-agarose resin. Phagocytosis assay was evaluated by FACS and Confocal Microscopy. *C. albicans* (NGY152 strain) were stained with FITC and then incubated with Helja for 45 minutes prior to BMDC stimulation. LPS (100 ng/ml) and 4°C incubation were used as controls. BMDCs phenotype was analyzed by FACS. Results: Confocal images and quantification of labeled-yeast revealed that the preincubation of fungal cells with Helja inhibited recognition by BMDCs and induced a statistically significant reduction on phagocytosis compared to control (n=2, ****$p<0.0001$ Helja-*Ca*+LPS-treated BMDCs vs *Ca*+LPS-treated BMDCs). This observation was confirmed by measuring FITC+ dots in the BMDC gate by FACS analysis (Helja-*Ca* treated BMDCs -53,7%- vs *Ca*-treated BMDCs -60.4%). Finally, *Ca* preincubated with Helja improved the upregulation of MHCII compared to untreated cells or *Ca*-stimulated BMDCs (n=3 **$p<0.01$). The same pattern was observed in the co-stimulatory molecule CD86, Helja-*Ca* treated BMDCs induced a significant increase in the percentage of CD86+ cells (54.1% vs 42.0% observed in *Ca*-treated BMDCS or 24.0% observed in untreated cells, n=3). Conclusions: These data suggest that the mannose-binding lectin blocks epitopes on *Candida* cell wall important for DC recognition. However, Helja promotes DCs maturation, a process required to activate adaptive immunity, highlighting its potential biomedical application.

212. (145) **A MANNOSE-BINDING LECTIN FROM *HELIANTHUS ANNUUS* RECOGNIZES GP120 FROM HIV-1 AND PROMOTES PHENOTYPE MATURATION IN DENDRITIC CELLS**

Melisa Radicioni[13#], Maia Chop[12#], Marianela Del Rio[13], Juan Sabatté[14], Mariana Regente[13&], Christian Rodriguez Rodrigues[12&].
1. CONICET, 2. Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. 3. Instituto de Investigaciones Biológicas, CONICET-UNMDP. 4. Instituto de Investigaciones Biomédicas en Retrovirus y Sida.
#& equal contribution

Background: Plant lectins have potential use in biomedicine as antimicrobial reagents_due to their binding specificity to glycoconjugates. Helja is a mannose-binding lectin isolated from sunflower seeds. Half of the molecular mass of the HIV-1 envelope glycoprotein (gp120) is constituted by high-mannose and complex N-glycans. The aim of this work is to analyze if Helja could recognize gp120 and induce dendritic cell maturation. Methods: Helja was purified from *Helianthus annuus* seeds on a D-mannose-agarose affinity chromatography. Potential molecular interaction between Helja and recombinant gp120 (M Group, B Subtype, SinoBiological, USA) was evaluated by dot blot and ligand blot approaches using anti-Helja antibodies. Bone marrow dendritic cells (BMDCs) were cultured in complete RPMI medium supplemented with FLT3-L. Cellular activation state was evaluated in Helja-treated BMDCs by measuring membrane proteins (CD86, CD40, MHCII, CD11c, CD80, Clec9a, Ly6G, SIPRα, CD103, SiglecH) by FACS. Results: The interaction of Helja with gp120 was initially detected in dot blot assays by immunodetection of lectin bound to viral protein immobilized on nitrocellulose membranes. This interaction was confirmed in ligand blot assays by detecting a signal whose molecular weight corresponds to gp120 (95 kDa). On the other hand, the presence of Helja (10 μg/ml) in BMDC culture induced an upregulation of MHCII, MHCI, CD80, CD40 and CD86 (n=3, **$p<0.01$ Helja-treated BMDCs vs controls, *$p<0.05$ Helja+LPS-treated BMDCs vs LPS control). No changes in Clec9a, Ly6G, SIRPα, CD103 and SiglecH were detected. **Conclusions:** These results suggest that Helja induces dendritic cell maturation, important to promote immune activation, and recognizes specific glycosidic residues and arrangements on the HIV-1 envelope protein which could have a potential biomedical use as a phyto-neutralizing agent in viral infection.

213. (216) **P2Y6 RECEPTOR ACTIVATION IS NECESSARY TO INDUCE PHAGOPTOSIS OF NEURONS BY B. ABORTUS-ACTIVATED MICROGLIA**

Autores: Fiorillo Evequoz Ariadna María, De Santis Arévalo Julia, Rodríguez Julia, Quintela Julieta, Giambartolomei Guillermo Hernán y Rodríguez Ana María
Instituto de Inmunología, Genética y Metabolismo (CONICET/UBA), Hospital de Clínicas "José de San Martín", Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

B. abortus-activated microglia kill neurons through primary phagocytosis or phagoptosis. Phagocytosis is a finely regulated process that involves the interaction of different receptors and their ligands. It has been shown that the purinergic pathway is involved in the modulation of different functions of phagocytes. The objective of this work was to investigate whether this signaling pathway is involved in the phagoptosis of neurons mediated by B. abortus-activated microglia. Primary cultures of neurons and microglia from Balb/c mice were infected. Neuron survival was assessed at 48 h by fluorescence microscopy. Co-cultures were treated with apyrase (an enzyme that degrades di and tri nucleotides), Reactive Blue 2 (RB2) (a P2X/P2Y purinergic receptor inhibitor), BBG (a P2X7 specific inhibitor) and MRS2578 (a P2Y6 specific inhibitor). Treatment of B. abortus-infected co-cultures with apyrase inhibited neuronal death, when compared to untreated cultures ($p<0.05$). Treatment of B. abortus-infected co-cultures with RB2 also prevented neuronal death ($p>0.05$). By using the specific inhibitors of P2X7 and P2Y6, we were able to demonstrate that the P2Y6, but not P2X7 purinergic receptor, is involved in the modulation of phagoptosis ($p>0.05$). In all cases microglia activation was not affected since TNF-α secretion was not significant different between treatments ($p>0.05$). These results demonstrate that the P2Y6 purinergic receptor and the nucleotides that activate it would be necessary for neuronal death mediated by microglia activated by B. abortus, describing new molecular mechanisms involved in the pathogenesis of neurobrucellosis.

214. (218) **Brucella abortus RNA DIMINISHES IFN-ɤ-INDUCED MHC-I MOLECULES IN DIFFERENT LINE REGULAR AND TUMOR CELLS**

Agustina Serafino, Yasmín Bertinat, Jorgelina Bueno, M. Ayelén Milillo# and Paula Barrionuevo#.
Laboratorio de Fisiología de los Procesos Inflamatorios, IMEX-CONICET, Academia Nacional de Medicina.
 # Both authors contributed equally to this work

Brucella abortus (*Ba*) is an intracellular pathogen capable of surviving inside macrophages. Since the disease is presented in multiple forms, many different cells are susceptible to be infected by *Ba*. We have previously demonstrated that *Ba* RNA diminishes the IFN-ɤ-induced MHC-I surface expression in human macrophages, retaining them within the Golgi Apparatus (GA). However, we acknowledged whether this event could be triggered in other cells able to be infected with *Ba*. So, we stimulated the lung epithelium cell line (Calu-6) and the endothelial microvasculature cell line (HMEC) with different doses of *Ba* RNA in the presence of IFN-ɤ. MHC-I expression was assessed by flow cytometry. *Ba* RNA (10 ug/ml) diminished the IFN-ɤ-induced MHC-I surface expression ($p<0.05$) in both cell lines. Next, by confocal microscopy, we observed colocalization of MHC-I and GA marker GM130 in CALU-6 cells treated for 48h, although to a lesser extent than in human macrophages ($p<0.05$). Conversely to what we expected, supernatants from Calu-6-*Ba* RNA-treated cells had higher IL-8 production compared to those from untreated cells ($p<0.05$). In addition, a decrease in MHC-I on the surface could be accompanied by an activation of Natural Killer (NK) cells. These cells are key for the defense against multiple tumors. Therefore, a question of great importance is: what is the relevance of MHC-I modulation in the context of other pathologies in which NK response is critical? For this we started by stimulating the human glioblastoma cells (U251) with different doses of *Ba* RNA in the presence of IFN-ɤ. MHC-I expression was assessed by flow cytometry. *Ba* RNA diminished the IFN-ɤ-induced MHC-I surface expression ($p<0.05$) in U251. Together these results show that *Ba* could persist successfully within the host, remaining unnoticed and evading $CD8^+$ T cell surveillance. On the other hand, the decrease in MHC-I could enhance the subsequent cytotoxic response against multiple tumors.

215. (240) **THE LACK OF NET INDUCTION BY *KLEBSIELLA PNEUMONIAE* IS NOT REVERSED WITH PLATELETS**

Federico Birnberg-Weiss[1], José R. Pittaluga[1], Joselyn E. Castro[1], Luis A. Castillo[1], Daiana Martire-Greco[1], Sonia A. Gómez[2], Verónica I. Landoni[1], Gabriela C.Fernández[1].
[1] Instituto de Medicina Experimental (IMEX)-CONICET/Academia Nacional de Medicina; Buenos Aires, Argentina. [2] Servicio de Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas Dr. Carlos G. Malbrán (INEI), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Buenos Aires, Argentina.

Klebsiella pneumoniae carbapenemase (Kp)-producing bacteria are associated with significant mortality in immunocompromised patients. We have previously reported that neutrophils (PMN) failed to release neutrophil-extracellular-traps (NET) when challenged with Kp belonging to ST258, whereas *Escherichia coli* (Eco) was a high NET inducer. It has been reported that PLT activation and PMN:PLT mixed-aggregate formation potrentiate NET induced by LPS and other soluble stimuli. Our aim was to study if the lack of Kp-mediated NET induction can be reversed by the presence of PLT. To assess the effect of both bacteria on PLT activation we measured P-selectin expression by flow cytometry. We found an increment in the percentage of P-selectin-positive PLT when challenged with Kp or Eco ($p<0,05$ vs. basal). Since PLT binding to PMN is associated with NET release we measured PMN-PLT mixed aggregates formation ($CD11b^+CD61^+$) by flow cytometry, using a PMN:PLT:bacteria ratio of 1:50:10. The percentage of mixed aggregates was increased when PLT were treated with Eco, but not with Kp ($p<0,05$). NET released by purified human PMN were quantified after 3 h using confocal microscopy after DNA and Elastase staining. Also, NET-associated Myeloperoxidase (MPO) and DNA were measured in the supernatants after controlled DNAse digestion. We observed that the presence of PLT did not affect NET formation neither with Kp nor with Eco ($p<0,05$). Our results indicate that PLT are not able to modulate the lack of NET formation when PMN faces Kp.

216. (242) **NEUTROPHIL BACTERICIDAL RESPONSE AGAINST *PROVIDENCI*A SPP**

Joselyn E. Castro[1], Federico Birnberg-Weiss[1], Jose R. Pittaluga[1], Sonia Gómez[2], Verónica I. Landoni[1], Gabriela C. Fernández[1].
[1] Laboratorio de Fisiología de los Procesos Inflamatorios, Instituto de Medicina Experimental (IMEX)-CONICET/Academia Nacional de Medicina, Buenos Aires, Argentina. [2] Servicio de Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas Dr. Carlos G. Malbrán (INEI), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Buenos Aires, Argentina

Antimicrobial resistance is a significant problem for the treatment of infectious diseases worldwide. *Providencia* spp. are Gram-negative opportunistic bacteria that are becoming extremely relevant in hospitalized patients. *P. stuartii* (Ps) and *P. rettgeri* (Pr) cause urinary tract infections, followed by pneumonia, septicemia, and others. The innate immune response against these pathogens has not been investigated. The aim of this study was to determine whether Ps and Pr are able to trigger the bactericidal response in neutrophils (PMN), one of the most relevant cells for the innate immunity. For this purpose, we evaluated two main mechanisms of PMN, the respiratory burst by flow cytometry using Dihydrorhodamine, and neutrophil extracellular trap (NETs) formation by release of double strand DNA and confocal microscopy in isolated human PMN (n=7) challenged with clinical multi-resistant isolates of Ps and Pr, and used E. coli (Ec) ATCC as a positive control (PMN:bacteria ratio of 1:10). We found that both Ps and Ec triggered the respiratory burst similarly, whereas Pr did not ($p<0.05$). None of Ps or Pr were able to induce NETs compared to Ec ($p<0.05$). However, degranulation of PMN, measured as myeloperoxidase release, was induced by Ps but not by Pr ($p<0.05$). Bacterial killing was determined as colony formation units (CFU) after incubation for 1 h in the presence of PMN. We found that PMN reduced CFU for Ps and Ec, but was less effective for Pr. All together, these results indicate differences in the ability of *Providencia* Spp. to induce bactericidal responses in PMN, and suggest an advantage of Pr over Ps.

217. (264) **PHENOTYPIC AND GENOTYPIC CHANGES IN *S. AUREUS* CLINICAL ISOLATES FROM PATIENTS WITH CHRONIC OSTEOMYELITIS HAVE AN IMPACT IN THE INDUCTION OF OSTEOCLASTOGENESIS.**

Ana-Katharina E. Gehrke[1,2], Cintia D. Gonzalez[1,2], Victoria Y. Lee[3], Daniel O. Sordelli[4], M. Victoria Delpino[5,6] and Marisa I. Gómez[1,2,5].
[1]Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD), Departamento de Investigaciones Biomédicas y Biotecnológicas, Universidad Maimónides, Buenos Aires, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [3]Facultad de Ciencias de la Salud, Universidad Maimónides, Buenos Aires, Argentina. [4]Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, Argentina. [5]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Argentina. [6]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, Argentina.

S. aureus undergoes phenotypic and genotypic changes as a consequence of adaptation and microevolution in the bone during chronic osteomyelitis. Although these changes can contribute to bacterial persistence, their impact in the pathogenesis of the disease has not been established. The study of 45 isolates from patients with chronic osteomyelitis (1 to 40 years of evolution) evidenced the loss of capsular polysaccharide (CP) expression and

the deletion of short sequence repeats (SSR) in the protein A (SpA) gene polymorphic region (Xr) over time. The aim of this study was to determine if CP expression and the number of SSR present in SpA would affect the ability of *S. aureus* to induce osteoclast differentiation. Using RAW 264.7 cells we showed that the CP- isolate HU25d (9 SSR), obtained from a patient with osteomyelitis of 8 years of evolution, induced significantly increased osteoclastogenesis and TNF-α production than the CP+ isolate HU25a (11 SSR), obtained from the same patient 7 years earlier ($p<0.001$). The impact of CP expression in osteoclastogenesis was confirmed using the strains Reynolds CP+ and the isogenic mutant CP- ($p<0.001$). Unstimulated cells were used as negative control in all the experiments and data was analyzed by ANOVA-Tukey's post-test. The CP+ isolates HU92a and HU92c were obtained simultaneously from a patient with osteomyelitis of 33 years of evolution. The isolate HU92c carrying 4 SSR induced significantly increased osteoclastogenesis than the isolate HU92a carrying 9 SSR ($p<0.05$). The ability of the SpA Xr region to modulate osteoclast differentiation was corroborated using *S. aureus* USA300 and the isogenic mutant ΔXr ($p<0.001$). In conclusion, our results indicate that the expression of CP as well as the length of the SpA Xr region modulate the capacity of *S. aureus* to induce osteoclast differentiation, suggesting that the changes that the bacteria undergoes in the bone microenvironment could contribute to the pathogenesis of the disease.

218. (277) **CHARACTERIZATION OF *Chlamydia trachomatis* MALE UROGENITAL INFECTION. ASSESSMENT OF ASSOCIATED INFLAMMATORY RESPONSE, SPERM QUALITY AND MOLECULAR EPIDEMIOLOGY**

Daniela Andrea Paira[1], Carolina Olivera[1], Rosa Molina[2], Andrea Tissera[2], Virginia Elena Rivero[1], Hector Alex Saka[1], Ruben Dario Motrich[1]

[1]CIBICI-CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. [2]Laboratorio de Andrología y Reproducción (LAR), Córdoba, Argentina. [3]Fundación Urológica de Córdoba para la Docencia e Investigación Médica (FUCDIM), Córdoba, Argentina.

Chlamydia trachomatis (CT) is the most prevalent sexually transmitted bacterial infection. Although the infection and associated pathology have been widely described in women, the study of male urogenital infection has been neglected. Herein, we analyzed the prevalence of CT male urogenital infection, the molecular epidemiology, semen inflammation and sperm quality in young-adult men. A cohort of 212 males aged 20-49 years old with couple's primary infertility or lower urinary tract symptoms was studied. Semen samples were collected by masturbation and semen analysis performed according to the WHO manual. CT and other uropathogens infections were assessed by PCR. Semen quality parameters, ROS, inflammatory cytokines, subpopulations of leukocytes were analyzed. The Kruskal-Wallis test and lineal regression were used for statistical analysis. A high prevalence of CT infection (32.1%) was found, presenting as mono-infection or co-infection with other uropathogens in 12.2% and 19.9% of cases, respectively. Interestingly, most infections were produced by non-previously reported CT genomic variants. Patients infected with CT only or co-infected with other uropathogens showed neither significant alterations in most of the sperm quality parameters analyzed nor increased inflammatory cytokines in semen. Remarkably, the infection with CT either alone or co-infecting with any other uropathogens inversely correlated with ROS levels and peroxidase-positive cell counts in semen. Moreover, the sole infection with CT associated with increased semen numbers of CD8+ T cells, macrophages and senescent T lymphocytes. Our results revealed a high prevalence of CT urogenital infection in men from our region. Interestingly, our data suggest that CT would skew the immune response away from inflammation thus remaining as a silent infection. Thus, men would provide a reservoir for continuous transmission of the infection.

219. (362) **SARS COV2 HOST IMMUNE RESPONSE AND CLINICAL MANIFESTATIONS: TLR3, TLR4 AND TLR7**

Martinez Marignac Veronica L. 1,2; Oertlin Gloria 1,2; Ghilini Daniel 1; Fleischman Erika 2; Richard Silvina M 3.

1. IBIOGEM, Laboratorio Interdisciplinario de Biología y Genética Molecular; Centro de Investigación Científica y de Transferencia Tecnológica a la Producción-CICYTTP (CONICET, Prov. Entre Ríos y UADER).
2. Laboratorio de Investigación en Enfermedades Infecciosas, Dr. Néstor Bianchi, Hospital san José de Diamante, Ministerio de Salud Provincia de Entre Rios.
3. Instituto Multidisciplinario de Biología Celular (IMBICE), La Plata, Buenos Aires.

Toll-like receptors (TLRs) may be involved in the initial failure of viral clearance and in the subsequent development of fatal clinical manifestations of severe COVID-19,essentially ARDS (acute respiratory distress syndrome).Here we characterized the expression of TLR 3, 4, and 7 in nasopharyngeal total RNA samples from 150 individuals positive for Covid19 by Neokit SA and 150 non detectable ambulatory patients; we compared with the symptomatology developed.We analyzed 4 cohorts: Viral genome detected patients with severe to high symptomatology(n=107);Viral genome detected patients without or low symptomatology(n=43);Viral genome non detected patients with severe to high symptomatology(n=109); andViral genome non detected patients without or low symptomatology(n=41).We showed significant differences of expression for TLR4 between detected and non-detected patients with severe or non-severe symptoms (Non Paired T–test pValue=0.04; and pValue=0.04).TLR3 showed significant differences between non detected and detected patients with severe symptoms(Non Paired T-test; pValue=0.003). The expression of TLR7 was not significant different between cohorts however when compared by Spearman's Correlation Coefficient there was a significant positive correlation between severity of symptoms in those patients without covid19 infection. The same significant positive correlation was observed for TLR4 and severe symptoms in no covid19 patients by Spearman's and Point-Biserial Correlation.On average all detected patients expressed these genes in a same way as non-detected patients with severe symptoms, been TLR4 the significant higher expressed gene in all patients with severe symptoms;we confirmed that the immune response can be associated with harm to the host due to persistent inflammation and tissue destruction; we confirmed TLR4 gene expression involvement in the pathogenesis of COVID-19 as well as other respiratory diseases;we suggest that effort must be done to focus therapies on TLR4 expression in inflammatory respiratory diseases.

220. (426) **AUTOCRINE AND PARACRINE IL-6 IS NECESSARY TO INDUCE PHAGOCYTOSIS OF VIABLE NEURONS BY *BRUCELLA ABORTUS*-ACTIVATED MICROGLIA**

Julia Rodríguez, Julia De Santis Arévalo, Ariadna M. Fiorillo Evequoz, Ana M. Rodríguez, Guillermo H. Giambartolomei

Instituto de Inmunología, Genética y Metabolismo (UBA-CONICET), Hospital de Clínicas "José de San Martín", Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Central nervous system infection by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. We have previously demonstrated that soluble mediators released by *B. abortus*-infected astrocytes induce inflammatory activation of microglia and phagocytosis of viable neurons. We have also demonstrated that neutralization of IL-6 in neurons/microglia co-cultures treated with supernatants from *B. abortus*-infected astrocytes inhibits neuronal death, and this effect is caused by a decrease in the phagocytic activity of microglia. Considering that both astrocytes and microglia are capable of secrete IL-6, we aimed to investigate the contribution of each cell type in this phenomenon. Astrocytes from wild type (WT) and IL-6 KO mice were infected or not with *B. abortus* for 24 h. After that, cell-free culture supernatants were used to stimulate primary murine co-cultures of WT and IL-6 KO microglia with neurons during 48 h. Neuronal density was evaluated by fluorescence microscopy. Treatment of WT co-cultures with supernatants from IL-6 KO infected astrocytes caused a partial inhibition of neuronal death ($p<0.05$). Similar results were obtained when neurons/IL-6 KO microglia co-cultures were treated with supernatants from WT- infected astrocytes ($p<0.05$). Neuronal loss was totally prevented in co-cul-

tures of neurons/IL-6 KO microglia treated with IL-6 KO infected astrocytes ($p<0.05$). Moreover, *B. abortus*-activated microglia from IL-6 KO mice were unable to induce neuronal death ($p<0.05$). These results indicate that both paracrine and autocrine IL-6 signaling in microglia can be sufficient to induce phagocytosis of viable neurons in the context of a *B. abortus* infection, and could highlight the relevance of this cytokine in neuropathological mechanisms caused by *Brucella* spp.

221. (455) ***TRYPANOSOMA CRUZI* INFECTION ENHANCED THE THYMIC OUTPUT AND INDUCE ALTERATIONS IN THE CENTRAL AND PERIPHERAL V-β TCR REPERTOIRE**

Brenda Dinatale[1], Florencia Belén González[1], Itauá Leston Araujo[2], Camila Bulfoni Balbi[1], María Florencia Pacini[1], Oscar Bottasso[1], Wilson Savino[2], Eduardo Roggero[1], Ana Rosa Pérez[1,3].

[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina, [2]Laboratorio de Pesquisas sobre o Timo, FIOCRUZ, Rio de Janeiro, Brasil, [3]Centro de Investigación y Producción de Reactivos Biológicos (CIPREB), Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina. E-mail: dinatale@idicer-conicet.gob.ar

The thymus is a central organ in the shaping of the peripheral T-cell repertoire. Earlier work in a murine model of Chagas disease revealed progressive thymus atrophy, characterized by $CD4^+CD8^+$ (DP) loss by apoptosis. Since alterations in the normal development of T-cells or in their repertoire could influence the outcome of this infectious disease, here, we evaluated the thymic output and the V-β repertoire after *T. cruzi* (Tc) infection. C57BL/6 mice (n=5/group) were infected with 1000 Tc and evaluated after 17 days post-infection (dpi). Non-infected (NI) mice act as controls. The thymic output was estimated by the quantification of recent thymic emigrants (RTEs) by intrathymic injection of fluorescein (FITC), and 24 h later, thymus and subcutaneous lymph nodes (SLN) were removed and monitored for $FITC^+$ cells occurrence. T-cell receptor excision circles (TRECs) were analyzed in blood by qPCR as RTEs markers. Repertoire screening was carried out by V-β family (5, 6, 8.1-8.2 and 14) detection by flow cytometry. In Tc mice, thymic depletion was coupled with adenomegaly. An increase in the counts of $FITC^+CD4^+$, $FITC^+CD8^+$, as well as immature and potentially autoreactive $FITC^+$DP T-cells was observed in the SLN after 17 dpi ($p<0.05$ vs NI). TRECs trend to increase after 17 dpi (median/range) NI: 114/(23-200), Tc: 184/(23-400). Given the enhanced influx of RTEs and the presence of DP cells in SLN, we examine the shaping of mature and immature T-cell repertoire. An increase in the frequency of all V-β families was detected after infection in the thymus ($p<0.05$ in all cases). Parallel, the frequency of V-β studied in the SLN of Tc animals not differed from NI, except for a decrease in V-β5. Nevertheless, V-β5 and V-β6 were over-represented in DP cells from SLN in infected animals ($p<0.05$ vs. NI). The abnormal thymic output of DP T-cells and the peripheral over-representation of some Vβ families, suggests that the intrathymic selection may be altered during the infection.

222. (472) **GAL-3 AND NEURAMINIDASE ACTIVITY ARE REQUIRED TO INDUCE PHAGOPTOSIS OF NEURONS BY *BRUCELLA ABORTUS*-ACTIVATED MICROGLIA**

Autores: De Santis Arévalo Julia, Fiorillo Evequoz Ariadna María, Rodríguez Julia, Rodríguez Ana María y Giambartolomei Guillermo Hernán

Instituto de Inmunología, Genética y Metabolismo (CONICET/UBA), Hospital de Clínicas "José de San Martín", Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

We have previously demonstrated that *B. abortus*-activated microglia kill neurons through primary phagocytosis or phagoptosis. Phagocytosis is a finely regulated process that involves the interaction of different receptors and their ligands. Galectin-3 (Gal-3) is a protein secreted by activated phagocytes and acts as an opsonin through the MERTK receptor. Gal-3 binds to desialylated proteins present on the cell's surface through the action of neuraminidases. The objective of this work was to investigate if Gal-3 is involved in the phagoptosis of neurons mediated by *B. abortus*-activated microglia. Primary cultures of neurons and microglia from wild type (Balb/c or C57/B6) or Gal-3 deficient mice were infected. Neuron survival was assessed at 48 h by fluorescence microscopy. Co-cultures were treated with Oseltamivir phosphate [neuraminidase (Neu) inhibitor], Tacrolimus (inhibitor of the phosphorylation and subsequent secretion of Gal-3). Neu activity on the cell membrane and TNF-α secretion were measured using commercial kits. *B. abortus*-infected microglia exhibit Neu activity on their cell membrane, which was significantly inhibited by Oseltamivir ($p<0.05$). Moreover, neuronal phagoptosis induced by *B. abortus*-activated microglia was inhibited by Oseltamivir ($p<0.05$) indicating that the desialylation of neuronal membrane sugars is involved in this phenomenon. Tacrolimus was also able to inhibit neuronal death induced by infected microglia ($p<0.05$). Microglia from Gal-3-deficient mice infected with *B. abortus* were unable to induce neuronal death, when compared with microglia from wild-type mice ($p<0.05$). In all cases microglia activation was not affected, since TNF-α secretion was not significantly different between treatments ($p>0.05$). These results demonstrate that Gal-3 and neuraminidase activity would be necessary for the neuronal death mediated by *B. abortus*-activated microglia, describing new molecular mechanisms involved in the pathogenesis of neurobrucellosis.

223. (516) **THE *VITA*-PAMP *BRUCELLA ABORTUS* RNA MODULATES LYMPHOCYTE RESPONSES**

Yasmín A. Bertinat, Jorgelina Bueno, Agustina Serafino, M. Ayelén Milillo, Paula Barrionuevo.

Laboratorio de Fisiología de los Procesos Inflamatorios, IMEX-CONICET, Academia Nacional de Medicina.

B. abortus (*Ba*), the causative agent of brucellosis, displays many strategies to evade T cell responses conducive to persist inside the host. We have previously showed that *Ba* can indirectly inhibit T cells by down-regulating MHC expression on macrophages and as a result, antigen presentation. Moreover, we demonstrated that *Ba* through its lipoproteins (structural component of bacteria) induces apoptosis of T lymphocytes. On the other hand, we have recently demonstrated that *Ba* RNA (PAMP related to pathogens' viability or *vita*-PAMP) is involved in macrophage immune-modulation. However, we did not know if *Ba* RNA was able to modulate T cell responses. To start with, we incubated cells of JURKAT T cell line with *Ba* for 1, 2, 3, 5 and 10 days. After each time point, the expression of the senescence marker CD28 was evaluated by flow cytometry. *Ba* diminished the expression of CD28 at 5 and 10 days ($p<0.05$). Then, we started to study the effect of *Ba* RNA. For this, we perform two experimental approaches: we evaluated the effect of different doses of *Ba* RNA on peripheral blood mononuclear cells (PBMCs) and we also evaluated the effect of *Ba* RNA on PBMCs previously stimulated with plate-bound anti-CD3. For both approaches, the stimulation was carried out for 1 and 5 days. After 24 h, the expression of the activation marker CD69 and CD28 was evaluated by flow cytometry gating on lymphocyte population. We also measured the expression of CD28 after 5 days. At 24 h, *Ba* RNA increased in a dose-dependent manner the percentage of $CD69^+$ lymphocytes ($p<0.05$) both in unstimulated or anti-CD3 pre-stimulated cells. At 5 days, *Ba* RNA diminished both the percentage of $CD28^+$ lymphocytes and the expression of CD28 ($p<0.05$) only in anti-CD3 pre-stimulated cells. Overall, our results show that at early time points RNA is a PAMP by which *Ba* is able to activate lymphocytes. However, later on *Ba* RNA could contribute to circumvent this activation and favor the establishment of a chronic infection.

224. (537) ***IN VIVO* INHIBITION OF MTOR PATHWAY DURING *TRYPANOSOMA CRUZI* INFECTION POLARIZES MACROPHAGES TOWARDS A PROINFLAMMATORY PROFILE WITH MITOCHONDRIAL ROS PRODUCTION**

Matias Vazquez, Ruth Eliana Baigorri, Florencia Hellriegel, Cinthia Stempin, Fabio Cerban

CIBICI-CONICET. Dpto. de Bioquímica Clínica, Fac. de Ciencias Químicas, Universidad Nacional de Córdoba.

Macrophages (Mo) are able to internalize the parasite that causes Chagas disease, *Trypanosoma cruzi*. However, the parasite can evade the microbicidal systems of Mo by intracellular modulation of the mTOR pathway. Rapamycin (Rapa) treatment of Mo previous to infection is able to inhibit mTOR pathway and to polarize Mo towards a proinflammatory profile, characterized by NLRP3 inflammasome activation and mitochondrial ROS (mROS) production. The objective of this work was to validate these results in an *in vivo* model. For this, Balb/c mice were treated with i.p. injections of 10 μg of Rapa while the control group received only the vehicle. Then, mice were separated from each group to be used as control non infected (NI-Rapa or NI-vehicle) or to be infected i.p. with 500 trypomastigotes (I-Rapa or I-vehicle). Rapa was administered every 72 hours beginning three days before infection and ending at 18 dpi. Mice were sacrificed at 19 dpi, blood and peritoneal lavage were obtained. We did not observe significant differences in parasitemia in infected mice between I-Rapa and I-vehicle groups. Cells from the peritoneum were analyzed by FACS to study the small (SPM, CD11b+, F4/80 low) and large peritoneal Mo (LPM, CD11b+, F4/80 high) populations. We observed that infection induced changes in the frequency of these populations (85%-15% LPM-SPM in the NI-vehicle and 77%-23% in the I-vehicle groups) but Rapa-treatment did not modify the proportion of these cells. Then, mROS production, NLRP3 expression and 4EBP1 phosphorylation were evaluated in these Mo peritoneal populations by FACS. mROS production was increased in infected compared to uninfected groups, and in those treated with Rapa compared to the vehicle group. NLRP3 expression and a tendency to decrease in 4EBP1 phosphorylation were observed in Rapa treated groups. Thus, it is possible to conclude that *in vivo* Rapa treatment validates previously *in vitro* results without altering the frequency of SPM/LPM populations.

225. (572) **THYMIC EXPORTATION AND EFFECTOR FUNCTION OF MEMORY-LIKE CD8⁺ T CELLS**

María Estefanía Viano[1], Eliana Baigorrí[1], Cinthia Stempin[1], Ana Rosa Perez[2], Brenda Di Natale[2], Florencia Pacini[2], Camila Bulfoni[2], Florencia Gonzalez[2], Nicolas L. Lidón[1], Fabio Cerban[1] and María Cecilia Rodríguez Galán[1]

1. Centro de Investigaciones en Bioquímica Clínica e Inmunología. CIBICI-CONICET. Facultad de Ciencias Químicas - UNC.

2. Instituto de Inmunología Clínica y Experimental de Rosario. IDICER-CONICET. Universidad Nacional de Rosario - UNR

Memory-like cells are a subset of CD8⁺ T cells that present a memory phenotype without previous antigen encounter. They are a heterogeneous group of cells compose of innate CD8⁺ T cells that arise in the thymus (T_{IM}) and are exported to secondary lymphoid organs (SLO) or, could develop directly in SLO where are named as virtual CD8⁺ T cells (T_{VM}). T_{VM}/T_{IM} rapidly respond to IL-12 and IL-18 and play a protective role during the early phase of bacteria, viral and parasite infections. We have previously reported that thymi from *Trypanosoma cruzi (Tc)*-infected mice are highly enriched on T_{IM}. T_{VM}/T_{IM} can be distinguished from conventional memory T cells (T_{MEM}) by their low expression of CD49d. While residing in the thymus, phenotypic analysis in OT-I mice demonstrate that OVA tetramer⁺ (OVAt⁺) single positive CD8 (SP8) cells (not specific for the parasite) expressed higher levels of CD44, CD122, CD5, CD69, Qa2 and decreased levels of CD24 compared to conventional polyclonal SP8 cells ($p<0,05$). These features are in accordance with a T_{IM} phenotype. Moreover, this pattern is even more pronounced after *Tc* infection ($p<0,05$). During exportation, our data demonstrate that CD8⁺ T cells generated in the thymus can accumulate in SLO up to 5 days after intrathymic staining with eFluor660 (eF) dye used for tracking ($p<0,05$). However, these experiments demonstrated that after *Tc* infection, a lower number of CD8⁺eF⁺ T cells can be found in SLO, although with a higher expression of CD44 and CD49d compared to control mice ($p<0,05$). Functionally, splenic T_{VM}/T_{IM} obtained at day 2 post-*Tc* infection produce high and similar levels of IFNγ *in vitro* in the presence of IL-12+IL-18 than T_{VM}/T_{IM} from control mice Surprisingly, splenic T_{MEM} from *Tc*-infected mice show significantly lower percentage of IFNγ⁺ cells than their control counterpart ($p<0,05$). Our data contribute to understanding the protective role of Ag-dependent and -independent CD8⁺ T cells early during *Tc* infection.

226. (602) **CHRONIC CHAGAS CARDIOMYOPATHY IS LINKED TO A DISRUPTED ACTIVATION OF THE HYPOTHALAMOUS-PITUITARY-ADRENAL AXIS AND A DECREASED 11B-HSD1 EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS**

Antonella Pacini[1], Silvina Villar[1], Rodolfo Leiva[2], Ana Rosa Pérez[1], Florencia Belén González[1].

[1]Instituto de Inmunología Clínica y Experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IDICER-CONICET), [2]Servicio de Cardiología, Sección Chagas, Hospital Provincial del Centenario, Rosario, Argentina. E-mail: apacini@idicer-conicet.gob.ar

Chronic Chagas cardiomyopathy (CCC) is a disease caused by *Trypanosoma cruzi* (Tc). We previously observed a disrupted activation of the hypothalamous-pituitary-adrenal (HPA) axis. The aim of this work was to evaluate possible difference in the immune-endocrine status between chronic chagasic patients with CCC (n=22), non-chagasic with cardiomyopathy (NCC, n=5) and healthy and seronegative individuals (Co, n=15). Systemic levels of Cortisol (GC) and DHEA-S concentration were determined in serum. The expression of genes involved in GC response as GC receptors (GRα functional receptor and GRβ inhibitor receptor) and 11β-HSD1 (which catalyzes the conversion of GC from inactive to active form) was assessed by qPCR. We also evaluated in peripheral blood mononuclear cells (PBMCs) the expression of genes regulated by GC and involved in the inflammatory response: IL-6, IFN-γ, IL-1β, TNF-α and tristetraprolin (TTP) by qPCR. As we previously shown, CCC individuals showed a disrupted activation of the HPA axis, characterized by decreased DHEA-S and GC levels together with an increased GC/DHEA-S ratio ($p<0,05$ vs Co). These alterations were not observed in NCC patients. In PBMCs from CCC patients, GRα expression did not differ from Co, while it was diminished in NCC individuals ($p<0,05$ vs Co and CCC), GRβ was not detectable in any of the groups and 11β-HSD1 was augmented in CCC patients respect to the Co group ($p<0,05$). All inflammatory cytokines trend to be increase in PBMCs that came from the CCC group, while TTP expression was diminished in CCC and NCC groups respect to the Co ($p<0,05$). These results suggest that an adverse endocrine milieu in CCC patients may predispose to an increased pro-inflammatory state compared with other types of cardiomyopathies, and this could be sustained by parasite persistence.

227. (604) **EFFECT OF METFORMIN TREATMENT ON THE EXPRESSION OF COSTIMULATORY AND INHIBITORY MOLECULES IN PERITONEAL AND SPLEEN MACROPHAGES OF *TRYPANOSOMA CRUZI* INFECTED MICE.**

Ruth Eliana Baigorri, Ma. Florencia Hellriegel, Ma. Belén Brugo, Matías Vázquez V, Ma. Estefanía Viano, Ma. Cecilia Rodríguez, Claudia Cristina Motrán, Cinthia Stempin, Fabio Cerbán

CIBICI CONICET - Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

Macrophages (Mφ) are antigen presenting cells (APC) that interact with primed CD4 T cells. This interaction is supported by surface and soluble mediators that promote a cellular or humoral response. Previously, we demonstrated in our *in vivo* model that peritoneal Mφ (PEM) and F4/80⁺CD11b⁺ spleen Mφ (SpM) exhibit high iNOS expression and NO release in the acute phase that could be reverted by Metformin (MF) treatment. MF is a diabetes drug that can modulate several pathways switching Mφ phenotype and function. In order to characterize the APC features of PEM and SpM we infected Balb/c mice i.p. with 500 trypomastigotes. At different times of infection we analyzed CD80, CD86, PD-L1 and PD-L2 expression by flow cytometry. We found an increase in CD80 and PD-L1 and less CD86 and PD-L2 positive cells in SpM in 23 dpi. PEM also increase the percentage of both CD80⁺ and PD-L1⁺ cells. Then, to evaluate

whether MF treatment of PEM could modulate costimulatory and inhibitory molecules and impact T cell activation, we cocultured total stimulated-splenocytes with infected PEM treated *ex vivo* with PBS or MF. We found a non-significant decrease in CD4 T cell proliferation assessed by CFSE dilution when splenocytes were cocultured with infected PEM. This effect was even higher in cocultures with infected and MF treated-PEM. Afterward, we evaluated expression of these molecules in an *in vivo* oral MF treatment of infected Balb/c mice (100 mg/kg beginning at day 6 until 18 dpi). We did not observe differences in PD-L1 expression of PEM or SpM. However, both populations of Mϕ showed a clear tendency to decrease in CD80 expression. In addition, we analyzed PD-1 expression in Treg, Tconv and CD8 T cells. Although MF treatment did not modify PD-1 expression in these cells we found an decrease in Treg cells in infected and MF treated animals compared with controls. These results suggest a potential role of Mϕ in T cell activation that could be modulated by MF during *T. cruzi* infection.

228. (612) **UV PHOTOTHERAPY, AS AN ADJUVANT TREATMENT FOR METHICILLIN- RESISTANT *STAPHYLOCOCCUS AUREUS* CUTANEOUS INFECTION, INDUCES REGENERATIVE SKIN CHANGES AND PROMOTES A PRO- INFLAMMATORY ENVIRONMENT**

Cela, EM[1,2]; Ledo, C[3,4]; Illescas, E[3]; Leoni, J[2]; Gómez, MI[3,4,5] and González Maglio, DH[1,2].

1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología. CABA, Argentina.

2. CONICET-Universidad de Buenos Aires. Instituto de Estudios de la Inmunidad Humoral (IDEHU), Facultad de Farmacia y Bioquímica. CABA, Argentina.

3. Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD). Universidad Maimónides.

4. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

5. Departamento de Microbiología, Parasitología e Inmunología. Facultad de Medicina. Universidad de buenos Aires.

Staphylococcus aureus is a colonizer of the human skin, a prevalent opportunistic pathogen in chronic wounds and a major cause of skin and soft tissue infections (SSTI). Antibiotic resistance and impaired wound healing are major concerns in SSTI and new therapies are needed. We have shown the potential of using Ultraviolet radiation (UVr) for skin immunomodulation during *S. aureus* infection. This study was aimed at optimizing the irradiation protocol including healthy skin around the abscess to improve the response elicited. BALB/c mice were subcutaneously inoculated with *S. aureus* USA300 LAC ($2x10^7$ CFU in 50 μl) and exposed to UVr (100 mJ/cm^2) 48 h later. Three experimental groups were included. G1: the irradiated area was limited to the abscess; G2: the irradiated area included 5 mm of healthy skin around the abscess; G3: non-irradiated animals as control. Abscess size and mice weight were monitored daily for 14 days. At different days post inoculation (dpi) mice were sacrificed for CFU, cytokines and chemokines quantification, and for histological evaluation. UVr significantly reduced the area of the skin lesion at 7 dpi, with a better performance in the group that included healthy skin for irradiation (53 % reduction in G2 vs. 38 % in G1 and G3, p<0,05). The differences were maintained at 14 dpi (92% reduction in G2 vs. 85% in G1 vs. 76 % in G3, p<0,01). Moreover, G2 presented a strong inflammatory response at 14 dpi (TNF-α, IL-1 β, KC; p<0,05, and IL-6 p=0,067 vs. G3). No differences in bacterial load were found among groups. However, circumscribed vs. diffused cellular infiltration and repaired vs. limited fibrosis were observed in G2 compared to G1 and G3 (respectively) at 10 and 14 dpi. G2 mice skin also showed regenerative changes, partially explaining the observed macroscopically tissue healing. Taken together, these results demonstrate the feasibility of using UVr to induce inflammatory mediators as well as skin repair and healing during *S. aureus* infection.

229. (626) **LEVELS OF CIRCULATING IGG ANTIBODIES AGAINST CFP10 AND ESAT6 PROTEINS DIFFERENTIATE ACTIVE TB PATIENTS AND RECENTLY INFECTED INDIVIDUALS FROM LATENTLY INFECTED SUBJECTS**

Agustín Vitti[1,2], Rocío Zuazo[1,2], Alejandro Castello[3], Estefanía Peri Ibañez[3], M. Paula Morelli[1,2], Lucía Donolli[1,2], Ana Bazan[1,2], Rita Armitano[4], Juan Stupka[4], Claudio Gallego[4], Domingo Palmero[5], Nancy Tateosian[1,2], Juan Iovanna[6], Verónica García[1,2], Nicolás Amiano[1,2]

1 Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), CONICET, 2 Departamento de Química Biológica, FCEN, UBA, 3 Laboratorio de Inmunología y Virología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes 4 Hospital General de Agudos Parmenio Piñero, 5 Hospital de Enfermedades Infecciosas Dr. Francisco J. Muñiz, 6 Centre de Recherche en Cancérologie de Marseille (CRCM), INSERM U1068, CNRS

Mycobacterium tuberculosis (*Mtb*), the agent responsible for the tuberculosis disease (TB) has been historically one of the deadliest infectious human pathogens. *Mtb* is characterized by its capacity to enter into a dormancy state that allows it to persist in an individual for years. This latent condition makes direct detection of bacterial components difficult and results in a significant obstacle for diagnosis. We have previously generated a fusion protein (Fp) of CFP10 and ESAT6 antigens that can be used for detecting *Mtb* infected individuals in an IGRA test. We have also demonstrated that the antigen Rv2626c allows to discriminate individuals with an established latent tuberculosis infection (LTBI) from tuberculosis patients (TBP), recently infected subjects (RI) and healthy controls (HC). Here, we analyzed levels of circulating IgG antibodies against Fp in these mentioned study groups. We used Fp to sensitize ELISA plates and measured IgG specific antibodies in plasma samples. We first observed that levels of IgG antibodies against ESAT6 and CFP10 could differentiate TBP and HC ($D.O._{TB}$ = 0.71 ± 0.10; $D.O._{HC}$ = 0.11 ± 0.03; ****p<0.0001) with ROC analysis showing 65.8% sensitivity and 70.0% specificity for a cut-off value of D.O.: = 0.125. Furthermore, anti-Fp IgG levels were able to distinguish asymptomatic individuals positive for QuantiFERON TB Gold Plus (QFT) or tuberculin skin test (TST) from TB patients. Surprisingly, RI QFT+ subjects showed significantly higher levels of IgG against Fp than LTBI ($D.O._{RI}$ = 0.45 ± 0.12; $D.O._{LTBI}$ = 0.12 ± 0.07; *p<0.05) and, in a similar manner, individuals with low and high cellular immune responses against Rv2626c could also be differentiated by levels of IgG against Fp. In summary, our findings suggest that Fp could be used to detect specific IgG antibodies in serodiagnostic tests to identify TBP and individuals at risk of developing active TB.

230. (643) **ROLE OF MYELOID CELLS IN BACTERIAL ENDOTOXINS INDUCED IMMUNOSUPPRESSION AND ITS RELATIONSHIP WITH IL10**

María Florencia Todero[1], Lautaro Maceira[1], Alan Bernal[2], Daniela Montagna[3], Gastón Dominguez[4], Adriana Fontanals[4], Bárbara Rearte[1].

[1]Laboratorio de Fisiología de los Procesos Inflamatorios, [2]Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, [3]Laboratorio de Oncología Experimental, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

[4]Fundación Instituto Leloir, Buenos Aires, Argentina.

Sepsis is characterized by a late immunosuppression (IS) establishment that promotes secondary reinfections, in which both anti-inflammatory cytokines such as IL10 and myeloid cell expansion are strongly involved. Among the etiological agents are gram-negative bacteria whose lipopolysaccharides (LPS) have a crucial role. Our aim is to characterize functional aspects of myeloid cells, their relationship with IL10 and understand their IS involvement. For this, BALB/c mice IL-10 deficient inoculated or not (KO) with increasing LPS doses (KOIS) were used. Clearance assessment: Intranasal instillation with *Pseudomona aeruginosa* ($5*10^7$ UFC/ml). Flow cytometry: Blood respiratory burst capacity and splenocytes proliferation in co-culture. In mice KOIS peripheral blood a significant increase of myeloid cells was observed (CD11b/Ly6G/Ly6C (absN°): KO=$2,8*10^6$±$3,7*10^6$; KOIS=$1,3*10^7$±$7,4*10^5$, p<0.001). However, respiratory burst capacity did not show differences into groups. Spleen lymphocytes proliferation showed a significantly decreased in mice KOIS (CFSE+Cell (%Ratio): KO=19,9±1,6;

KOIS=11,7±1,4, p<0.01), jointly with a significant increase of myeloid cells in spleen (CD11b/Ly6G/Ly6C (absN°): KO=9,6*10^5±2,7*10^5; KOIS=1,4*10^7±3,9*10^6, p<0.01). In addition, bone marrow (BM) from KOIS mice showed a high percentage of myeloid cells (CD11b/Ly6G/Ly6C (% cells/femur): KO=40,7±2,4; KOIS=61,8±0,6, p<0.001). In co-culture assays to control splenocytes plus BM cells from IS mice, a decreased proliferative capacity was observed. This could be associated with the inhibitory effect of BM myeloid cells on lymphocyte proliferation. A diminished clearance capacity in KOIS mice was observed, reflected in higher lung bacterial counts. Our results show that in a context of IS and in IL-10 absence the myeloid cells expanded present functional alterations that are detrimental to immune homeostasis. Further approaches such as the restoration of IL-10 will be developed in the future.

231. (650) **KINETICS OF SYSTEMIC IMMUNE CELLS POPULATIONS AFTER AN INTRAMAMMARY EXPERIMENTAL INFECTION OF DAIRY COWS WITH TWO *sTAPHYLOCOCCUS AUREUS* STRAINS WITH distinct adaptation genotypes**

Engler Carolina[1], Pirola Silvana[1], Beccaría Camila[1], Silvestrini Paula[1], Simonutti Valeria[1], Baravalle Celina[1,2], Calvinho Luis[2,3], Dallard Bibiana[1,2], Renna María Sol[1,2].

[1]Instituto de Ciencias Veterinarias del Litoral (ICIVET-Litoral), Universidad Nacional del Litoral (UNL)/Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. [2]Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. [3]Estación Experimental Agropecuaria Rafaela, Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela, Santa Fe, Argentina.

The aim of this study was to evaluate whether systemic immune cells populations undergo changes after an experimental intramammary infection with two *S. aureus* strains with different adaptation genotypes (low and high) to the bovine mammary gland. Three groups of clinically healthy cows, 4 cows/group, were inoculated by the intramammary route in two mammary quarters with *S. aureus* 806 strain (non persistent-NP), *S. aureus* 5011 strain (persistent-P) and pyrogen-free saline solution (control group). Peripheral blood samples were collected at 0, 0.5, 1, 2, 3, 4, 7, 14 and 21 days post-inoculation (pi) from all groups to evaluate the frequency of monocytes, LB, LTCD4 and LTCD8 by flow cytometry. A Generalized Linear Model was used to analyze the results. No effect of infection was observed between groups in the percentages of monocytes over time (p=0.842). When only day 2 pi was analyzed, a decrease in the percentages of monocytes was observed in animals inoculated with NP strain (p=0.033). A significant effect of infection was observed between groups in the percentages of LB over time (p=0.025). On day 3 pi, a decrease in the percentages of LB was observed in animals inoculated with P strain (p<0.001). No effect of infection was observed between groups in the percentages of LTCD8 over time (p=0.408). When only day 21 pi was analyzed, a decrease in the percentages of LTCD8 was observed in animals inoculated with NP strain while an increase was observed in animals inoculated with P strain (p<0.001). No effect of infection was observed between groups on LTCD4 percentages over time (p=0.115). When only day 3 pi was analyzed, an increase in the percentages of LTCD4 were observed in animals from NP and P groups (p=0.016). A better understanding of the host immune response mechanisms that develop against specific *S. aureus* genotypes may contribute to discovering the complex interactions that determine the establishment and chronicity of intramammary *S. aureus* infections.

232. (655) **MURINE MACROPHAGES SURFACE N-GLYCANS PARTICIPATE IN YopP-MEDIATED IMMUNOPATHOGENESIS OF *Yersinia enterocolitica* INFECTIONS**

Juan Agustín Garay[1], Juan Eduardo Silva[1,2], José Varas[1], Samanta Funes[1,2], Brenda Jofré[1,2], María Silvia Di Genaro[1,2], Roberto Carlos Davicino[1,2]

[1]Universidad Nacional de San Luis, Argentina. [2] Instituto Multidisciplinario de Investigaciones Biológicas de San Luis, CONICET, Argentina.

Yersinia enterocolitica (Ye) outer membrane protein P (YopP) is able to manipulate macrophages (MΦ) by inducing apoptosis and suppressing signaling molecules production. Although YopP has been shown to interact in a carbohydrate-dependent manner with host proteins, its interaction with glycans remains unclear. The aim of our work is to explore the influence of MΦ surface N-glycans on the immunomodulatory effects triggered by YopP. Murine peritoneal MΦ were obtained by intraperitoneal lavage, plated at a 1x10^6 cells/mL and purified by incubation for 2 h at 37°C in DMEM medium in an atmosphere with 5% CO_2. Then, MΦ were incubated with 5 IU/mL of PNGase F in DMEM medium without Fetal Bovine Serum for 4 h (MΦd) and cultured overnight in supplemented DMEM medium. Subsequently, MΦ were infected with Ye serotype 0:8 (pYV+, WA-314) (Ye wt) or with Ye WA-314 deficient in YopP (pYV+, WA-C pYVNalrKanr) (Ye Δ*yopP*) at MOI 10:1. Then, MTT, lactate dehydrogenase (LDH), Griess and Urease assays were performed. Apoptosis was evaluated by flow cytometry using Annexin V-propidium iodide, and TNF-α was measured by ELISA. Our results shows that N-deglycosylation does not affect viability. Besides, we observed no significant apoptosis in Ye wt-infected MΦd compared to uninfected counterpart, suggesting a critical impact of N-glycans in the well-known triggering role of Ye on apoptosis. On the other hand, we showed that YopP has a dual effect: in MΦ inhibits NO production (p<0.05) but in MΦd stimulates NO production (p<0.05), suggesting an influence of N-glycans in the NO production by YopP. Surprisingly, urea was increased in uninfected MΦd and in infected MΦ or MΦd (p<0.01). Interestingly, TNF increased in MΦd (p<0.01); however, N-deglycosylation did not interfere the regulatory effect of YopP on TNF. This work highlights an unexplored participation of N-glycans in macrophages during Ye infection.

233. (686) **POSTIMMUNOBIOTICS INCREASE ANTIVIRAL IMMUNITY IN ALVEOLAR MACROPHAGES: IMPACT ON THE RESISTANCE AGAINST RESPIRATORY SYNCYTIAL VIRUS**

Fernanda Raya Tonetti[1], Ramiro Ortiz Moyano[1], Stefanía Dentice Maidana[2], Mariano Elean[1], Leonardo Albarracín[1], Haruki Kitazawa[3], Julio Villena[1,3].

[1]Laboratory of Immunobiotechnology, Reference Centre for Lactobacilli (CERELA-CONICET), Tucuman, Argentina. [2]Laboratory of Antimicrobials, Institute of Microbiology, Faculty of Biochemistry, Chemistry and Pharmacy, National University of Tucuman, Tucuman 4000, Argentina. [3]Food and Feed Immunology Group, Laboratory of Animal Food Function, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

Beneficial microbes with immunomodulatory capacities (immunobiotics) and their non-viable forms (postimmunobiotics) could be effectively utilized in the prevention of respiratory viral infections. In this study, we evaluated the ability of 36 *Lactobacillus gasseri* strains to modulate the immune response of porcine alveolar macrophages (3D4/31 cells) to the stimulation with the Toll-like receptor 3 (TLR3) agonist poly(I:C). By determining the expression of *IFN-β* and *RNAseL* we selected *L. gasseri* TMT36, TMT39 and TMT40 as the strains with immunomodulatory potential. Postimmunobiotics (HK) derived from the heat treatment of those strains were also evaluated in macrophages. In our hands, HK36, HK39 and HK40 enhanced the expression of *IFN-β*, *IFN-λ1*, and the antiviral factors *RNAseL* and *Mx2* (p<0.05) in porcine alveolar macrophages indicating their capacity to improve the innate antiviral immune response in this respiratory immune cell population. We further evaluated the capacity of the postimmunobiotics to improve the resistance against the respiratory challenge with RSV *in vivo*. Thus, 3-week-old BALB/c mice were nasally primed with HK36, HK39 or HK40 for two consecutive days (10^8 cells/mouse/day) and then infected with 10^6 PFU of RSV by the nasal route. Postimmunobiotic treatments significantly reduced RSV titers and lung damage in mice compared to the control group (p<0.05). The treatments also increased the levels of IFN-β, IFN-λ, IL-1β and IL-6 (p<0.05) in the respiratory tract. In addition, the expression levels of the antiviral factors *Mx1*, *RNAseL* and *OAS1* in alveolar macrophages from mice treated with postimmuno-

biotics were significantly up regulated when compared to controls ($p<0.05$). Although our findings should be deepened and expanded, the results of the present work provide a scientific rationale for the use of nasally administered HK36, HK39 or HK40 to beneficially modulate TLR3-triggerd respiratory innate immune response.

234. (703) **MOTHER-TO-CHILD ANTI-NEF PASSIVE IMMUNIZATIO PREVENTS AIDS DISEASE PROGRESSION IN HIV-1 VERTICALLY-INFECTED CHILDREN.**

Navarro Romina S[1], Marino Silvia A[2], Bellazzi Mónica P[1], Bruque Carlos D[1], Corró Guillermo[1].
1) UCT-HP, Hospital El Calafate SAMIC, El Calafate, Santa Cruz, Argentina. 2) Laboratorio de Pesquisa Neonatal, Hospital de Pediatría "Garrahan" SAMIC, Buenos Aires, Argentina.

HIV-1 Nef protein has been strongly associated with uninfected T-cell death (bystander effect). This protein exerts a cytotoxic effect upon CD4+ cells though some authors found no correlation between Nef concentration in plasma and T-cell counts. On the other hand, we have previously observed that high titers of anti-Nef antibodies were found in plasmas of long-term non-progressor children but not in typical or rapid-progressors. The aim of this preliminary study was to evaluate the protective effect that may have the pre-existent anti-Nef antibodies at acute or primary infection. 32 HIV-1 vertically-infected children that had not received any ARV prophylaxis who had been clinically assisted at Garrahan Hospital from Buenos Aires were retrospectively included in the study. Plasma samples at 1-3 months post-birth were evaluated for anti-Nef IgG. The children were then classified into 3 groups according to the anti-Nef antibody levels: IgG- (anti-Nef-IgG titer <50) n=9, IgG+ (anti-Nef-IgG titer range 200-7500) n=20 and LTNP-like (anti-Nef-IgG >11000) n=3. We observed that the children of the last group remained AIDS-free for more than 10 years while the children that had low titers or no anti-Nef Antibodies progressed to AIDS within 2 years post-birth. Our preliminary data and results suggest that pre-existent anti-Nef antibodies may have a protective effect, preventing AIDS disease progression in HIV-1 vertically infected children. Although this is a preliminary study, our observations would be of great interest for a therapeutic vaccine design as well as alternative therapies development.

235. (706) ***TRYPANOSOMA CRUZI* INFECTED MICE EXHIBIT FOLLICULAR CYTOTOXIC CD8+ T CELLS WHICH CONDITION B CELL DIFFERENTIATION RESPONSE *IN VITRO***

Gazzoni Yamila1, Almada Laura1, Gareca Julio C1, Montes Carolina L1, Acosta-Rodríguez Eva V1, Gruppi Adriana1.
1.Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, X5000HUA, Córdoba, Argentina.

Chagas disease is a chronic infection caused by *T. cruzi* in which early plasmablast(PB) response and delayed Germinal Center(GC) reaction occur. Previously, we identified a subset of $CD8^+$T cell in spleen and inguinal lymph nodes called follicular cytotoxic $CD8^+$T cells(Tfc, CD8+CXCR5+PD-1+) whose peak of response is at 18 day post infection(dpi), as PB response did. Tfc share gene signature with Tfh by expressing ICOS, CD40-L and Bcl-6 and are an effector subset since produce high levels of IFN-γ, Granzyme B and TNF. To study Tfc function on B cell response, C57BL6 mice were intraperitoneally infected with 5000 tps Tulahuen strain. By immunofluorescence of spleen sections, we observed at 18 dpi, $CD8^+$T cells inside follicles(FO); at 23 and 28 dpi they were outside. $CD8^+$T cells were in close contact with PNA^+GC B cells, extrafollicular PB and parasites. In those FO where $CD8^+$T cells and parasites were high, PNA^+ cells were low, suggesting that $CD8^+$T cells could condition B cell differentiation. These CD8+T cells cannot be identified as Tfc, but their presence in FO suggests CXCR5-expression. To evaluate if Tfc influence B cell response we performed *in vitro* co-culture assays. Purified B cells from uninfected mice were cultured with CpG and anti-CD40 for 24 h. After that, sorted Tfc or Non-Tfc obtained at 18 dpi from spleen of infected mice were added to the culture in a 2:1 proportion(2CD8+:1B cell) for 20 h. We observed by FACS, a higher frequency of $B220^+CD138^+Blimp\text{-}1^+$ and $B220^+CD138^{neg}$-$Blimp\text{-}1^+$ cells in the co-culture of activated B cells with Tfc versus with Non-Tfc($p<0,05$). High levels of IgG2c and IgA in the supernatants of B cell cultured with Tfc was detected by LEGENDplex. Non-Tfc did not increase any immunoglobulin isotype. IgM and IgG1 were decreased in B cell co-cultures with both subsets($p<0,05$). To summarize, we identified Tfc in *T. cruzi* infected mice which are able to influence B cell response by promoting PB differentiation and IgG2c and IgA production.

236. (729) **ANTIBODY RESPONSE TO VACCINATION AGAINST SARS-COV2 AND PROTECTION FROM THE INFECTION**

Noelia Flor[1], María Inés García[1], Ana Molineri[2], Camila Miotti[2], Oscar Bottasso[3], Carolina Veaute[1], Cristina Diez[4]
[1]Laboratorio de Inmunología Experimental. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe, Argentina
[2] Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Ruta 34 Km 227, Rafaela, Santa Fe, Argentina
[3] Instituto de Inmunología Clínica y Experimental de Rosario. Rosario, Santa Fe. Argentina
[4] Laboratorio de Biología Molecular e Inmunología Aplicadas. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe. Argentina

This study aimed to assess the response to vaccination against SARs-CoV2 in the FBCB-UNL community. 188 individuals (53 male, 135 females, aged 18-67 ys) were recruited from December 2021 to July 2022, and 3 blood samples were taken 1 month apart. Demographic and epidemiological data were registered for each sampling time. Anti-S antibody concentration (UI/mL) was quantitated by ELISA. Anti-N antibody levels were measured by ECLIA and expressed as Positivity Ratio (PR). A PR>1 indicates exposure to the antigen and was used to diagnose previous infections. Statistical analysis was done using a generalized linear mixed model and non-parametric tests. Most of the participants had two (49%) or three (50%) doses of the SARS-CoV2 vaccine at the start of the study. Only 1% received no dose either before or during the study. At the end of the project, 20% of the participants had two, 69% three, and 10% four doses. Regarding the first two doses, 72 individuals received Sinopharm, 71 Astrazeneca, 39 Sputnik V, 1 Moderna, and 2 Pfizer, with no significant differences in the levels of anti-S antibodies among the different schedules. In the third and fourth doses, Moderna and Pfizer predominated, followed by Astrazeneca, Sputnik, and Johnson & Johnson in descending order. Although individuals who had two doses maintained positive anti-S antibody levels 5-6 months after the last dose, such values were significantly lower than the ones seen after 30-60 days among those receiving the third dose ($p<0.001$-Kruskal-Wallis test). In addition, anti-S antibodies in the first sample were higher in individuals who did not subsequently become infected compared to those who did ($P=0.003$). Regarding the booster, the number of infections observed before the second sample was lower in individuals who had received the third dose before the first sample ($P<0.001$). The protective capacity of anti-S antibody levels as well as that of the third dose of the anti-SARS-CoV2 vaccine is highlighted.

237. (730) **DYNAMICS OF ANTI-NUCLEOCAPSID AND ANTI-SPIKE ANTIBODIES ELICITED BY SARS-COV2 INFECTION IN A UNIVERSITY SETTING**

Noelia Flor[1], María Inés García[1], Ana Molineri[2], Camila Miotti[2], Oscar Bottasso[3], Cristina Diez[4], Carolina Veaute[1]
[1] Laboratorio de Inmunología Experimental. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe, Argentina
[2] Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Ruta 34 Km 227, Rafaela, Santa Fe, Argentina
[3] Instituto de Inmunología Clínica y Experimental de Rosario. Rosario, Santa Fe. Argentina
[4] Laboratorio de Biología Molecular e Inmunología Aplicadas. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe. Argentina

This study assessed the dynamics of the SARS-CoV2 infection in workers and students at the FBCB-UNL. 188 individuals (53 male, 135 females, aged 18-67 y) were recruited from December 2021 to July 2022, and 3 blood samples were taken 1 month apart. Demographic and epidemiological information was registered for each sampling time. Anti-S antibody concentration (UI/mL) was quantitated by ELISA, whereas anti-N antibody levels were measured by ECLIA and expressed as Positivity Ratio (PR). A PR>1 indicates antigen exposure being used to identify individuals with previous infections. For statistical analysis, nonparametric tests were applied. Results are shown as median and interquartile range. Forty-two percent of the individuals reported being diagnosed before or during the study; however, anti-N levels indicated that 80% of the participants had been in contact with the virus. At recruitment, 60 individuals vaccinated with 3 doses were previously infected, 3 of them being reinfected throughout the study. Among individuals with 2 doses, 39 had the infection before recruitment, 4 became infected once, and 1 had 2 consecutive infections during the study. After 3-5 months of the second dose, the infected individuals with 2 doses of any vaccine had higher anti-S levels compared to those with no infection (43346 [25110-99586] vs. 9135 [6714-18689] p<0.0001). The anti-N levels were also higher in infected compared to non-infected individuals vaccinated with 2 doses of Sinopharm (129.3 [88.11-172] vs 3.72 [1.25-9.00] p<0.0001). When analyzing only individuals vaccinated with Spike-based vaccines, anti-N antibodies remained positive 200 days after the infection (7.45[2.61-28.72]). Anti-N antibodies persisted at least 6 months after infection and allowed the detection of a high proportion of asymptomatic infected individuals. Infection behaved as a booster in vaccinated individuals. Although with some degree of variability, the studied population had immunity against SARS-CoV2.

238. (731) ***STAPHYLOCOCCUS EPIDERMIDIS* INFECTS AND REPLICATES IN ULTRAVIOLET IRRADIATED-KERATINOCYTES INDUCING A PRO- INFLAMMATORY ENVIRONMENT**

Mirada C[1]; Leoni, J[2]; González Maglio, DH[1,2] and Cela, EM[1,2].
1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología. CABA, Argentina.
2. CONICET-Universidad de Buenos Aires. Instituto de Estudios de la Inmunidad Humoral (IDEHU), Facultad de Farmacia y Bioquímica. CABA, Argentina.

The skin constantly interacts with commensal microorganisms, usually in a tolerogenic way. However, when the epithelium is disrupted, these microorganisms reach areas without tolerance, producing alterations in the dialogue with the immune system. Moreover, the skin may also be exposed to ultraviolet radiation (UVr), generating different effects on exposed cells. We aimed to study the effects of keratinocytes' UVr exposure on the dialogue between innate immunity and *Staphylococcus epidermidis* (a component of skin microbiota). HaCaT cells ($1x10^5$) were seeded into a 24-well plate for 24 h, then washed and UV irradiated (0 or 25 mJ/cm^2). Then, plates were infected with *S. epidermidis* (MOI: 0, 10 and 100), centrifuged, incubated for 2 h (37 °C, 5% CO2) and finally washed with PBS. For CFU quantification, some wells were immediately treated with Triton X-100 (attachment + internalization), others were incubated with 50 μg/mL gentamicin (1h) and then lysed (internalization) and the rest were maintained in DMEM without antibiotics (12h) and lysed (replication). From the last wells (12h), culture supernatants were harvested for cytokine measurement and LDH cytotoxicity assay. Other plates were stimulated with same concentrations of heat-killed bacteria and incubated for 24h to evaluate cytokine production.
S. epidermidis infected control and irradiated cells (MOI 10 and 100) equally, although the last presented more attached bacteria (p<0,05) and intracellular replication (MOI 10 and 100, p<0,05 vs control). Bacteria also induced IL-6, IL-8, IL-1β and TNF-α production (p<0,05) synergically with UVr. On the contrary, HKB did not modify IL-6 levels and reduced IL-8 production (MOI 10 and 100, p<0,05). There was also a strong synergism in cell death with UVr and live bacteria. *S. epidermidis* was able to infect and replicate in UV irradiated HaCaT cells, increasing cell death and pro-inflammatory cytokine production. These effects are dependent on the bacteria viability.

239. (746) ***TRYPANOSOMA CRUZI* INFECTION: ROLE OF WNT SIGNALING IN THE MODULATION OF FIBROBLAST PHENOTYPE AND FUNCTION**

María Belén Brugo [1,2], Juan Nahuel Quiroz [1,2], Ruth Eliana Baigorri [1,2], Melisa Herrera [1,2], Ximena Volpini [1,2], Claudia Cristina Motrán [1,2]
1. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Argentina. 2. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Argentina.

Chagas disease is one of the most neglected tropical diseases, with cardiomyopathy being the main cause of death in *T. cruzi*-infected patients. As the parasite actively replicates in cardiomyocytes, the heart remains a key target organ in the pathogenesis. Cardiomyocytes occupy approximately 75% of normal myocardial tissue volume, but they account for only 30–40% of cell numbers. The remaining cells are predominantly fibroblasts (FB). We have reported that *in vivo* inhibition of Wnt signaling by treatment with IWP-L6 (an inhibitor of Wnt proteins secretion) during the acute phase of *T. cruzi* infection controls the parasite load, inhibits the development of fibrosis-prone Th2-type immune response, and prevents the development of chronic Chagas disease's cardiac abnormalities. To evaluate whether *T. cruzi* infection induce in FB the expression of genes involved in Wnt signaling, NIH3T3 cells were infected with *T. cruzi* and the expression of Ctnnb1, Wnt3a, Wnt5a, Wisp1 and Axin1 transcripts assessed at different times post infection (pi), using uninfected cells as control. The evaluation by means of q-PCR of the transcripts corresponding to Wnt3a and the target genes Wisp1 and Axin1 revealed that at 3 hpi there is an increase in the transcription of this genes. In addition, increased transcription of Wnt5a and Ctnnb1 transcripts was observed at 6 hpi. Then, we studied whether the inhibition of Wnt signaling by block Wnt proteins secretion using IWP-L6 or LGK9 regulates FB function by modulating the secretion of IL-1β, IL-6, and the production of NO and cytoplasmatic (c) or mitochondrial (m) ROS. Block of Wnt signaling increased the % of cells producing NO (DAF-FM+ cells) (p<0.05) and the production of IL-1β and IL-6 (p<0.05), decreased the production of mROS (mitoSOX+ cells) (p<0.05) and did not modify the cROS (H2DCFDA+ cells). Our data suggest that Wnt signaling is involved in the regulation of FB phenotype and function during *T. cruzi* infection.

240. (803) **EARLY TREG CELL DEPLETION PROMOTES INITIAL CONTROL OF *TRYPANOSOMA CRUZI* BUT LEADS TO A DELETERIOUS OUTCOME IN THE CHRONIC PHASE DURING THE EXPERIMENTAL INFECTION**

Cintia Liliana Araujo Furlan, Santiago Boccardo, Constanza Rodriguez, Carolina Lucía Montes, Adriana Gruppi, Eva Virginia Acosta Rodríguez
CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

We reported that after *Trypanosoma cruzi* (Tc) infection, Tregs undergo a marked reduction in frequency. To assess its biological relevance, we evaluated the effect of specific Tregs depletion on parasite control and the response of different immune cell populations at different time points post-infection (pi). For this, DEREG mice were infected with Tc and injected with diphtheria toxin (DT) to eliminate Tregs or PBS as control at days (d) 5 and 6pi. At d7pi, DT treatment (Tx) showed a marginal effect on APC activation but increased the numbers of splenic Tconv cells by d11pi, which displayed an activated/effector phenotype. At d20pi, DT Tx resulted in the expansion of anti-parasite specific CD8+T cells in blood, spleen and liver, which was accompanied by reduced parasitemia levels. To investigate the Tregs suppressive mechanism operating, we focused on CD39, a molecule highly upregulated by Tregs after infection. Thus, we transferred in vitro differentiated Tregs from WT or CD39KO mice to DT-treated mice. Mice that received WT Tregs but not KO Tregs-transferred animals reverted at least in part the effect

of Treg depletion, shown by numbers of total, Tconv and CD8+T cells at d22pi in the spleen. Finally, we examined whether the Treg response in the acute phase had an effect over the chronic phase. At d100pi, DT Tx led to increased activity of plasmatic markers of tissue damage as well as increased parasite burden in skeletal muscle (SM). Concomitantly, DT-treated mice showed decreased leucocyte infiltrate but higher frequency of CD8+T cells in SM. Altogether, our results indicate that during Tc infection Tregs suppress CD8+T cell immunity and impairs Tc control at the acute phase likely involving the modulation of APCs and Tconvs by a mechanism mediated by CD39 expression on Tregs. However, limiting the Treg response to achieve a better control of Tc replication in the acute phase does not necessarily improve parasite burden nor tissue damage in the chronic phase.

241. (876) **B-LYMPHOCYTE DYNAMICS IN SMALL INTESTINE DURING *TRYPANOSOMA CRUZI* INFECTION**

Gareca Julio C1, Gazzoni Yamila1, Almada Laura1, Brunotto Valentina1, Acosta-Rodríguez Eva V1, Montes Carolina L1, Gruppi Adriana1.

1.Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, X5000HUA, Córdoba, Argentina.

The acute phase of experimental *Trypanosoma cruzi* infection results in splenomegaly and expansion in lymph nodes. *T. cruzi* infected mice exhibit polyclonal B cell activation, hypergammaglobulinemia and unspecific antibodies (Abs) in sera. On the other hand, parasite-specific IgG isotypes are also present in the infected mice and have been described as an important mechanism to control parasitemia. The contribution of the different B cells subpopulations from the secondary lymphoid organs to this infection are diverse and not completely understood. In this work, we proposed to evaluate the gut response during *T. cruzi* infection since intestine is the tissue with the highest number of B cells. For that, 8-12 weeks old C57BL/6 mice were intraperitoneally injected with 5000 trypomastigotes of *T. cruzi* Tulahuén strain or with PBS (control mice). At different days post infection (dpi) small intestine, Peyer's patches (PP) and mesenteric lymph node were obtained. By macroscope evaluation we observed a decrease in the number and size of PP at the time of highest parasitemia (18 dpi). This decrease in PP was transient since at 82 dpi PP recovered the normal size. By immunofluorescence (IF), at 18 dpi, we observed a decrease in B and $CD4^+$ and $CD8^+$T cells in PP and infiltrating T cells in the muscle layer of ileum sections. Accordingly, by FACS, we observed that PP had a marked decrease in the number of T and B cells, being the greatest reduction in B cell population. IF of mesenteric lymph nodes of infected mice showed that CD169+ metallophilic macrophages disappeared from the subcapsular sinus and clustered around the follicles. On the other hand, follicles were disorganized and infiltrated by $CD4^+$ T cells. Based on the results we hypothesize that the small intestine could be contributing lymphocytes to secondary lymph nodes.

242. (894) **CYTOQUINE MODULATION OF AUTOPHAGY IN NEUTROPHILS FROM TUBERCULOSIS PATIENTS WITH DIFFERENT SEVERITY OF THE DISEASE**

María Paula Morelli[1], Candela Martin[1], Lucia Donnoli[1], Camila Martinena[1], Nancy Tateosian[1], Nicolas Oscar Amiano[1], Lorena Ciallella[2], Rosa María Musella[2], Adriana Rodríguez Mieres[2], Graciela M. Gragnolini de Casado[2], Domingo Palmero[2], Verónica Edith García[1].

[1]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), CONICET-UBA, Buenos Aires, Argentina. [2]Hospital de Enfermedades Infecciosas Dr. Francisco J. Muñiz, Buenos Aires, Argentina.

Introduction: Human immune responses against *Mycobacterium tuberculosis* (*Mtb*) involve different cell types and immune mediators. Underlying tuberculosis (TB) clinical manifestations, individual immunological profiles can be identified in patients according to their immune responses during infection. *Mtb* infected neutrophils predominate in patient´s lungs but their functions remain poorly understood. Here we investigated if the two interrelated cytokines IFNα and IL-1β regulate autophagy in patients with different immune responses to *Mtb.* Methods: TB patients were classified as low and high responders (LR and HR, respectively) according to their immune responses to sonicated *Mtb* (*Mtb*-Ag). Neutrophils were isolated from heparinized blood and suspended at 2×10^6cell/mL in supplemented media. Cells were then stimulated with *Mtb*-Ag (10 µg/mL) ± IL-1β (10 µg/mL) or INFα (5 µg/mL) and LC3 levels were determined by flow cytometry and confocal microscopy. P values of <0.05 were considered statistically significant. Results: A positive correlation between neutrophil counts and the percentages of PMNs $LC3B^+$ was detected in LR and HR TB patients ($p<0.05$). However, HR patients showed the strongest linear correlation ($r=0.8791$). Moreover, stimulation with *Mtb*-Ag significantly augmented the percentage of LC3A/B-II^+ neutrophils in HR patients as compared to LR ($p<0.0001$). Interestingly, stimulation with *Mtb*-Ag plus IL-1β or IFNα significantly decreased the levels of autophagy as compared to *Mtb*-Ag stimulation alone. In contrast, in LR patients, *Mtb*-Ag+IFNα augmented autophagy as compared to *Mtb*-Ag ($p<0.05$), whereas IL-1β treatment had no effect on this process. Conclusions: Our present findings suggest that IL-1β and/or IFNα might promote or impair neutrophil autophagy induced by *Mtb*, suggesting that manipulation of key cytokines according to the immune status of the TB patient might collaborate to improve the anti-mycobacterial human response in active TB.

243. (897) **IL-1 β, PGE2 AND IFNα REGULATE THE AUTOPHAGY PROCESS IN MONOCYTES FROM TUBERCULOSIS PATIENTS**

Candela Martin [1], María Paula Morelli [1], Nancy L.Tateosian [1], Nicolas Oscar Amiano [1], Lucia Donnoli [1], Camila Martinena[1], Lorena Ciallella [2], Rosa Musella [2], Graciela Casado [2], Domingo Palmero [2], Verónica Edith García [1] .

[1] Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), CONICET-UBA, Buenos Aires, Argentina. [2] Hospital de Enfermedades Infecciosas Dr. Francisco J.Muñiz, Buenos Aires, Argentina.

Introduction: Autophagy plays a crucial role in immunity against intracellular pathogens. We previously reported that both IFN-γ and IL-17A influenced autophagy during human tuberculosis (TB). Both IL-1 and IFNα/β represent two main cross regulated inflammatory cytokines during *Mycobacterium tuberculosis* (*Mtb*) infection. Moreover, IL-1 and IFNα/β are functionally connected via eicosanoids like PGE2. Here we studied the potential role of these immune mediators on the autophagy of *Mtb* in monocytes from TB patients. Methods: Tuberculosis (TB) patients were classified as low and high responders (LR and HR, respectively) according to their immune responses to sonicated *Mtb* (*Mtb*-Ag). Control individuals were healthy donors (HD) from the community. Monocytes were obtained from heparinized peripheral blood and cultured (2x10'6cs/ml) with an *Mtb* lysate (*Mtb*-Ag, 10µg/ml) ± IL-1β (10ng/ml), IFNα (10ng/ml) o PGE2 (2nM). Flow cytometry was used to evaluate autophagy levels. P-values<0,05 were considered significantly different. Results: Our data showed that *Mtb*-Ag stimulation increased autophagy levels in HD and HR TB patients but IL-1β stimulation significantly decreased the autophagy process in the 2 groups of individuals ($p<0.05$). In contrast, Ag stimulated monocytes from LR TB patients did not display detectable autophagy levels of *Mtb*, either in the presence or absence of IL-1β. Instead, treating monocytes with *Mtb*-Ag plus either PGE2 or IFNα markedly augmented autophagy in HD and HR TB, but had no effect on LR TB patients. Conclusions: Our present findings indicate the existence of a differential response between HR and LR TB patients upon stimulation of their monocytes with IL-1 β, PGE2 e IFNα, which might be critical to propose new effective host directed therapies (HDT) for TB.

244. (917) **EVALUATION OF GLUCOCORTICOID SENSITIVITY IN PERIPHERAL BLOOD MONONUCLEAR CELL FROM PATIENTS WITH TUBERCULOSIS**

Georgina Gallucci[1], Estefanía Massa1, Matilde Imhoff[1,2,] Magdalena Diab[1], Ariana Díaz[1,2], Bettina Bongiovanni[1], Natalia Santucci1,2, Marisa Derio[1], Diego Bértola[2,3], Ricardo Di Masso[2,] Oscar Bottasso[1,2], María Luisa Bay[1,2], Luciano D'Attilio[1,2.]

[1]Institute of Clinical and Experimental Immunology of Rosario

(IDICER), (National University of Rosario-CONICET), Rosario, Argentina.
[2]School of Medical Sciences, National University of Rosario, Rosario, Argentina.
[3]Department of Clinical Medicine, Centennial Provincial Hospital, Rosario, Argentina.

Tuberculosis (TB), is a major worldwide health problem, caused by Mycobacterium tuberculosis (Mtb). Previous studies in patients with pulmonary TB showed an immuno-endocrine imbalance characterized by a disease-severity associated increase in plasma levels of proinflammatory cytokines and glucocorticoids (GCs). Moreover, a decreased ratio of glucocorticoid receptor transcript isoform (GRα/GRβ), unfavourable to cortisol action, was detected. This may be in line with certain degree of GC endogenous resistance, mainly in cases with progressive disease. In chronic diseases as TB, cortisol plays a crucial role modulating immune response and diminishing tissue damage. To date, it has never been assessed a functional assay of GC sensitivity in TB patients. Hence, GCs sensitivity in peripheral blood mononuclear cell (PBMCs) from TB patients (n=8) and Healthy controls (HCo) (n=8) stimulated with Mtb irradiated (Mtbi) in the presence of different doses Cortisol (5.10^{-5}-10^{-8}M) was evaluated. The GC effect was measured by quantifying Mtbi-induced-Interleukin-6 (IL-6, ELISA) inhibition in 24h cultures supernatant. Doses-response curves from different groups were analysed as two-way repeated measures analysis of variance (ANOVA). As expected, cortisol produced a significant dose-dependent inhibition of IL-6 production [F= 11.4; p<0.0001]. Also, there was a significant effect of cell origin (from TB or HCo) against the cortisol effect over IL-6 production [F= 29.1; p< 0.0001]. Suggesting that PBMC from TB patients were less sensitive to *in vitro* GC treatment compared with controls. In concordance with previous results, this assay points out to certain degree of resistance to immunomodulatory cortisol capacity. Taken together these results indicate that endocrine environment of PBMC from TB patients provide a signature that may influence specific immune response.

INFECTOLOGY AND PARASITOLOGY I

Thursday, November 17, 9-10:30 hr
Chairs: Elsa Zotta - Claudia Silberstein

245. (13) **MOLLUSK HEMOCYANIN AS A SOURCE OF ANTIMICROBIAL PEPTIDES AND IMMUNOSTIMULANT ELICITOR**

Chiumiento Ignacio R., Cortez M. Fernanda, Brola Tabata R., Ituarte Santiago, Tricerri Alejandra M., Heras Horacio and Dreon Marcos S.
Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata. 60 y 120, La Plata (1900) Argentina

Hemocyanins (HCs) are large respiratory proteins widely distributed among invertebrates in nature. Particularly, mollusk HCs have attracted biomedical interest as non-specific immunostimulants, some of them being already employed in antitumoral therapies. In addition, HCs also stood out as precursors of antimicrobial peptides (AMPs), considered to be generated upon the action of bacterial proteases over Hc during infection. To contribute to the understanding of molluscan HCs as bioactive compounds, specially focusing on the searching for derived AMPs, we studied the HCs of invasive South American freshwater snail *Pomacea canaliculata* (PcH). First, *in silico* analysis were conducted for searching potential AMPs in the previously deduced PcH protein sequence. A list of predicted candidates was obtained from Anti-BP and CAMPr3 tools, and further analyse in APD-3 server. The four best-scored candidates were synthesized *de novo* and employed in diffusion agar assays against *Staphylococcus pseudintermedius* and *Escherichia coli*. Subsequently, Minimum Inhibitory Concentration tests were carried out, retrieving values between 6mM and 3mM. AMPs structures were predicted using PEP-FOLD 2.0, and peptide sequences mapped into a PcH Cryo-EM model previously resolved. To explore the immunostimulant capability of PcH, THP-1 monocytes were exposed to PcH and showed a Th1-like cytokine secretion pattern. Moreover, a marked cellular adhesion, indicative of monocyte´s activation, was observed by Fluorescence Microscopy when THP-1 cells were directly incubated with PcH. In conclusion, we characterized four PcH-derived AMPs, reinforcing the idea of molluscan HCs as AMP sources. Additionally, we demonstrated PcH may trigger a proinflammatory response and induces monocytic differentiation. Although more work is needed to elucidate the mechanisms implied in AMPs generation and immunostimulation, we propose PcH as a promising biomedical compound, easy to purify from a readily available source.

246. (100) **FENOFIBRATE MODULATES CARDIAC MACROPHAGE ACTIVATION PROFILE AND HEART INFLAMMATORY RESPONSE AND IMPROVES VENTRICULAR FUNCTION IN *T. CRUZI*-INFECTED MICE**

Azul Pieralisi[1], Carolina Poncini[2], Aldana Sequeyra[1], Ágata Cevey[1], Martín Donato[3], Gerardo Mirkin[2], Nora Goren[1] and Federico Penas[1]
1. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, UBA-CONICET. Facultad de Medicina-Universidad de Buenos Aires.
2. Instituto de Investigaciones en Microbiología y Parasitología Médica, UBA-CONICET. Facultad de Medicina-Universidad de Buenos Aires.
3. Instituto de Fisiopatología Cardiovascular, UBA. Facultad de Medicina-Universidad de Buenos Aires.

Monocyte-derived macrophages (Mϕ) are one of the main infiltrating leukocytes in response to heart infection in Chagas disease. Due to their functional and phenotypic plasticity, manipulating Mϕ subsets can be crucial in collaborating with vital cardiovascular functions, such as tissue repair and defense against the infection. Previous works of our group showed that Fenofibrate (Fen) improves cardiac function and inflammatory parameters in a chronic Chagas model. Here, to deepen into the study of Fen, C57BL/6 mice were infected with *Trypanosoma cruzi* (*Tc*) and treated with 100 mg/kg/day of Fen for 14 days. Fen treatment neither changes parasitemia nor body weight or survival of *Tc*-infected mice. Next, we found that Fen decreases iNOS, IL-6, TNF-α and CCR2 mRNA expression and increases mRNA expression of M2 markers (MR, YM1, PPARα, FIZZ, and IL-10) in heart (RT-qPCR, p<0.05). In this sense, *Tc*-infected and Fen-treated hearts showed decreased iNOS and increased Arg I expression (WB, p<0.05). Fen also decreases cardiac inflammatory foci (hematoxilin–eosin staining, p<0.05). We evaluated Mϕ ($CD11b^+LY6C^+F4/80^+$) from ficoll-purified cardiac myeloid cells and we observed that Fen decreases M1 profile ($CD206^-$) and increases M2 profile ($CD206^+$) in comparison with untreated infected mice (FACS, p<0.05). Besides, Fen down-regulates CCR2 and iNOS and increases Arg I expression in cardiac myeloid cells (WB, p<0.05). Finally, our results show that Fen is able to restore the abnormal ventricular function displayed by *Tc*-infected mice (echocardiography, p<0.05). In summary, Fen modifies the profile of cardiac macrophages and modulates the inflammatory response, while improving the left ventricle functionality in an acute model of infection. The in-depth knowledge of the mechanisms of action of Fen could provide a rational framework for the therapeutic approach of chagasic cardiomyopathy by combination of an anti-inflammatory therapy together with the anti-parasitic treatment.

247. (109) **SHIGA TOXIN TYPE 2 (STX2) INCREASES CELL VOLUME OF HUMAN GLOMERULAR ENDOTHELIAL CELLS (HGEC) THAT EXPRESS AQUAPORIN 1 AND AQUAPORIN 4 (AQP1 AND AQP4)**

Fernando Gomez[1], Julieta Reppetti[2], Daniel Claudio Girón Reyes[1], Flavia Sacerdoti[1], Cristina Ibarra[1], Nora Martínez[2], Alicia Damiano[2], Gisela Di Giusto[3], María Marta Amaral[1].
1. Laboratorio de Fisiopatogenia, IFIBIO-Houssay (UBA-CONICET) Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
2. Laboratorio de Biología de la Reproducción, IFIBIO-Hous-

say (UBA-CONICET) Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
3. Laboratorio de Biomembranas, IFIBIO-Houssay (UBA-CONICET) Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Hemolytic Uremic Syndrome associated to Shiga toxin–producing *Escherichia coli* (STEC-HUS) infection is the major cause of acute renal injury in pediatric age groups in Argentina. Previously, we demonstrated that Stx2 inhibited the net water transport (Jw) across HGEC monolayers, possibly as a consequence of direct alterations in the Jw mechanisms. In addition, we showed that Stx2 increased the cell area of HGEC and AQPs inhibitors were able to reverse this effect. Therefore, in this work we evaluate changes in cell volume of monolayers of HGEC treated with Stx2 as well as we analyze the expression of both AQP1 and AQP4 by these cells. Immunohistochemical and immunofluorescence studies were performed in HGEC that were grown on gelatinized glass coverslips (12 mm) and then treated or not with Stx2 for 40 minutes. Cell images were acquired and digitized using a confocal microscope Olympus FluoView (FV1000). A z-stack consisting of a series of 0.5 μm optical 2D slices was taken from top to bottom of the cells and then 3D reconstructed. The measure of total cell volume and the analysis of AQP1 or AQP4 expression and localization in HGEC cells images were processed using the Surface tool of Imaris 7.1.0 software. Our results show that HGEC treated with Stx2 exhibited an increase in the cell volume (Stx2: 9374 ± 992 μm^3 vs CTRL 4411.15 ± 470 μm^3, n=6, $p<0.05$). Moreover, both AQP1 and AQP4 were localized in the cell membrane of HGEC, but when they were treated with Stx2, a significantly lower expression of AQP1 was detected with respect to control cells (AQP1 Intensity: Stx2: 409 ± 51 vs CTRL: 801 ± 82 vs n= 6, $p<0.05$). These results demonstrate for the first time that Stx2 increases the HGEC cell volume and AQP1 and/or AQP4 may be involved in this event. Additionally, the drop in the expression of AQP1 may be related to a cell protection mechanism to prevent the cell volume increase triggered by Stx2.

248. (148) **SUNFLOWER HULLS POLYPHENOLIC EXTRACT EXHIBITS ANTIFUNGAL PROPERTIES AGAINST *C. albicans***

Marianela Del Rio, Nahuel Pasqual*, Guadalupe Rodríguez*, Melisa Radicioni, Guadalupe Martínez, Mariana Regente.
**equal contributions.*
Instituto de Investigaciones Biológicas-Universidad Nacional de Mar del Plata (IIB-UNMdP), CONICET

Candida albicans is an opportunistic fungus causing superficial and systemic infections. The search for safe and efficient antifungal compounds is a challenge for researchers. Sunflower hulls (SH) is an agroindustrial by-product rich in phenolic compounds with bioactive properties potentially applicable as natural therapeutic agents. The objective of this work was to explore the antifungal activity of polyphenol extract from SH on *C. albicans.* Phenolic extracts (EPC) were prepared by maceration of SH in 80% ethanol and total polyphenols were quantified with the Folin-Ciocalteu method. Fungal growth inhibition assays were performed in liquid medium in the presence and absence of EPC and OD reading at 630 nm was determined. EPC 0.25 mg/ml and 0.5 mg/ml showed 0-3.5% and 51-83% inhibition of yeast growth, respectively. Subsequently, cell viability was studied by colony count in solid medium, and 25.8-38% and 78-80% death was recorded in the yeasts treated with EPC 0.25 mg/ml and 0,5 mg/ml, respectively. In addition, the incorporation of Evans blue in the yeasts treated with EPC 0,5 mg/ml was visualized by microscopy analysis, indicating changes in the permeability of the plasma membrane. Through cell wall (congo red) and plasma membrane (SDS) disruptive agent assays, it was shown that EPC 0.25 mg/ml strongly inhibits fungal growth in the presence of SDS, demonstrating that the plasma membrane is its target of action. On the other hand, the effect of EPC on morphological switching, a virulence factors of *C. albicans*, was studied. The incubation of yeasts under conditions of pseudohyphae induction in the presence of EPC showed an inhibition of dimorphism between 52.8% and 100%, for each dose respectively, keeping the yeasts in their blastospore form. Thus, our results highlight the polyphenolic extract from sunflower hulls as a potential tool for the treatment of fungal diseases.

249. (154) **LOCAL CD4+/FOXP3+ T CELL RESPONSE IN TONSIL OF CHILDREN WITH EBV INFECTION**

Amarillo María Eugenia[1], Moyano Agustina[1], Ferressini Natalia[1], De Matteo Elena[2], Preciado María Victoria[1], Chabay Paola[1].
[1]IMIPP, Molecular Biology Laboratory, Buenos Aires, Argentina; [2]IMIPP, Pathology Division, Buenos Aires, Argentina.

Our aim was to study CD4+ T cells in tonsils from children with Epstein Barr virus (EBV) infection. We studied 37 patients undergoing tolsillectomy. EBV infection status was defined by serology and viral load was assessed by qPCR and expressed as copy/ug DNA. Immunohistochemistry (IHC) was performed for IL-10, LMP1 and EBNA2 proteins. CD4+ T cells were characterized by Foxp3 and CD4 double IHC. IL-10, Foxp3, CD4 and double staining counts were differentiated between germinal center (GC) and interfollicular (IF) regions of the tonsil. Results were expressed as positive cells/mm^2. 13 patients were primary infected (PI), 6 with reactivation (R), 14 healthy carriers (HC) and 4 no infected (NI). There was a significant difference in the CD4+, Foxp3+ and CD4+/Foxp3+ cell count between the GC and the IF regions of the tonsil in the entire cohort ($p<0.0001$). No differences were observed in CD4+, Foxp3+ and CD4+/Foxp3+ cell number among PI, R and HC, as well as between infected and NI patients ($p>0.05$). Significant lower CD4+ cell count was proved at IF region in PI, R and HC compared to NI children ($p<0.05$). Significant lower CD4+ cell count was observed in latency II-III grouped profiles compared to latency 0-I ($p=0.0444$). In contrast, CD4+/Foxp3+ cells showed a statistical positive correlation with viral load in latency II-III grouped profiles ($r=0,4671$;$p=0.0284$). Remarkably, CD4+ cells displayed a statistical positive correlation with IL-10+ cells in pediatric patients who expressed latency III antigens ($r=0.6180$;$p=0.0244$). Conclusion: the recruitment of CD4+, Foxp3+ and double positive T cells prevails outside the GC. The lower CD4+ cell count in infected patients may indicate decreased recruitment of this cells in the context of EBV infection. The lower recruitment of CD4+T cells might be involved in LMP1 and EBNA2 expression. Immune-regulatory environment may contribute to higher viral load and broader expression of latent proteins.

250. (158) **HANTAVIRUS PULMONARY SYNDROME IN TIMES OF SARSCOV2. ANDEAN REGION EXPERIENCE**

Ayelén A. Iglesias[1], María S. Macías Aguirre[1], Cecilia V. Rizzo[1], Isabel A. Dimenech[1], Quimey Jaskolowski[1], Constanza R. Anselmo[2], Camila P. Olivera[2], Jorge D. Diaz[3], Carlos E. Biondo[3], Lorena C. Lewis[2], Yésica R. Espasandin[1].
1. Hospital Área Programa San Carlos de Bariloche "Dr. Ramón Carrillo".
2. Hospital Zonal de Esquel.
3. Departamento de Programas Sanitarios, Área Programática Esquel.

Hantaviruses are emerging human pathogens. These zoonotic viruses are responsible of two different clinical presentations, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Hantaviruses predominantly infect microvascular endothelial cells causing capillary leakage. The hallmark of hantavirus diseases is the vascular permeability, which leads to pulmonary edema in HPS patients. Andes virus (ANDV) is endemic in southern Argentina and Chile where it was associated to high case fatality rates and several person-to-person transmission outbreaks. To evaluate the effect of SarsCov2 pandemic in HPS diagnostic we analyzed the number of SarsCov2 cases confirmed by real time RT-PCR, total HPS confirmed cases and fatality rates in the Andean region. HPS cases were confirmed by ELISA, 18 close contacts were followed up by real time RT-PCR every 5 days during 45 days. Furthermore, in order to evaluate patient's evolution we analyzed clinical picture and laboratory findings. A total of 48,884 patients were studied for SarCov2 by real time RT-PCR between March 2020 and July 2022, of which 32.4% tested positive and the fatality rate was 1.33%. In the same period 37 suspected cases were ana-

lyzed for HPS resulting in 11 confirmed diagnoses. All HPS patients presented a severe form of disease with expected symptoms. The fatality rate was 45.4%. ANDV was not detected among close contacts monitoring. Co-infection among ANDV and SarsCov2 was not detected in the Andean region. However, because of similitude of prodromal symptoms all patients were firstly treated as Covid suspected case. The fact that HPS patients were previously treated as SarsCov2 suspicious case delayed the appropriated diagnosis, this could have affected clinical evolution of patients because with early HPS diagnosis, Ribavirin treatment is approved in the region. HPS fatality was within the expected rate. There was no evidence of person-to-person transmission.

251. (208) **DETECTION OF NEURAL EXTRACELLULAR VESICLE PROTEINS IN BLOOD AS PREDICTIVE EARLY BIOMARKERS OF ENCEPHALOPATHY ASSOCIATED WITH HEMOLYTIC UREMIC SYNDROME (HUS)**

Ana B. Celi[1], Natalia Szpilbarg[2], Romina Glisoni[3], Analia López Díaz[4], Adriana Cangelosi[5], Patricia A. Geoghegan[5], Alipio Pinto[1], Jorge Goldstein[1]

(1) Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), Instituto de Fisiología y Biofísica ''Houssay" (Ifibio), Laboratorio de Neurofisiopatología, Facultad de Medicina, Argentina.

(2) Universidad de Buenos Aires, Consejo Nacional De Investigaciones Científicas y Técnicas (Conicet), Instituto de Fisiología y Biofísica ''Houssay" (Ifibio), Laboratorio de Biología de La Reproducción, Facultad de Medicina, Argentina.

(3) Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), Departamento de Tecnología Farmacéutica, Instituto de Nanobiotecnología (Nanobiotec), Facultad de Farmacia y Bioquímica, Argentina.

(4) Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), Instituto de Fisiología Y Biofísica ''Houssay" (Ifibio), Facultad de Medicina, Argentina.

(5) Centro Nacional de Control de Calidad de Biológicos (Cnccb), Anlis Dr. Carlos G. Malbrán", Ciudad Autónoma de Buenos Aires, Argentina.

Shiga toxin (Stx) producing *E. coli* (STEC) is the principal etiologic agent causing HUS, characterized by hemolytic anemia, thrombocytopenia and renal failure. Neurological alterations may occur, and consequently a poor prognosis and increased mortality rate are recorded. STEC is a gram-negative bacterium, and in addition to Stx also releases LPS, involved in proinflammatory-related events which contributes significantly to the development of the disease. The detection of serum biomarkers associated with neural injury during the first days of bloody diarrhea onset, and prior to HUS signs and symptoms, could be determinant to start pharmacological strategies to prevent neural damage. The aim of this work was to determine whether neuronal tau and astrocyte GFAP proteins can be considered early serological biomarkers of encephalopathy in the context of HUS. For this purpose, NIH-Swiss male mice were intravenously injected with vehicle, LPS (800ng), Stx2 (3.5ng, $1LD_{100}$) or a combination of Stx2+LPS (same previous amounts). After 1- and 2- days, blood samples were collected to detect tau protein by Elisa (Invitrogen, Viena, Austria) and extracellular vesicles (EVs) were obtained from plasma by differential centrifugation. These EVs were characterized in identity, quantity and size using western blot, Bradford and Dynamic Light Scattering (DLS) techniques. One way ANOVA and Tukey post-hoc tests were employed for statistical analysis. A significant two-fold increase of tau protein was determined after 2 days in the Stx2+LPS group ($p<0.05$) with respect to the vehicle. The presence of EVs TSG101 marker containing GFAP in Stx2+LPS treated mice was confirmed by western blot analysis from a pellet obtained from differential centrifugation. We conclude that in addition to the immunodetection of free circulating tau by ELISA, the isolation of plasma EVs may be a valuable tool for the detection of different neural biomarkers, like GFAP, in the context of HUS.

252. (224) **IDENTIFICATION OF *Chlamydia trachomatis* LGV L2 MALE UROGENITAL INFECTION. A CASE REPORT OF CHRONIC EPIDIDYMITIS**

Daniela Andrea Paira[1], Carolina Olivera[1], Andrea Daniela Tissera[2], José Javier Olmedo[3], Rosa Isabel Molina[2], Héctor Alex Saka[1], Ruben Darío Motrich[1].

[1] CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. [2] Laboratorio de Andrología y Reproducción (LAR), Córdoba, Argentina. [3] Fundación Urológica Córdoba para la Docencia e Investigación Médica (FUCDIM), Córdoba, Argentina.

Chlamydia trachomatis (CT) is an obligate Gram-negative intracellular bacterium being the most prevalent sexually transmitted bacterial infection worldwide. Although specific antimicrobial therapy is highly effective, a high rate of CT male urogenital infections are asymptomatic, leading patients not to seek treatment and allowing continuous transmission of the infection. *Lymphogranuloma venereum* (LGV) is caused by CT genotypes L1-L3. No confirmed cases of CT-LGV had been reported in Argentina until 2017, when 33 cases of CT-LGV infection were detected in a group of men who had sex with men, 90% HIV positive, and presenting with broad clinical manifestations. In the present work, we report the case of a 36 y.o. heterosexual men, HIV negative, who presented in August 2018 referring symptoms of chronic epididymitis (more than 6 months of evolution), with right testicular cord swelling and extremely painful upon palpation. Semen analysis revealed oligoasthenoteratozoospermia without leukocytospermia. The screening of several common uropathogens by culture and PCR showed the sole infection with CT. Interestingly, *ompA* gene sequencing revealed a CT-LGV L2 genotype. Moreover, molecular epidemiological analysis by multi-locus sequence typing (MLST) Uppsala strategy confirmed CT-LGV L2 genotype as ST 144. Strikingly, the patient did not present external lymphadenopathies, which are commonly observed in CT-LGV2 infections. After treatment with doxycycline 100 mg/12h for 7 days, the signs and symptoms gradually remitted until resolution. A new semen analysis performed 2 months later revealed complete resolution of the infection and a significant improvement in sperm quality. From the epidemiological point of view, this would be the first report of CT LGV-L2 detection in Argentina, in a male, heterosexual, HIV-negative patient, with clinical symptoms of chronic epididymitis, constituting valuable epidemiological information with a significant impact on public health.

253. (303) **PHARMACOKINETIC PARAMETERS AND THERAPEUTIC EFFICACY OF NOVEL MEBENDAZOLE FORMULATIONS IN MICE OF THE CBi-IGE MODEL OF TRICHINELLOSIS, RESISTANT TO THE PARASITE**

Ana V. Codina[1,2], Rocío Pistelli[1], Ariana Rosales[3], Paula Indelman[4], María C. Lamas[3,5], Lucila I. Hinrichsen[1,2]

[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, UNR; [2]CIC-UNR; [3]Depto. de Farmacia, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR; [4]Área Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR; [5]IQUIR-CONICET

Mebendazole (MBZ) is a benzimidazole widely used in oral chemotherapy against intestinal parasites due to its broad-spectrum activity and low cost. Its primary disadvantages are poor water solubility and low bioavailability. Two novel formulations were designed to improve MBZ solubility, a nanoparticulate system (*Np*) and a β-cyclodextrin citrate inclusion complex (*Comp*). This work aimed to analyze whether these systems improve MBZ pharmacokinetics and *in vivo* efficacy against *T. spiralis* encysted larvae in CBi-IGE mice resistant to the parasite. CBi/L adult mice of both sexes were given a single oral dose of MBZ, *Np*, or *Comp* (15 mg MBZ/kg bw). Next, blood samples were collected at different times to quantify MBZ plasma concentration by HPLC analysis to estimate bioavailability. Mice given *Np* showed an increased Cmax (peak plasma concentration), not observed for *Comp*, compared with those receiving pure MBZ (♂ $P=0.04$; ♀ $P=0.01$). However, both formulations showed a higher AUC (area under the concentration-time curve) than MBZ (percent increase, *Np*: ♂50%, ♀57%; *Comp*: ♂67%, ♀54%; $P>0.05$). The *in vivo* therapeutic efficacy was studied in mice orally infected with

2 L1 *T. spiralis* larvae/g bw (n=6/treatment and sex). Control (*C*) and treated mice receiving a daily oral dose of MBZ, *Np*, or *Comp* on days 27, 28, and 29 post-infection were euthanized 7 days after the last dose. Muscle worm burden (MWB, number of L1 larvae/g muscle weight) and number of dead larvae (NDL) were determined. The formulations significantly decreased MWB compared with ***C*** or MBZ-treated mice (percent decrease, *Np*: ♂92%, ♀91%; *Comp*: ♂93%, ♀95%; P<0.05). Though not significantly, mice treated with *Np* or *Comp* increased NDL percentage. The formulations' higher Cmax and AUC were reflected in an improvement in therapeutic efficacy in the chronic phase of the infection. These results suggest that both systems would allow the use of lower doses of MBZ, thus reducing the possible toxic effects of the treatment.

254. (391) **IMMUNOSUPPRESSIVE THERAPY AND RISK OF CHAGAS DISEASE REACTIVATION**

Matías Ildebrando Marchetto 1, Maria Belén Piñero 1, Silvia Repetto 1,2, Catalina Dirney Alba Soto 1.
1 Facultad de Medicina. IMPAM (UBA-CONICET); 2 División de Infectología, Hospital de Clínicas José de San Martín

The risk of reactivation of chronic *T. cruzi* infection following treatment with immunosuppressive drugs is not fully understood yet, thus we developed an experimental model to evaluate this risk, and to identify host factors that determine reactivation. We studied the course of *T. cruzi* infection in mice at chronic stage following treatment with conventional immunosuppressive drugs used for autoimmune diseases or transplanted individuals. Balb/c female mice at 120 dpi with *T. cruzi (*K98 strain) were treated with increasing oral doses of Leflunomide (LE), Tacrolimus (TA), Mycophenolate mofetil (MMF) and Azathioprine (AZA). Control infected animals were administered vehicle alone (Carboxymethylcellulose; CMC 1%) and control uninfected animals were treated with each drug. Reactivation was detected in 60% (3/5) of mice treated with MMF (25dpi) and AZA (39 dpi), being cumulative parasitemia significantly higher with MMF (<0.05) with respect to AZA treated and controls (CMC). MMF and AZA (20.66% and 15.44%) caused a significant weight reduction. Treatment with TA and LE reduced peripheral blood leukocyte counts mainly at the expense of CD4+ LT compared to controls, but not AZA and MMF treatment. MMF decreased the relative number and size of germinal centers regardless of infectious status (p<0.01) yet infected MMF treated mice presented significantly greater splenomegaly (p<0.01). AZA treatment increased fibrosis in heart tissues of infected animals. In line with observations in transplant recipient patients with chronic Chagas disease, the experimental model shows that the first-choice drug to prevent transplant rejection, MMF, carries a higher risk of reactivation compared to AZA. Our results also show that LE and TA in individual treatments, even at high doses, do not trigger an increase in parasitemia. This model allows to estimate the individual risk of immunosuppressive drugs that are usually used in multi-treatment schemes.

255. (398) **F1b AND F4 HEPATITIS B VIRUS SUBGENOTYPES REPLICATION MODULATE RNA GRANULES AND LIPID DROPLETS IN HUMAN HEPATOCYTES**

Melisa Micheletti[1], Mercedes Elizalde[2], Diego Flichman[2], Luciana Barbini[1]
[1]Departamento de Química y Bioquímica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET, Buenos Aires, Argentina; [2]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires-CONICET, Buenos Aires, Argentina

Hepatitis B virus (HBV) is classified in different subgenotypes (sgts) with different biological activities, being F1b and F4 of main circulation in Argentina. We previously reported the modulation of RNA granules (stress granules, SGs and processing bodies, PBs) and lipid droplets (LDs) by HBV replication, and colocalization of surface antigen (HBsAg) in a system of stable transfected hepatic cells with gt D. The aim of this study was to evaluate the impact of the most prevalent F1b and F4 replication in human hepatocytes on these structures. Materials and methods: Huh-7 cells were transfected with full-length F1b and F4 genomes, achieving an efficient replication. The simultaneous detection of HBsAg and SGs, PBs or LDs was performed by indirect immunofluorescence using antibodies to HBsAg and SGs (TIA-1, TIA-1/R, G3BP1), PBs (DCP1a) or LDs (ADRP). The images were analyzed with ImageJ. Results: HBV replication of both sgts increased the number of SGs (+) cells, with differences between sgts according to the detected protein (TIA-1 100% F1b, 80% F4; TIA-1/R 49% F1b, 52% F4; G3BP1 53% F1b, 27% F4). Also, the number of SGs/cell increased by replication, showing differences between sgts (TIA-1 7% F1b, 20% F4; TIA-1/R 45% F1b, 18% F4; G3BP1 24% F1b, 50% F4). The number of PBs was not modified in transfected cells for both sgts. Indeed, an increased number of LDs was observed in cells with replication, showing F4 a greater increase (377% F1b, 683% F4), with no differences in LDs/cell. When analysing HBsAg presence in these structures, a partial colocalization was observed in SGs, PBs or LDs, with similar results between sgts. The presence of HBsAg in the granules may be necessary at any step of the viral cycle. In conclusion, both F1b and F4 replication in human hepatocytes modulates RNA granules differentially and affects LDs quantities. These changes may contribute to disturb hepatocyte functions and participate in pathogenesis mechanisms of chronic HBV infections.

256. (455) ***TRYPANOSOMA CRUZI* INFECTION ENHANCED THE THYMIC OUTPUT AND INDUCE ALTERATIONS IN THE CENTRAL AND PERIPHERAL V-β TCR REPERTOIRE**

Brenda Dinatale[1], Florencia Belén González[1], Itauá Leston Araujo[2], Camila Bulfoni Balbi[1], María Florencia Pacini[1], Oscar Bottasso[1], Wilson Savino[2], Eduardo Roggero[1], Ana Rosa Pérez[1,3].
[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina, [2]Laboratorio de Pesquisas sobre o Timo, FIOCRUZ, Rio de Janeiro, Brasil, [3]Centro de Investigación y Producción de Reactivos Biológicos (CIPREB), Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina. E-mail: dinatale@idicer-conicet.gob.ar

The thymus is a central organ in the shaping of the peripheral T-cell repertoire. Earlier work in a murine model of Chagas disease revealed progressive thymus atrophy, characterized by $CD4^+CD8^+$ (DP) loss by apoptosis. Since alterations in the normal development of T-cells or in their repertoire could influence the outcome of this infectious disease, here, we evaluated the thymic output and the V-β repertoire after *T. cruzi* (Tc) infection. C57BL/6 mice (n=5/group) were infected with 1000 Tc and evaluated after 17 days post-infection (dpi). Non-infected (NI) mice act as controls. The thymic output was estimated by the quantification of recent thymic emigrants (RTEs) by intrathymic injection of fluorescein (FITC), and 24 h later, thymus and subcutaneous lymph nodes (SLN) were removed and monitored for $FITC^+$ cells occurrence. T-cell receptor excision circles (TRECs) were analyzed in blood by qPCR as RTEs markers. Repertoire screening was carried out by V-β family (5, 6, 8.1-8.2 and 14) detection by flow cytometry. In Tc mice, thymic depletion was coupled with adenomegaly. An increase in the counts of $FITC^+CD4^+$, $FITC^+CD8^+$, as well as immature and potentially autoreactive $FITC^+DP$ T-cells was observed in the SLN after 17 dpi (p<0.05 vs NI). TRECs trend to increase after 17 dpi (median/range) NI: 114/(23-200), Tc: 184/(23-400). Given the enhanced influx of RTEs and the presence of DP cells in SLN, we examine the shaping of mature and immature T-cell repertoire. An increase in the frequency of all V-β families was detected after infection in the thymus (p<0.05 in all cases). Parallel, the frequency of V-β studied in the SLN of Tc animals not differed from NI, except for a decrease in V-β5. Nevertheless, V-β5 and V-β6 were over-represented in DP cells from SLN in infected animals (p<0.05 vs. NI). The abnormal thymic output of DP T-cells and the peripheral over-representation of some Vβ families, suggests that the intrathymic selection may be altered during the infection.

257. (602) **CHRONIC CHAGAS CARDIOMYOPATHY IS LINKED TO A DISRUPTED ACTIVATION OF THE**

HYPOTHALAMOUS-PITUITARY-ADRENAL AXIS AND A DECREASED 11B-HSD1 EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS

Antonella Pacini[1], Silvina Villar[1], Rodolfo Leiva[2], Ana Rosa Pérez[1], Florencia Belén González[1].
[1]Instituto de Inmunología Clínica y Experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IDICER-CONICET), [2]Servicio de Cardiología, Sección Chagas, Hospital Provincial del Centenario, Rosario, Argentina. E-mail: apacini@idicer-conicet.gob.ar

Chronic Chagas cardiomyopathy (CCC) is a disease caused by *Trypanosoma cruzi* (Tc). We previously observed a disrupted activation of the hypothalamous-pituitary-adrenal (HPA) axis. The aim of this work was to evaluate possible difference in the immune-endocrine status between chronic chagasic patients with CCC (n=22), non-chagasic with cardiomyopathy (NCC, n=5) and healthy and seronegative individuals (Co, n=15). Systemic levels of Cortisol (GC) and DHEA-S concentration were determined in serum. The expression of genes involved in GC response as GC receptors (GRα functional receptor and GRβ inhibitor receptor) and 11β-HSD1 (which catalyzes the conversion of GC from inactive to active form) was assessed by qPCR. We also evaluated in peripheral blood mononuclear cells (PBMCs) the expression of genes regulated by GC and involved in the inflammatory response: IL-6, IFN-γ, IL-1β, TNF-α and tristetraprolin (TTP) by qPCR. As we previously shown, CCC individuals showed a disrupted activation of the HPA axis, characterized by decreased DHEA-S and GC levels together with an increased GC/DHEA-S ratio ($p<0,05$ vs Co). These alterations were not observed in NCC patients. In PBMCs from CCC patients, GRα expression did not differ from Co, while it was diminished in NCC individuals ($p<0,05$ vs Co and CCC), GRβ was not detectable in any of the groups and 11β-HSD1 was augmented in CCC patients respect to the Co group ($p<0,05$). All inflammatory cytokines trend to be increase in PBMCs that came from the CCC group, while TTP expression was diminished in CCC and NCC groups respect to the Co ($p<0,05$). These results suggest that an adverse endocrine milieu in CCC patients may predispose to an increased pro-inflammatory state compared with other types of cardiomyopathies, and this could be sustained by parasite persistence.

258. (693) **ENTEROAGGREGATIVE *Escherichia coli* (EAEC) PRODUCES DOUBLE LAYERED VESICLES IN STATIONARY PHASE, CONTAINING CHROMOSOMAL DNA AND THE DNA-PROTECTING PROTEIN Dps**

Marta Almirón1, María Josefina Varas2, María Agustina Rocca2, Gustavo Javier Cabrera2, Norberto Sanjuan2.
1Cátedra de Microbiología y Parasitología, Facultad de Odontología, Universidad de Buenos Aires, Argentina, 2Laboratorio de Microscopía Electrónica y Patología Experimental, Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

The production and extracellular shedding of membrane vesicles has been described in eukaryotes, Gram-negative and Gram-positive bacteria. While investigating adhesins in the EAEC 17-2 strain by transmission electron microscopy (TEM), we made the serendipitous observation that it produced outer membrane vesicles different from those described previously. The purpose of this work was to characterize them by ultrastructural, biochemical and molecular biology methods. Bacteria were grown in 1,000 ml of LB broth for 8 days and harvested at different time-points post-inoculation. Cells were spun down at 10,000 X g for 10 min and supernatants were passed through a 0.45 μm membrane filter under sterile conditions. After ultracentrifugation at 150,000 X g for 1 h, pellets were resuspended in 50 mM HEPES with 20% glycerol and kept at -20°C. Separation of pellets´ contents in a discontinuous sucrose gradient allowed the obtention of different bands. In one of them, rounded, double layered, 100-120 nm- width vesicles were observed by transmission electron microscopy using thin sections and negative-staining techniques only in late stationary phase. Classic molecular biology procedures, the shadowing Kleinschmidt method and Random Amplification of Polymorphic DNA (RAPD) demonstrated that vesicles contained chromosomal DNA. We hypothesized that vesicle DNA had, somehow, to be protected after these structures were released. Thus, we explored and demonstrated by Western blotting the presence of the DNA-protecting protein Dps associated to the DNA. Furthermore, other bacterial proteins such as flagellin, FliC and several adhesins were detected by two-dimensional polyacrylamide gel electrophoresis inside the purified vesicles. We conclude that shedding of extracellular, double layered vesicles containing chromosomal DNA and a DNA-protecting protein only in late stationary phase could be a mechanism of resistance and genomic DNA preservation in non-sporulated bacteria.

259. (720) **PHOTODYNAMIC INACTIVATION FROM ALA, ON BACTERIAL INFECTIONS AND SKIN WOUNDS IN AN *IN VIVO* MOUSE MODEL**

Roberto Tomás[1], Gabriela Di Venosa[1], Fernanda Buzzola[2], Adriana Casas[1], Leandro Mamone[1].
[1]CIPYP, Hospital de Clínicas José de San Martín, UBA, CONICET, Buenos Aires, Argentina, [2]IMPAM, UBA, CONICET, Buenos Aires, Argentina.

Photodynamic Inactivation (PDI) is a treatment that uses a photosensitizing compound (PS) that after absorbing visible light in the presence of molecular oxygen generates reactive oxygen species, which cause damage and inactivation of microorganisms. 5-aminolevulinic acid (ALA) is a precursor in the biosynthesisof porphyrins, some of which can act as PSs in both eukaryotic cells and bacteria. The objective of this work was to evaluate the effect of ALA-PDI (visible light irradiation after topical ALA treatment) on the progression of wounds caused by *Staphylococcus aureus* infection, in an *in vivo* model in mice. CF1 mice were injected subcutaneously with a suspension of *S. aureus* RN6390. After 48 h, 20 mg/ml ALA solution was applied to the skin, in the area of infection, 4 h later, fluorescence emitted by the synthesized porphyrins was detected. The PDI was performed employing a 635 nm laser device. The effect of ALA-PDI was determined by measuring the area of wound (caused by infection) during four weeks after treatment. Porphyrins were identified by fluorescence spectroscopy. The effects of ALA-PDI on the bacteria present in the infection were determined by measuring CFUs from skin homogenates. Infected skins showed a higher production of porphyrins than non-infected. The main porphyrin synthesized was protoporphyrin IX. Wounds treated with ALA-PDI began to reducearea immediately after irradiation(in contrast to untreated or only irradiated controls). Time required to fully closure wounds in the ALA-PDI group was significantly less ($p<0.01$) than in untreated controls (14 vs 27 days). In addition, it was observed that irradiated controls without ALA, also reduced the area of the wounds earlier than untreated controls. By employing an alternative model of uninfected skin wound, this effect of laser irradiation on wound healing was corroborated. Results suggest that PDI employing ALA as a precursor of PSs porphyrins is a promising option to treat superficial infections.

260. (754) ***Lactobacillus plantarum* CIDCA 83114 REDUCES THE PATHOGENIC POTENTIAL OF SHIGA TOXIN (Stx)-PRODUCING *Escherichia coli* (STEC) O157:H7**

Romina J. Fernández Brando[1], Daniela Carballo[1], Alan M Bernal[1], Fernando N Sosa[1], María Victoria Ramos[1], Analía G Abraham[2], Graciela Garrote[2], Marina S Palermo[1].
1- Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina, Bs As.
2- Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) -CONICET-UNLP-CIC.PBA

Shiga toxin (Stx)-producing Escherichia coli (STEC) O157:H7 is a foodborne pathogen, which can lead to the life-threatening Hemolytic Uremic Syndrome (HUS). There is no treatment available in order to reduce HUS outcome up to date. Probiotics are live microorganisms that promote beneficial health effects on the host. *Lactobacillus plantarum* CIDCA 83114 (CIDCA 83114) was previously isolated from kefir and reduced the cytotoxic activity of Stx on Vero cells. The aim of this work was to evaluate the effect of CIDCA 83114 on the pathogenic potential of a clinically isolated STEC strain. We evalu-

ated the inhibition of STEC growth by CIDCA 83114 by co-culturing both bacterial strains in ratios 1:1, 1:10 and 1:100 (STEC: CIDCA 83114) and counting colony forming units by plating in specific media. Besides, we also tested the competition and exclusion exerted by CIDCA 83114 on STEC adhesion to Caco-2 cells. Results were statistically analyzed by Kruskal-Wallis test. We observed that CIDCA 83114 completely inhibited STEC growth by 24 h co-culture in all the ratios tested ($p<0.005$). Then we carried out epithelial cell adhesion assays in which CIDCA 83114 was added simultaneously with or 1 h before STEC for competition and exclusion assays respectively. CIDCA 83114 did not reduce STEC adhesion to Caco-2 cells by competition. However, it was able to significantly reduce STEC adhesion by exclusion after 3 h culture ($p<0.05$). In conclusion, CIDCA 83114 was able to completely inhibit STEC growth *in vitro* after 24 h co-culture and reduce STEC adhesion to Caco-2 cells by an exclusion mechanism in all ratios tested. Given that CIDCA 83114 was also proved to reduce Stx activity *in vitro*, these results encourage us to test the ability of this strain to reduce HUS outcome after STEC infection *in vivo*.

INFECTOLOGY AND PARASITOLOGY II

Saturday, November 19, 9-10:30 hr

Chairs: María Marta Amaral - Julia Loos

261. (15) **CLINICAL CHARACTERISTICS AND PROGNOSTIC FACTORS OF PATIENTS WITH FEBRILE NEUTROPENIA**

Roberto Parodi[1-2], Mariana Lagrutta[1-2], Agustina Genesio[1], Waldemar Grosselli[1], Melina Ramallo[1], Mercedes Martin[1], Julieta Moreno[1], Lisandro Sommer[1], Alcides Greca[1-2], Oscar Bottasso[3].

[1] 1° Cátedra de Clínica Médica y Terapéutica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario. Argentina

[2] Servicio de Clínica Médica, Hospital Provincial del Centenario, Rosario (Santa Fe). Argentina

[3]Instituto de Inmunología Clínica y Experimental de Rosario (UNR-CONICET)

Our study investigated the clinical and epidemiological features of febrile neutropenia episodes at a high-complexity hospital in Rosario city, in addition to ascertaining biochemical variables (erythrocyte sedimentation rate -ESR-), C reactive protein -CRP-, red blood cell distribution width -RDW-). Some relationships between such variables with disease outcomes may eventually contribute to better patient management. We conducted a descriptive, analytical, observational, prospective study of consecutive inpatients with febrile neutropenia (March 2016-March 2020) who were followed up during such complications. Sixty-one episodes of febrile neutropenia were recorded; 28 women/33 men, with a median age of 40 years; 36 of them following antineoplastic chemotherapy. There were 9 deaths. Blood cultures were positive in 19.6% of episodes (12 positive cultures). Statistical analysis (univariate and adjusted methods) showed an association between mortality, lower ESR values ($p<0.001$), and leukocyte counts on admission ($p<0.045$). It was also found that the greater the decrease in RDW values during patient evolution the lower the mortality ($p<0.035$). For instance, an RDW decrease ≥0.07 coexisted with significantly lowered mortality, whereas the risk of mortality in cases with an augmented RDW was high [OR 10.2 (95% CI: 1.07-97.41), $p<0.035$]. Increased CRP was associated with higher mortality risks [Odds ratio=12 (95% confidence interval: 1.14 -126.12), $p<0.03$]. Biochemical variables and their changes throughout the disease course seem to behave as prognostic indicators for the fatal course in this patient series, particularly lower ESR and leukocyte count values on admission along with CRP and RDW values during evolution.

262. (32) **THE NOVELTY OF IDENTIFYING SARS-CoV2 VARIANTS IN POOLED SAMPLES USING DROPLET DIGITAL PCR**

Sofía Belén Heckel[1,2,3], Antonella Pacini[2,4], Guadalupe Ibarra[1,2,3], Franco Paredes[1,3], Maria Victoria Petreli[1,3], Marilina Perez[1], Natalia Adriani[1], Juliana Sesma[1,2,3,4].

[1]Biología Molecular, Hospital Provincial de Rosario, Argentina, [2]Instituto de Inmunologia Clínica y Experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IDICER-CONICET), [3]Facultad de Ciencias Bioquímicas y Farmaceuticas, Universidad Nacional de Rosario (FCM-UNR), [4]Facultad de Ciencias Medicas, Universidad Nacional de Rosario (FCM-UNR).

Considering that new SARS-CoV-2 variants continue emerging and monitoring their circulation through genomic surveillance remains important, our aim is to demonstrate the feasibility of differentiating SARS-CoV-2 variants in grouped positive samples.

One of the most important applications of droplet digital PCR (ddPCR) is rare mutation detection. Here the challenge is the discrimination between two highly similar sequences: the variant present at a very low frequency in a vast pool of wild-type (WT). This is possible due to the partition of the sample into thousands of droplets that work in parallel, increasing sensitivity and resistance to inhibitors.

Previously, we validated the use of ddPCR for testing SARS-CoV-2 in pooled samples by combining up to 34 samples per pool. Now, we are extending ddPCR use to identify SARS-CoV-2 variants in a pool of SARS-CoV-2 positive samples. A negative test result will indicate that all individuals in the pool are WT for that specific mutation while a positive result will indicate that at least one individual within the pool carries that mutation. Test were performed from July 2021 to March 2022 with de-identified samples. SARS-CoV-2 positive samples were identify by RT-PCR and later variants were genotyped by RT-PCR. Pools of different sizes (1 variant in a group of 5, 10 or 15 WT samples) were designed and ddPCR was performed. Data was analyzed with Quanta Soft analysis software (Bio-Rad). In order to detect two SARS-CoV-2 variants of concern: Delta and Omicron, the TaqMan SARS-CoV-2 Mutation Panel from Thermo Fisher was used for both RT- and ddPCR. In the present work, we demonstrated that for pools of up to 10 samples, ddPCR present 100% sensitivity (n=24) and >98% specificity (n=50) when analyzing 3 different probes for delta and/or omicron SARS-CoV-2 variants. Therefore, we reported the novel diagnostic technology of SARS-CoV-2 pool genotyping by ddPCR to achieve quick results (< 24 hs), high testing throughput and low costs.

263. (437) **SEARCHING FOR A SCAFFOLD FOR THE IN-SILICO DESIGN OF AN ANTIBODY FOR AN EPITOPE FROM ECHINOCOCCUS GRANULOSUS HISTONE H4**

Andrea Maglioco[1,2], Facundo Ariel Agüero[1,2], Margot Paulino[3], Alicia G Fuchs[1,4]

[1]Universidad Abierta Interamericana (UAI), Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS), Buenos Aires, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [3]Departamento de Experimentación y Teoría de la Estructura de la Materia y sus Aplicaciones, Facultad de Química, Bioinformática DETEMA- Udelar, Universidad de la República, Montevideo, Uruguay. [4]Instituto Nacional de Parasitología "Dr Mario Fatala- Chaben", ANLIS-Malbrán, Buenos Aires, Argentina.

Cystic echinococcosis (CE) is a worldwide parasitic disease caused by *E. granulosus*. The first line of diagnosis is based on cyst imaging and the epidemiological status. Serology is based on the detection of antibodies (Ab); however, it has false positives and negatives due to the antigen (Ag) used and the cyst localization and stage. Furthermore, Ab can persist in circulation even in the absence of the infection. The determination of circulating Ag would allow a more accurate diagnosis of the presence of the infection. We have recently identified that histone H4 (H4) from *E. granulosus* could be a relevant Ag for the detection of CE. We obtained a validated model for H4 and predicted its linear (Lep) and conformational epitopes (Maglioco et al, 2022). The aim of this work was the selection of single-chain fragment variable (scFv) for the in-silico design of an antibody for H4 epitope. The scFv from humans, with heavy (VH) and light chains (VL), available in PDB with a resolution of 1-2 Å (7VYT, 2YC1, 4UT7, 6J9O) were used. The CDRs were determined with Parapred. The docking of scFvCDRs with H4 (Lep 134-149,

H4Lep) was obtained by ZDOCK. The part of H4 that did not correspond to H4Lep was blocked and the binding of the CDRs to H4Lep was carried out. The highest scoring pose of each scFvCDRs:H-4Lep was analyzed in MOE 2019 software. Three hydrogen bonds (VL-Ser31:H4-Phe145, VL-Gly50:H4-Asp148 and VH-Ser56:H4-Arg136) were obtained for 7VYT; 2 hydrogen bonds (VL-Ser163:H4-Arg140 and VH-Val101:H4-Phe145) and an ionic interaction (VL-Asp182:H4-Arg140) were obtained for 2YC1; 2 hydrogen bonds (VH-Tyr100A:H4-Arg144 and VH-Tyr100F:H4-Ser138) and an ionic interaction (VL-Lys53:H4-Asp148) were obtained for 4UT7 and no interaction was found for 6J9O. We concluded that 7VYT, 2YC1, and 4UT7 could be good scaffolds to continue the in-silico design of an Ab for H4Lep. Further experiments will be required to analyze the relevance of the described interactions for H4 recognition.

264. (438) **FUNCTIONAL AND EVOLUTIONARY CHARACTERIZATION OF IRE-XBP1 PATHWAY IN *ECHINOCOCCUS* SP**

Malena Díaz[1,*]; Camila Ledo[1,*], María Celeste Nicolao[1,2], María Silvina Fornasari[2,3], Andrea C. Cumino[1,2]
*[1]IIPROSAM, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. [2]Conicet, [3] Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. *equal contribution.*
E-mail: camila.ledo85@gmail.com; celestenicolao@hotmail.com.

The alterations in protein folding and assembly can induce the endoplasmic reticulum (ER) UPR. IRE1 is the most evolutionarily conserved ER stress transducer, which upon activation, undergoes dimerization-dependent auto-phosphorylation, and allosterically induces its cytosolic endoribonuclease activity. Successively, IRE leads to unconventional splicing of the XBP1 mRNA. The translation of this spliced transcription factor results in the transcriptional induction of genes expressing chaperones, ERAD components, and autophagy regulators. Previously, we have identified orthologues of IRE2, XBP1, and ATF6 in the genome of *E. granulosus*, the causative agent of human echinococcosis. In this work, we evolutionary and functionally characterized the IRE/XBP pathway in *Echinococcus* sp. Based on computational assays, we studied the evolutionary relationship of *Echinococcus*-IRE/XBP proteins among metazoans and performed a phylogenetic tree showing their position. Also, we showed that *Echinococcus*-IRE has considerable similarity in secondary and tertiary structures to human IRE (3p23.1A homodimer, 35% identity and 0.49 QMEANDisCo). Moreover, we determined that the level of total *xbp*1 transcript increased under ER-stress inducers treatment and, consequently provokes the enhancement of *grp*78 mRNA expression indicating the occurrence of ER stress in the parasite. Splicing of the *xbp*1 mRNA was analyzed to assess IRE1 activation. Through RT-PCR and sequencing, we corroborated the presence of unspliced- and spliced- *xbp*1 in the parasites. Additionally, since XBP-1 mediates the activation of TFEB, a transcription factor that promotes autophagy, we verified that IRE1/XBP1 activation induces autophagy, with overexpression of *Echinococcus* atg6, *atg*8 and *tfeb* genes. The crosstalk between the IRE1/XBP1 branch and autophagy highlights the importance of both mechanisms in parasite survival. Therefore, targeting the UPR-induced autophagy response may lead to novel therapeutic approaches.

265. (470) **MOLECULAR BIOLOGY TECHNIQUES APPLIED TO THE DIAGNOSIS AND CHARACTERIZATION OF CHILDREN SYPHILIS CASES**

Luciana N García [1,2] , Nicolas Morando[3], Fernanda Lascano [1,2], Samanta Moroni[1], Guillermo F Moscatelli [1,2] , Nicolás Gonzalez [1] ,Margarita Satostegui[1],Griselda Ballering [1], Maria Angeles Pando[3], Jaime M Altcheh [1,2]
1 Servicio Parasitología- Chagas. Hospital de Niños Ricardo Gutiérrez, Capital Federal, Buenos Aires, Argentina.
2 Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP), CONICET- GCBA, Buenos Aires, Argentina
3 Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), CONICET-UBA,Buenos Aires, Argentina

Syphilis is caused by *Treponema pallidum pallidum* (TPA). The scarce data about the biology of TPA during the complex clinical stages and the multiple routes of transmission allows syphilis to cause a large proportion of morbidity in children. The use of molecular biology techniques (MBT) in the diagnosis of syphilis is in the beginnings. In 2019 we initiate a multicenter clinical study evaluating the implementation of MBT for the diagnosis of syphilis in children. Our aim is evaluate the performance of real time PCR (qPCR) in blood and swab samples for the detection of *dnaA* gen and the characterization of TPA by multilocus sequence typing (MSLT). A strict evaluation detected 4 cases of congenital syphilis, 16 cases of acquired secondary syphilis transmitted through nonsexual contact and 12 cases of acquired secondary syphilis transmitted through sexual contact in teenagers (total=42). Of these, 99 samples were processed for DNA extraction (QIAGEN, Germany) followed by qPCR usingTaqman probes. The global efficiency per patient was: Sensitivity (Se)=78.1, Specificity(Sp)=100, Positive predictive value(PPV)=100, Negative predictive value(NPV) = 69.6; with lowest values in blood samples (16.1/100/100/38.1)and highest in swab samples (88.5/100/100/70). So far, 8 patients have been studied for MSLT. Edition and alignment were performed compared to the reference sequences of SS14 (CP004011.1), Nichols (CP004010.2) and Escherichia coli 23s rRNA genes at the positions 2058 and 2059 (V00331) for azithromycin (AZ) resistant mutation. All samples except one, predicted to be AZ sensitive based on 23S A2058 allele. Nichols-like samples (n=5) belong to the 9.7.3 allelic profile while SS14-like samples (n=3) belong to the 1.1.1 allelic profile. These preliminary data support the use of qPCR methods as confirmatory techniques in children syphilis and bring important data of TPA genome in these populations, which is an urgent need for prevention policies and vaccine development.

266. (557) **EFFECT OF ATP ON THE ADHESION OF RED BLOOD CELLS TO ENDOTHELIAL CELL ELICITED BY PLASMODIUM FALCIPARUM**

Renata Blanco[1], Emilia Belén Sousa[1], Nicolás Saffioti[2], Pablos Julio Schwarzbaum[1], Cora Lilia Alvarez[1]
1- Instituto de Química y Físico-Química Biológicas "Prof. Alejandro C. Paladini", Universidad de Buenos Aires (UBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Farmacia y Bioquímica, Junín 956, C1113AAD Buenos Aires, Argentina. 2- Instituto de Nanosistemas. Universidad Nacional de San Martín. Avenida 25 de Mayo 1021, Buenos Aires, Argentina.

Plasmodium falciparum, the most dangerous parasitic agent causing malaria, invades human red blood cells (RBCs) causing hemolysis, adhesion of infected RBCs (iRBCs) to endothelium and microvascular obstruction. ATP and its byproducts are important extracellular ligands modulating purinergic signaling within the intravascular space. In RBCs extracellular ATP regulation depends on the balance between ATP release by specific transporters and extracellular ATP hydrolysis by ectonucleotidases. Previously, we demonstrated that, in a suspension of iRBCs at low parasitemia, not only iRBCs but also non-infected RBCs (cultured in the presence of iRBCs (niRBCs)) presented activation of ATP release via Pannexin 1, and of extracellular ATP degradation by ectoATPase activity. This activation might be involved in microvascular obstruction caused by malaria, thus we studied whether extracellular ATP affects the adhesive properties of iRBCs and niRBCS. *P. falciparum* was cultured in RBCs. iRBCs and niRBCs were purified by magnetic columns. Adhesion to a surface covered with poly-Lysine was measured by a colorimetric method (crystal violet). Adhesion to a monolayer of confluent endothelial cells (EA.hy 926) growing on coverslips was measured by cell counting. Suspensions of iRBCs and niRBCs were incubated with endothelial cells for 30 minutes at 37 °C, washed to remove non-adhered RBCs, and fixed with 3% glutaraldehyde. Then, RBCs were counted by bright field optical microscopy. Results show that the adhesion of iRBCs is greater than that of the niRBCs. There were non-statistical differences between niRBCs and control RBCs *i.e.* RBCs cultured in the absence of *P.falciparum*. Preliminary results suggest that exposure to μM concentrations of ATP, ADP,

AMP and ADO did not affect adhesion of either iRBCs or niRBCs. Future studies will also assess the effect of purinergic signaling in RBCs aggregation and deformability to understand it's role in microvascular obstruction in malaria.

267. (605) **BEHAVIOR ANALYSIS FOR PREDICTIVE PURPOSES OF COVID-19 IN THE ARGENTINE REPUBLIC, SANTA FE PROVINCE AND ROSARIO CITY**

Azul María Milacher, María Eugenia Cabral, Fabricio Nazareno Trabachino, María Clara Civetta, Juan Ignacio Vansteenkiste, María Delamorclaz, Lucia Nannini, Abril López, Azul María Hernandez , Andrea Rafaella Barbosa Lima, Jeremías Ruiz, Jazmín Morales, Gianluca Miele, Ana Paula García, Martín Ruiz Diaz, Amanda Carolina Mendonça kato, Juan Pablo Trabachino, Franco Facciuto, Damián Lerman Tenenbaum , Jorge Luis Molinas

IMOFyS Morphological-Functional and Systemic Research Team. Physiology SM-UNR. School of Medicine (SM), National University of Rosario (UNR). National University of Rosario (UNR)

COVID-19 temporal occurrence frequency would reproduce a fractal rhythm. It behavior can be analyzed by mathematical algorithms that determine Fractal Dimension (FD) and predictive determination coefficient (R^2). FD would express virus temporal repetition and R^2 express response capacity to environment demands, its ranges between 0 to 1, greater than 0.5 would indicate persistence of the system. The aim of this work was to analyze temporal distribution of COVID-19 using Higuchi Algorithm (HA) for predictive purposes in the Argentine Republic (AR), Santa Fe Province (SFP) and Rosario City (RC) according to the epidemiological week (EW). Observational, longitudinal and prospective study. COVID-19 positive cases (PC) were considered by the Ministry of Health daily reports, from the first PC (10^{th} and 11^{th} EW 2020) to 34^{th} EW (2022). HA was applied to EW. Median (M) and standard deviation (±) were obtained from FD and R^2, and Pearson›s correlation coefficient (r) between FD and R^2 according to territory. Results: FD(AR): M=2.30±0.44-R^2(AR): M=0.95±0.08; FD(SFP): M=1.67±0.37- R^2(SFP): M=0.79±0.12; FD(RC): M=1.58±0.45- R^2(RC): M=0.77±0.13. Correlation for AR: r=0.64 ($p<0.004$), SFP: r=0.73 ($p<0.0001$), RC: r=0.63 ($p<0.0007$). Conclusion: COVID-19 behavior in AR shows a growing and sustained temporal manifestation and system-environment interaction, while in SFP and RC it has found limitations. COVID-19 could be sustained over the time in the three territories if current conditions continue. PC decrease would not be accompanied by an environment adaptability decrease of COVID-19, it suggests the study of health measures impact and COVID-19 population coexistence.

268. (606) **PREDICTIVE ANALYSIS OF DENGUE VIRAL DISEASE THROUGH HIGUCHI'S ALGORITHM IN THE CITY OF ROSARIO**

María Delamorclaz, María Eugenia Cabral, Juan Ignacio Vansteenkiste, Julia Catalá, Juliana Baleani, Fabricio Nazareno Trabachino, Tomás Rosso, Larissa Bettega, Adrián Nicolás Martínez, Lautaro Marbán, Priscila Ornella Roth, Azul María Milacher, Franco Facciuto, Damián Lerman Tenenbaum, Jorge Luis Molinas

IMOFyS Morphological-Functional and Systemic Research Team. Physiology SM-UNR. School of Medicine (SM), National University of Rosario (UNR). National University of Rosario (UNR)

Dengue Viral Disease (DVD) is transmitted by the Aëdes aegypti mosquitoes, theses find in Rosario City (RC) the climatic, urban and migratory conditions for its appearance and expansion; therefore, preventive measures focus on vector control. DVD develops an endemic cycle (EC) during epidemiological weeks (EW) between November to May of the following year. DVD would reproduce a fractal rhythm, feasible to be analyzed by mathematical algorithms that determine the Fractal Dimension (FD) and the Predictive Determination Coefficient (R^2). FD would express temporary repetition of DVD and R^2 express DVD's adaptive capacity, its range between 0 to 1, values greater than 0.5 would indicate persistence of DVD. The aim of this work was to analyze temporal frequency of DVD in RC by Higuchi's Algorithm (HA) with predictive purposes. Observational, longitudinal and prospective study. Positive cases (PC) of DVD were considered by RC's government reports from 44th EW to EW to 22nd EW of the following year (from 2017 to 2022). HA was applied to PC diary registry. FD and R^2 were obtained by EC and Pearson correlation coefficient (r) was determined by fractal parameters. Results by EC: 2017-2018 EC: FD= 0.38, R^2=0.43; 2018-2019 EC: FD= 0.25, R^2=0.08; 2019-2020 EC: FD= 0.7, R^2=0.3; 2020-2021 EC: FD= 0.92, R^2=0.77 and 2021-2022 EC: FD= 0.91, R^2=0.69. Correlation: r= 0.91 ($p<0.0307$). Conclusion: HA shows that DVD vector encountered environmental limitations in 2017-2019 EC, while in 2020-2022 EC it achieved greater environment interaction. It would indicate that if current conditions persist, preventive measures should be adjusted to avoid DVD transmission in next EC.

269. (644) **SEROPREVALENCE AND RISK FACTORS ANALYSIS OF HEPATITIS E VIRUS INFECTION IN HUMAN POPULATION FROM TANDIL, BUENOS AIRES, ARGENTINA**

Mariana Alejandra Rivero[1], Lorena Arce[2], Silvina Elena Gutiérrez[1], Adela Tisnés[3], Juan Antonio Passucci[1], Florencia Sánchez[3], Julia Analía Silva[1], Julia Matias Brancher[2], Macarena Haure[3], Silvia Marcela Estein[1], María Guadalupe Vizoso Pinto[2].

1. Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA); Centro de Investigación Veterinaria de Tandil (UNCPBA-CICPBA-CONICET), Tandil, Buenos Aires, Argentina

2. Facultad de Medicina, INSIBIO (CONICET-UNT), Universidad Nacional de Tucumán, Argentina.

3. Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA)

4. Facultad de Ciencias Humanas (UNCPBA)- CIG- IGEHCS- CONICET. Tandil, Buenos Aires, Argentina.

Background: Hepatitis E virus (HEV) infection is a common cause of acute clinical hepatitis worldwide and an emerging disease in Argentina, primarily transmitted via the fecal-oral route due to contaminated water and food. It is also a zoonotic disease, being swine the main reservoir. Prevalence of HEV infection in humans in several regions of Argentina remains unknown, mainly because of lack of awareness and proper diagnosis.

Objectives: i) to determine the prevalence of HEV infection in humans from Tandil, ii) to evaluate its association with demographic and socioeconomic variables, and iii) to describe and analyze spatial patterns related to the infection. Methods: Blood samples were obtained from 969 individuals aged 1-80 years (median 44) by a random spatial sampling method. Socioeconomic information (socioeconomic status, overcrowding, educational level, health insurance coverage, access to drinking water, and appropriate sewage disposal) was obtained by a survey. The address of the participants was georeferenced. Anti-HEV IgG was detected by a validated indirect in-house ELISA, developed in INSIBIO (CONICET-UNT). Associations between the variables and seropositivity were evaluated by Chi square and Wilcoxon tests. Scanning for clusters with high rates of positivity was carried out using the Bernoulli model. Results: Anti-HEV antibodies were detected in 4.34% (IC 95% 3.23-6.01) of samples, more frequently in men (6%) than women (4%), although not statistically significant (p-value 0.0542). The median age of seropositive individuals was higher than seronegative (52 vs 42, p-value 0.0002). Socioeconomic variables were not associated with seropositivity. No significant cluster of positivity was detected by the purely spatial analysis. Conclusions: HEV is prevalent in the population under study. The importance of considering HEV infection in the differential diagnosis of hepatitis in inhabitants belonging to different socioeconomic groups is highlighted.

270. (776) **ENVIRONMENTAL ENRICHMENT IMPROVES SOCIAL BEHAVIOR IN A MURINE MODEL OF CHRONIC TOXOPLASMOSIS**

Tadeo Amato[1], Florencia Andrés[1], Nadia Justel[2], Valentina Martín[1], Vanesa Sánchez[1]

[1]Laboratorio de Inmunología Vacunas y Alergias (ITECA-CESyMA-ECyT-UNSAM), [2]Laboratorio Interdisciplinario de Neurociencia Cognitiva (LINC, CINN, UP, CONICET)

At the chronic stage of T. gondii infection, tissue cysts are located mainly in SNC. There are no treatments able to eliminate these resistant structures or to reduce the adverse effects associated with the infection. Currently, accumulated evidence links chronic infection with different pathologies, including neurocognitive and behavioral conditions. Herein, we study the effect of environmental enrichment (EE) as a non-invasive therapy against chronic toxoplasmosis and the impaired social behavior associated. Methods: The EE therapy involves increasing the available space and the addition of novel elements in the habitat. A three-chamber sociability device where a naive mouse (stranger) was placed was used to evaluate social abilities of C57BL/6 chronically infected treated (TE) or untreated (T) mice. The data was analyzed with t-student test. Brain cyst burden was evaluated at the end of the assay. Results: The results indicate that the EE treatment on infected mice improved its social ability measured as time of active contacts between the test mouse and novel mouse (TE vs T; p=0,0276). Indeed, TE mice showed higher exploration of the novel subject than T mice (up to 1,7-fold higher; p=0,0033). Moreover, 75% of TE mice showed a first impulse to approach that area while only 25% of T mice showed this behavior. Chronically infected treated mice showed a significantly better discrimination index compared to T group (p = 0,0443). All these data evidence that EE treatment improves social abilities in mice. Nevertheless, TE brain parasite load was similar to the T group. Conclusions: This environmental enrichment therapy showed a positive impact in social behavior, showing its potential to deal with the harmful effects of chronic toxoplasmosis, improving well-being of the affected individuals and the social environment that surrounds them and in which they develop. This type of non-invasive therapy could be easily incorporated into translational medicine approaches.

271. (805) **SKIN WOUND HEALING EFFECTS AND ACTION MECHANISM OF LACTIPLANTIBACILLUS PLANTARUN**

Nicolás Argañaraz[1], Candelaria Fanjul[1], Yanina Kolling[1] ,Cecilia Werenitsky[2], Maximiliano De Boeck[3], Pablo Valdecantos[3], Nadia Gobbato[1], J. Carlos Valdez[1] y Mirta Rachid[1]
1. Cátedra de Inmunología- Fac. de Bioquímica, Qca. y Farmacia-U.N.T.
2. Cátedra de Bacteriología- Fac. de Bioquímica, Qca. y Farmacia-U.N.T.
3. Instituto de Investigaciones Biológicas (INSIBIO)-U.N.T.

The aim of this study was to investigate the wound healing effect of *Lactiplantibacillus plantarum* (Lp) and a posible underlying mechanism involved in its action using *in vitro* and *in vivo* models. Balc/c mice were used in the study and fulll-thickness excisional punch wound were created through the skin. Mice were divided into: group 1 (uninfected control), group 2 (infected on the wound with Methicillin resistant *Staphylococcus aureus* strains 1x10^7 UFC/ml) and group 3 (infected and treated with topical applications of whole culture of Lp. Mice were sacrificed on day 4, 6 and 10 post-treatment. We investigated: a) Wound area, b) Histopathological study (H&E), c) Re-epithelialization and d) Microbial evaluation (CFU/g of tissue). *In vitro* studies: The fibroblast cell line L 929 was used. On day 2 after seeding (80% confluence), cells were incubated with 50, 100,150 and 200 ul of viable Lp, not viable Lp and supernatant for 24, 30 and 48hr and studied: 1) MTT assay for cell viability determination, 2) Scrath assay, 3) Collagen-I contraction assay and 4) Assessment of collagen deposition and quantitative analisys by Sirius red staining. H&E staining of skin biopsies showed trat Lp acelerated closure wound and complete re-epithelialization by day 6. In addition, the presence of granulation tissue favors the healing process. Mice in the group 2 displayed slower wound closure over time. Bacteria load was significantly reduced in the group 3 compared to the group 2 (10^3UFC/ml vs 10^7 UFC/ml p< 0.01). Viable Lp, not viable Lp and supernatant (50ul at 24hr) significantly promoted cell proliferation. This increase was significant (p< 0.05) compared to those without treatment. Lp and supernatant increased the content of soluble collagen in the supernatant in a concentration-dependent manner without reduction of the cell viability (300ug/ml vs 129ug/ml p <0.01). Lp promotes the healing process, reduced infection and promotes the development of granulation tissue.

272. (849) **GENOTYPIC CHARACTERIZATION OF CARBAPENEM-RESISTANT ISOLATES FOR EPIDEMIOLOGICAL SURVEILLANCE IN A PUBLIC HOSPITAL IN ROSARIO**

Andreina Reschia [1] ,Sebastian Cogliatti [1],Carolina Martinez [1] ,Gustavo Rossignol [1] ,Luis Caprile [1] ,Nadia Gerhardt [1,4] ,Aranza Sorribas [1,4] ,Juliana Sesma [1,2,3]
1 Hospital Provincial de Rosario
2 IDICER-CONICET
3 Facultad de Ciencias Médicas- Universidad Nacional de Rosario (FCM-UNR)
4 Facultad de Ciencias Bioquímicas y Farmacéuticas (FCByF-UNR)

Multi-resistant Gram-negative bacilli constitute a problem of great importance, as they can generate outbreaks making difficult the infection control. The characterization of a resistance mechanism in rectal swabs is a powerful tool to provide resources for the future implementation of prophylaxis treatments in case the patient develops symptoms. Objectives: To implement molecular biology techniques to evaluate the epidemiology of rectal colonization of multiresistant microorganisms in patients during their hospitalization in Intensive Care (IC). Material and Methods: 420 samples of rectal swabs from patients from IC, were cultured in selective and differential media. Isolated colonies were identified by MALDI-TOF, genotypically characterized by multiplex PCR by Malbrán Institute protocols, detecting the presence of genes coding for serino-carbapenemases (KPC, OXA-48) and metallo-β-lactamases (NDM, IMP, VIM). Results: Of the 420 samples, 157 were positive for Gram-negative (G(-)) bacilli resistant to carbapenems. Of these 157 samples, 108 were *Klebsiella pneumoniae*, (104 were KPC positive, 2 were NDM positive, and 2 were KPC/NDM double mechanism positive), 3 were *Pseudomonas putida* VIM positives, 4 were *Pseudomonas aeruginosa* and 42 were *Acinetobacter baumanii* MDR, both last two without presenting any of the resistance genes analyzed. The genotypic determinations of the samples were verified phenotypically, obtaining 100% correlation. Conclusions: During the last year, the percentage of colonization by G(-) bacilli resistant to carbapenems in swabbed patients was 37%, being KPC the most frequent mechanism detected. The prompt determination of resistance mechanisms by PCR is relevant for an early and effective diagnosis. The few therapeutic options to treat infections by multiresistant microorganisms make it necessary to implement an active surveillance by constantly searching for colonized patients to reinforce isolation measurements.

273. (860) **ADVANCED EXPERIMENTAL CYSTIC ECHINOCOCCOSIS: REPROGRAMMING THE INTERMEDIARY CARBON METABOLISM IN THE PARASITE UNDER METFORMIN TREATMENT *IN VIVO***

Julia A. Loos[1], Perla Negro[2], Graciela L. Salerno[* 3], Andrea C. Cumino[*1]
[1]IIPROSAM, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata.
2 Parasitología y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Santa Fe, Argentina, Boulevard Ovidio Lagos 1000, (2170) Casilda, Santa Fe, Argentina.
*3 Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET) and Centro de Investigaciones Biológicas (CIB-FIBA), Mar del Plata, Argentina. *equal contribution, E-mail: acumino@gmail.com*

Cystic echinococcosis is a progressive and chronic disease caused by the larval stage of the species complex *Echinococcus granulosus sensu latu.* New treatment options are needed especially for advanced disease. Since in these cestodes glycogen is the main energy storage molecule and glucose the major fermentative substrate, our approach was to target the metabolic pathways of the parasite involved in energy production, focusing on the AMPK/TOR pathway. Here, the aim was to assess the *in vivo* efficacy of met-

formin as an indirect AMPK agonist, in an advanced disease model (1year post-infection in mice), employing the highest dose of assayed drug (250 mg kg^{-1} day^{-1}). Metformin-treated mice exhibited a reduction of cellular integrity of the germinal layer of cysts, registering a drug concentration of 1.7 mM (which inhibits mitochondrial respiratory chain complex I), a reduction in intracystic glucose with an increase in lactate concentration, consistent with the rise in the glycogen breakdown and in the LDH activity. Interestingly, the fraction of reducing soluble sugars decreased by 3 times in the cystic fluid and germinal cells after of drug-treatment. However, non-reducing soluble sugars, such as sucrose and trehalose, were consumed in the cystic fluid but showed a significant increase at the intracellular level in presence of the drug. It is surprising that trehalose and sucrose biosynthesis was upregulated during starvation induced by metformin. That is, a futile cycle of non-reducing sugars synthesis and glycogen catabolism during starvation. Function of these disaccharides as stress protectants during starvation provides some resolution to this paradox, as it also occurs in others invertebrates and plants. In the same line evidence, fasting and starvation induce hepatic gluconeogenesis in mammalians. Thus, in this parasite metformin affects glucose-starvation-induced AMPK activation and restructures carbohydrate metabolism prior induction of cell death.

274. (890) **THE POTENTIAL USE OF CANNABIDIOL IN *MYCOBACTERIUM TUBERCULOSIS* INFECTION**

Camila B. Martinena[1*], Merlina Corleto[2,4*], Candela Martin[1], Lucía B. Donnoli[1], Rocío Zuazo[1], Ana J. Bazan Bouyrie[1], Agustín Vitti[1], María Paula Morelli[1], Rosa M. Musella[3], Lorena Ciallella[3], Graciela M. Cragnolini de Casado[3], Domingo J. Palmero[3], Nicolás Amiano[1], Verónica García[1], Paulo C Maffia[2,4Φ], Nancy L Tateosian[1Φ].

1. Instituto de Química Biológica de la Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, IQUIBICEN-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica.

2. Laboratorio de Aplicaciones Biotecnológicas y Microbiología, Universidad Nacional de Hurlingham.

3. División de Tisioneumonología, Hospital F. J. Muñiz, Buenos Aires, Argentina.

4. Concejo Nacional de Investigaciones Científicas y Técnicas, CONICET.

Cannabidiol (CBD), the main non-psychoactive ingredient of the cannabis plant, is one of more than 100 cannabinoids that can be extracted from the *Cannabis sativa* L., many of which have been shown to be biologically active. Besides the scientific research, the societal acceptance of CBD oil for medical application has increased in recent years. However, in spite of the increasing research, all of CBD biological effects are not completely understood. The purpose of this work was to investigate the antimicrobial and immunomodulatory effects of CBD in *Mycobacterium tuberculosis* (*Mtb*) infection. Results: First, we evaluated the anti-mycobacterial effect of different concentrations of CBD (150µg/mL, 15µg/mL, 1.5µg/mL 0,15µg/mL and 0,015µg/mL) by colony forming units (CFU) counts after2hours and 24hours of treatment. We found that CBD treatment produced a decrease in *Mtb*H37Rv viability in a dose dependent manner at 2hours and 24hours (ANOVA test, $p<0,05$). Then, we investigated the anti-mycobacterial effect of CBD in macrophage infected cells with *Mtb*H37Rv (MOI 10). We observed a reduced intracellular *Mtb*H37Rv viability after CBD treatment (CFU counts, 24hours) at concentrations that displayed no cytotoxic effect on THP-1 cell lines determined by Trypan Blue assay (CBD: 150µg/mL $p<0,05$). Finally, we investigated de modulatory effect of CBD on healthy donors (HD) and tuberculosis patients (TB) PBMC´s IFN-γ and IL-17A secreted levels. After 5 days of PBMC´s *Mtb*-Ag stimulation, CBD treatment (15µg/mL) decreased the IFN-γ and IL-17A levels detected by ELISA in HD and TB patients. Conclusion: Overall, the data support the notion that CBD is immune modulator and antimicrobial for tuberculosis human infection.

275. (909) **ANTIVIRAL AND IMMUNOMODULATORY ACTIVITY OF A SYNTHETIC STEROID ANALOG AGAINST ZIKA VIRUS INFECTION IN OCULAR CELLS**

María Ximena Guerbi [1,3], Alejandro Berra [1,2], Flavia M. Michelini [1,2]

1, CEMET-HEC, F.Varela, Argentina; 2, CONICET; 3, CIC.

Zika virus (ZIKV) is a mosquito-transmitted arbovirus in humans, with other modes of transmission including sexual, perinatal, and via blood transfusion. Most of infected patients are asymptomatic, but infection can be associated with viral neurotropism and Guillain-Barre syndrome. Conjunctivitis, uveitis, and unilateral acute maculopathy have been reported in adults after acute infection. Congenital Zika syndrome (CZS) is the distinctive phenotype of babies infected with ZIKV in utero, with neurological, ocular, hearing, and skeletal abnormalities. There are no vaccines or specific antiviral agents to treat ZKV infections. Virest, (22S, 23S)-22,23-dihydroxystigmast-4-en-3-one, is a steroidal analogue with stigmastane structure, with antiviral activity against diverse clinically relevant human virus with different structures and replicative strategies. In the present study we evaluated a potential direct antiviral activity of Vires against ZIKV, through virus yield reduction assays, and an indirect inhibitory activity by an immunomodulation of the infected cells, through the quantification of the secreted cytokines with enzyme linked immunoassays, in human cell lines derived from ocular tissue and in macrophages. Virest significantly reduced viral yields in retinal, conjunctival, and corneal cells, and it induced an increase in proinflammatory cytokines secretion. However, the compound induced a significative reduction of cytokine secretion in infected macrophages. ANOVA followed by Tuckey test, $p<0,001$ were performed ($n>2$). Virest exhibited antiviral activity against ZIKV in ocular epithelial cells and modulated the inflammatory response in infected epithelial cells and macrophages.

276. (927) **CELLULAR PHYSIOLOGICAL IMPROVEMENT BY SPINOCHROMES FROM SEA URCHIN EGGS. A POTENTIAL ALLY IN IMPROVING THE SECUELAES OF COVID-19**

Marisa Avaro[1]; Rubilar, Tamara[1,2],Silvana Maidana[3], Alejandra Romera[3], Elena S. Barbieri[1,2], Augusto Crespi-Abril[1,2];-Valeria Brichetti[4]; Julieta Tejada[4]; Fernando Saldarini*[4] y Gabriela de Larrañaga[5*].

[1] Instituto Patagónico del Mar (IPaM), Facultad de Ciencias Naturales y de la Salud (FCNyCS), Universidad Nacional de la Patagonia San Juan Bosco (UNP), Puerto Madryn, Argentina.

[2] Laboratorio de Oceanografía Biológica (LOBIO), Centro para el Estudio de Sistemas Marinos (CESIMAR), CCT CENPAT-CONICET, Puerto Madryn, Argentina.

[3] Instituto de Virología e Innovaciones Tecnológicas IVIT (INTA-CONICET), N. Repetto y Los Reseros S/N, CC25 (B1712WAA), Castelar, Buenos Aires, Argentina.

[4] Servicio de Tisioneumonología, Hospital Donación Francisco Santojanni, CABA, Argentina.

[5] Laboratorio de Hemostasia y Trombosis, Hospital de Infecciosas F. J. Muñiz, CABA, Argentina.

**hemostasia@gmail.com*

Spinochromes are marine polyphenols known for their antioxidant properties, enhance glutathione metabolism and reduce cellular inflammation. They are naturally concentrated in the eggs of sea urchins and there are two therapeutic pharmacological formulations marketed in Russia and Germany. In Argentina, a nutraceutic with Echinochrome A (EchA) has been formulated as a product equivalent to that used in Russia and is being used in a multicenter clinical trial focused on patients with sequalae of COVID-19 (Long COVID-19), under the hypothesis of decreasing cellular inflammation and increasing cell viability. Patient recruitment is closed, 54 patients have been recruited, with an average age of 51 years (60% women). The patients consume 6 ml of 0.025 mg/ml of EchA per day for 90 days and clinical, biochemical (biomarkers of inflammation and thrombosis) and psychological parameters are analyzed; all interconnected through a SKYMED telemedicine platform. In parallel, EchA was evaluated to demonstrate cell viability activity in Madin Darby Canine Kidney (MDBK) and Vero cells with neutral red in a concentration range of 0.0375 ug/ml to 1000 ug/ml with exposure for 2 hours and 72 hours. Differences in cell viability were found in cell

type, exposure time and EchA concentration (p<0.0001), showing higher survival of MBDK with longer exposure time and lower concentrations. However, no trial reports less than 80% of survival, and at 72 hours, it is observed that cell viability exceeds the control by 20%. These results support the hypothesis that EchA generates a cellular physiological improvement reflected in a percentage equal to or greater than the control. Although the results of the clinical trial are in the stage of analyzing the results and opening the double blind, EchA is expected to generate an improvement in the symptoms, signs, and biomarkers of Long Covid due to its ability to increase cell viability.

277. (928) **EVALUATION OF A SYNTHETIC STIGMASTANE ANALOGUE AGAINST DENV AND ZIKV INFECTION IN EPITHELIAL AND INFLAMMATORY CELLS**

Johanna B. Díaz Sierra[1,2], Carlos Bueno[1,2]. José Peña Carcamo[1,2], Laura Alché[1,2], Cybele García[1,2], Flavia Michelini[1,2]
1, Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Virología, Buenos Aires, Argentina; 2, CONICET - Universidad de Buenos Aires. Instituto de Quimica Biológica de la Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina.

Dengue virus (DENV) is the most prevalent arbovirus around the world, with 2.5 million of infections in Latin America. On the other hand, flavivirus Zika (ZIKV) have rapidly propagated in the last years and has been associated with congenital malformations in newborn and other neurological disorders. There is no specific treatment against these viruses, thus, new antivirals would be useful in the treatment of these infections. Stigmastane analogue Virest (22S, 23S)-22,23-dihydroxystigmast-4-en-3-one, has antiviral activity against diverse human pathogen viruses and it modulates the inflammatory response of epithelial and inflammatory cells after viral and non-viral stimuli. In this study we evaluated the antiviral activity of Virest against DENV and ZIKV, through viral yield reduction assays and immunofluorescence, and the immunomodulatory effect of the compound, through the quantification of secreted pro inflammatory cytokines in epithelial and inflammatory cells. Virest significantly reduced viral yields of DENV and ZIKV. The inhibitory effect was not due to a virucidal effect and was evidenced after virus entry to the cells. Virest induced an increase in cytokines secretion in infected epithelial cells, but it reduced their secretion in infected inflammatory cells. ANOVA followed by Tuckey test, p<0,001 were performed (n>2). Virest shows antiviral and immunomodulatory activities against DENV and ZIKV infection.

INNATE IMMUNITY *Thursday, November 17, 14-15:30 hr*

Chairs: Luisina Onofrio - Soledad Gori - Eliana Cela Andrés Alloatti - Florencia González - Luciano D´attilio - Ariana Díaz - Juliana Sesma - Ayel®n Milillo - Victoria Ramos - Gabriela Fernández - Samanta Funes - Carolina Amezcua

278. (135) ***Minthostachys verticillata*** **ESSENTIAL OIL MODULATES IL-1β AND IL-6 SYNTHESIS IN BOVINE EPITHELIAL CELLS CHALLENGED WITH A *Staphylococcus aureus* STRAIN**

Sofia Arsaute(1), Ivana Dalila Montironi(2), María Eugenia Cecchini(1), Elina Reinoso(1), Laura Noelia Cariddi(1).
(1) INBIAS, CONICET- UNRC, Río Cuarto, Córdoba, Argentina. (2) Cátedra de Farmacología, FAV, UNRC, Río Cuarto, Córdoba, Argentina.

In previous studies we showed that the essential oil (EO) of *Minthostachys verticillata*, a native plant species, has the ability to modulate the immune response. The aim of this work was to evaluate the effect of EO on the synthesis of proinflammatory cytokines in bovine mammary gland epithelial cells (MAC-T) in the presence or absence of a *Staphylococcus aureus* strain (Sa) isolated from cows with mastitis. The cells were treated with EO (25, 50 and 100 μg/mL), Sa ($5x10^6$ CFU/mL) and pretreated with EO and then challenged with Sa at different times (2, 6, 24 and 48 h). Cells without stimulation were used as control. IL-1β and IL-6 cytokines were quantified in the cell supernatant by sandwich ELISA. MAC-T cells stimulated with Sa produced higher levels of IL-1β and IL-6 than untreated cells (p<0.001) up to 48 h. MAC-T cells also responded to EO stimulation (25, 50, and 100 µg/mL) with increased IL-1β levels between 2 and 6 h (p<0.05) and increased IL-6 synthesis between 6 and 48 h (p<0.01 and p<0.05) compared to untreated cells. A decrease in both cytokines in cells treated with EO was observed between 24 and 48 h without differences compared to untreated cells. In cells pretreated with EO and then challenged with Sa, increased levels of both cytokines were observed in first hours. However, after 6 h a decrease in IL-1β was observed compared to cells treated with Sa alone (p<0.01) being the lowest values at 48 h (p<0.001). After 24 h, a decrease in IL-6 was observed compared to cells treated with Sa alone (p<0.05), being the lowest values at 48 h (p<0.01, p<0.001 and p<0.001). These results suggest that after 24 h, EO would stimulate the production of anti-inflammatory cytokines and these would inhibit the production of IL-1β and IL-6 in cells pretreated with EO and challenged with Sa. In further assays will quantify anti-inflammatory cytokines.

279. (167) **STUDY OF γδ T LYMPHOCYTES RESPONSES TO BACTERIAL AND DAMAGE ASSOCIATED MOLECULES IN THE PRESENCE OF SHIGA TOXIN TYPE 2.**

Nadia Carolina Melillo[1], David Antonio Rosso[1], Micaela Rosato[1], Carolina Maiumi Shiromizu[1], Irene Angélica Keitelman[1], Florencia Sabbione[1], Analía Trevani[1,2], María Marta Amaral[3], Carolina Cristina Jancic[1,2]
[1]Instituto de Medicina Experimental – CONICET – Academia Nacional de Medicina. Buenos Aires, Argentina, [2]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina, [3]Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay – CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

The hemolytic uremic syndrome (HUS) mainly affects children younger than 5 years old, who have a higher risk of developing severe consequences such as acute or chronic renal failure. HUS associated with diarrhea, hemolytic anemia, and thrombocytopenia is a consequence of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) infection. Stx type 2 (Stx2)-producing strains are associated with severe cases of HUS in Argentina. γδ T cells are a specialized subset of T cells, which act as early sensors of cellular stress and infection. They can exert cytotoxicity against infected cells and produce cytokines and chemokines. Previously, we demonstrated that Stx2-treated human glomerular endothelial cells modulate the γδ T cell functions. In this work, we studied the activation of human peripheral blood γδ T cells in response to Stx2 (0.01ng/ml), in the presence of different agonists to emulate an inflammatory microenvironment that could be developed at the gut epithelial barrier. For that purpose, we used: 1) the phosphoantigen HMBPP (1μM), a potent γδ T cell bacterial agonist; 2) lipopolysaccharide (LPS: 100ng/ml), an integral component of Gram-negative bacteria; and 3) monosodium urate crystals (MSU: 200μg/ml) which act as molecular associated damage pattern. To evaluate γδ T cell activation, we analyzed CD69 expression by flow cytometry, and cytokine production by ELISA, after 24 h incubation with the agonist alone or in combination with Stx2. As result, we observed an increase in CD69 expression, IFN-γ, and TNF-α production (p<0.05), in HMBPP and LPS-activated γδ T cells. No differences were noticed in the presence of MSU crystals. Nevertheless, when each stimulus was treated in combination with Stx2, there was a slight but no statistically significant decrease in γδ T cell activation. Our results suggest that the combination of Stx2 with bacterial agonists or damage-associated molecular patterns may not be a powerful modulator of γδ T cell activation.

280. (229) **RNA FROM *PSEUDOMONAS AERUGINOSA* ATTENUATES NEUTROPHIL FUNCTIONS**

Pittaluga JR, Birnberg-Weiss F, Castro J, Castillo LA, Serafi-

no A, Martire-Greco D, Fernández GC and Landoni VI.
Laboratorio de Fisiología de los Procesos Inflamatorios. Instituto de Medicina Experimental (Academia Nacional de Medicina-CONICET).

Pseudomonas aeruginosa (PAE) is a well-known opportunist microorganism capable of cause multiples diseases. When immune cells arrive to the site of infection, they are exposed to RNA from bacteria. We have previously documented that RNA from *Escherichia coli* (RNA_{ECO}) or RNA from *Klebsiella pneumoniae* (RNA_{KPN}) were able to activate neutrophils (PMN), whereas RNA from PAE (RNA_{PAE}) failed to induce PMN activation. The aim of this work was to further characterize the effects of RNA_{PAE} on PMN response. PMN were isolated from healthy donor and incubated with purified RNA from bacteria (1μg/mL) or live bacteria (MOI 1:1) for 30 minutes. Activation of PMN was measured by flow cytometry (CD11b mean intensity), PMN migration was determined using a Boyden chamber and IL-8 was determined with ELISA KIT. Results showed that RNA_{PAE} failed to induce IL-8 secretion or chemotactic activity compare to RNA_{Eco} and RNA_{KPN} ($p \leq 0.05$). Surprisingly, activation of PMN observed with RNA_{ECO} or RNA_{KPN} was diminished when RNA_{PAE} was present ($p \leq 0.05$), suggesting that RNA_{PAE} modulates negatively PMN activation by others prokaryotic RNA. We next wondered if RNA_{PAE} was able to modulate PMN response to bacteria. Therefore, when live PAE were used as stimulus, activation, chemotaxis and bactericidal ability of PMN was significantly reduced when RNA_{PAE} was present ($p \leq 0.05$). None of the RNA were able to decrease the PMN response against ECO and KPN. In conclusion, these results revealed that RNA_{PAE} diminishes PMN response to PAE. Further studies will allow to determine if RNA_{PAE} constitutes a mechanism to attenuate PMN response favoring PAE survival in an infectious focus.

281. (234) **GALECTIN-8 C-TERMINAL CARBOHYDRATE RECOGNITION DOMAIN IS RESPONSIBLE FOR ANTIGEN UPTAKE ENHANCEMENT IN DENDRITIC CELLS**

Cecilia Arahí Prato*[1,2]; Laura Victoria Borbolla[1,2]; Oscar Campetella[1,2], and María Virginia Tribulatti[1,2].
1. Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).
2. Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín.
San Martín, Buenos Aires, Argentina.
**arahi.prato@gmail.com*

Galectin-8 (Gal-8) is a mammalian lectin capable of stimulate adaptive immune responses by acting on both CD4+ T cells and antigen-presenting cells (APCs). Recently, we found that Gal-8-glycan interaction at the dendritic cell surface results in an augmented antigen attachment and internalization, a crucial step during the initiation of a given immune response. Since Gal-8 is composed by two distinct carbohydrate recognition domains (*N*-CRD and *C*-CRD) fused by a linker peptide, we aim to characterize this activity at the molecular level by analyzing the involvement of each isolated CRD and the requirement of the "heterodimeric" structure. For this, we generated single *N*- and *C*-CRD as well as chimeric recombinant proteins consisting of two covalently fused *N*-CRD (*N*-*N*) or *C*-CRD (*C*-*C*). Then, mouse bone marrow-derived dendritic cells (BMDC) were incubated in the presence of fluorescently labeled ovalbumin (OVA) together with each single domain (*C*-CRD or *N*-CRD), the equimolar mixture of both (*C*-CRD + *N*-CRD) or each chimera (*C*-*C* or *N*-*N*). Antigen internalization was determined by flow cytometry. Only the presence of the *C*-CRD (alone, in mixture with *N*-CRD or in the chimera *C*-*C*) was able to recapitulate the Gal-8 effect on antigen attachment and internalization on APCs. Moreover, he addition of galectin inhibitor, lactose, inhibited the effect induced by *C*-CRD, confirming the participation of lectin-glycan interactions with the glyconjugates at the BMDCs surface. In accordance, BMDCs pulsed with OVA in the presence of isolated *C*-terminal (but not the *N*-terminal domain) enhanced antigen-presentation to cognate CD4+ T cells, as efficiently as native Gal-8. Taken together, these findings demonstrate that only the *C*-terminal domain is involved in Gal-8-induced antigen internalization on APCs, and that the "heterodimeric" structure is not required, providing a new insight to advance in the use of Gal-8 as an adjuvant in vaccine formulations.

282. (239) **ANALYSIS OF THE INTERACTION BETWEEN ©™ T CELLS AND GLIOBLASTOMA CELL LINE-DERIVED MICROVESICLES**

Micaela Rosato[1], Carolina Maiumi Shiromizu[1], David Antonio Rosso[1], Nadia Carolina Melillo[1], Alejandra del Pilar Infante Cruz[1], Gabriela Salamone[1,2], Carolina Cristina Jancic[1,2]
[1]Instituto de Medicina Experimental – CONICET – Academia Nacional de Medicina. Buenos Aires, Argentina. [2]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina.

Glioblastoma multiforme (GBM) is the most aggressive malignant cerebral tumor in adults and has a median survival of less than a year after diagnosis. GBM is refractory to standard treatments because of its infiltration nature. GBM immunotherapy based on γδ T cells has been proposed to treat this pathology. γδ T cells are non-conventional T lymphocytes that recognize malignant cells and can trigger their apoptosis. Furthermore, the tumor cells can secrete extracellular vesicles (EV), among them, microvesicles (MV), containing molecules that regulate tumor microenvironment to allow their growth and progression. The interplay between EV and target cells can be mediated by membrane receptors, and by the internalization or fusion of EV with cells. Previously, we demonstrated that MV released by GBM cell line U373 activated γδ T lymphocytes. In this work, we aimed to analyze the interaction between MV and γδ T cells. For that, peripheral blood γδ T lymphocytes were purified by using an anti-TCR γδ MicroBead isolation kit, and MV were obtained from U373 cell line conditioned medium by differential centrifugation. MV were then stained with the lipophilic fluorescent dye PKH26, and incubated with γδ T cells for 30 and 120 min. Afterward, the fluorescence on γδ T lymphocytes was evaluated by flow cytometry (n=4) and confocal microscopy (n=5). The analysis of γδ T cells by flow cytometry showed that after 30 min of incubation they were PKH26+ ($p<0.05$). Moreover, the observation by confocal microscopy revealed that γδ T cells and PKH26+ MV were associated. Interestingly, at 30 min MV displayed a localized and dotted distribution on γδ T cells, while at 120 min it was diffuse and homogeneous along the cells. Also, there was an increase in the PKH26+ γδ T lymphocytes at 120 min, suggesting the progression in the interaction along the time ($p<0.05$). Our finding suggests that the GBM-derived MV interact with γδ T cells, and they may fuse with the cellular plasma membrane.

283. (241) ***KLEBSIELLA PNEUMONIAE* AFFECTS NEUTROPHIL ELASTASE DYNAMICS**

Federico Birnberg-Weiss[1], José R. Pittaluga[1], Joselyn E. Castro[1], Luis A. Castillo[1], Daiana Martire-Greco[1], Federico Fuentes[1], Fabiana Bigi[2], Sonia A. Gómez[3], Verónica I. Landoni[1], Gabriela C. Fernández[1].
[1] Instituto de Medicina Experimental (IMEX)-CONICET/Academia Nacional de Medicina; Buenos Aires, Argentina. [2] Instituto de Agrobiotecnología y Biología Molecular (IABIMO), Instituto Nacional de Tecnología Agropecuaria (INTA), CONICET, Buenos Aires, Argentina. [3] Servicio de Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas Dr. Carlos G. Malbrán (INEI), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Buenos Aires, Argentina.

The importance of neutrophil elastase (NE) in the bactericidal response of neutrophils (PMN) relies on different mechanisms. NE contained in azurophilic granules (AzG) can kill bacteria after fusion with phagosomes. Also, NE translocation from AzG to the nucleus is a key step for neutrophil-extracellular-traps (NET) formation, and depends on NE activity. *Klebsiella pneumoniae* carbapenemase (Kp)-producing bacteria are associated with significant mortality in immunocompromised patients. We have previously reported that PMN failed to release NET when challenged with Kp, whereas *Escherichia coli* (Eco) was a high NET inducer. Our aim was to deepen

the study of the lack of NET formation in the presence of Kp focusing on NE dynamics. To determine whether Kp directly affects NE activity, we used a PMN lysate as a source of NE, and determined NE activity using a commercial kit after 1 h incubation with Kp or Eco. We found that neither Kp or Eco affected NE activity ($p<0,05$). To evaluate translocation of NE from AzG to the nucleus we incubated purified PMN with Kp or Eco (MOI 10), or the NET inducer PMA (20 nM) for 1 h. Cells were observed by confocal microscopy after DNA and NE staining, and the percentage of nucleus with NE was quantified. We found that NE translocates to the nucleus in both PMA and Eco treatments, but Kp failed to induce NE translocation ($p<0,05$). Finally, we evaluated the presence of NE in phagosomes containing bacteria. PMN were challenged with GFP-Kp or -Eco (MOI 10) for 30 min. The number of PMN containing NE-positive intracellular bacteria was determined by confocal microscopy. Kp containing phagosomes did not co-localize with NE while those from Eco did ($p<0,05$). In summary, Kp affects the translocation of NE to the nucleus without interfering with NE activity. Moreover, the lack of intracellular co-localization of Kp with NE suggests a possible alteration of AzG mobilization mediated by Kp. Our results indicate that Kp interferes with NE dynamics.

284. (260) **INHIBITION OF CLASS I SIRTUIN ACTIVITY PROMOTES NEUTROPHIL ACTIVATION AND SURVIVAL**

Lucia Bleichmar[1], Ignacio Mazzitelli[1], Claudia Melucci[1], Jorge Geffner[1], Fernando Erra Diaz[1].
[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA – INBIRS – CONICET UBA

Sirtuins are a family of NAD^+ dependent enzymes that control the acetylation state of a wide variety of histone and non-histone proteins. Due to their deacetylase activity, Sirtuins modulate many cellular functions and are known to participate in the regulation of cell metabolism and aging. Sirtuin family comprises SIRT1 to SIRT7 and it can be divided into four classes: class I (SIRT1-SIRT3), class II (SIRT4), class III (SIRT5) and class IV (SIRT6, SIRT7). Here we studied the effect of the class I Sirtuin inhibitor EX-527 on human neutrophils. Neutrophils were purified from peripheral blood by conventional procedures (% purity >97%). By qPCR we found that human neutrophils express *SIRT2*, *SIRT3, SIRT5* and *SIRT6 genes.* We also found that the class I SIRT inhibitor, compound EX-527 (100 μM), significantly increased ($p=0.0313$, $n=6$) the production of ROS evaluated by dihydrorhodamine oxidation and flow cytometry, induced by fMLP (100 nM), at 20 min of incubation: % increase = 220 ± 62. It also modestly increased the survival of resting neutrophils (Annexin V/propidium staining and flow cytometry) evaluated at 18hs of culture: % viable neutrophils 24 ± 3.4 and 30 ± 3.8 ($n=12$, $p=0.043$), for cells cultured in the absence or presence of EX-527, respectively. Interestingly, this anti-apoptotic effect was further increased when neutrophils were cultured at pH values similar to those found in inflammatory tissues (pH 6.0): % viable neutrophils 40 ± 4.1 and 71 ± 3.3, for neutrophils cultured without or with EX-527 ($n=12$, $p=0.0005$). Moreover, we found that EX-527 significantly ($p<0.01$) increased survival of neutrophils treated with pro-apoptotic agents such as Zymosan, 2-Deoxy-D-glucose, UV radiation and hyperthermia. Our observations suggest that class I Sirtuins might be involved in the regulation of neutrophil activation and survival.

285. (263) **MODULATION OF THE IMMUNE RESPONSE BY A NOVEL AMNIOTIC MEMBRANE DERIVATIVE IN A HUMAN MONOCYTIC CELL LINE**

María Ximena Guerbi[1,5], Maia Elizagaray[3], Laura Carballo[2], Griselda Moreno[3,4], Flavia Michelini[1,4],, Alejandro Berra[1,4,6].
1, CEMET-HEC, F.Varela, Argentina; 2, HEC, F.Varela, Argentina; 3, IIFP, CONICET, UNLP, La Plata, Argentina; 4, CONICET; 5, CIC; 6AMNIOS-BMA, Argentina.

Macrophages can acquire distinct functionalities, depending on the type of activating stimuli they are exposed to. They might be activated in a proinflammatory way (M1), or might undertake an alternative activation pathway in order to promote tissue remodeling and repair (M2). Amniotic membrane has been used as a biological dressing. We found that the healing time of complex skin wounds has been surprisingly shortened in patients treated with a novel lyophilized and sterilized amniotic membrane patch. To understand the mechanisms involved, we characterized the cytokines profile secreted by THP-1 monocytes differentiated to macrophage during 72 h treatment with 50 ng/ml PMA followed by 100 ng/ml LPS activation, with or without the addition of rehydrated patches supernatants to the culture wells. After 6 and 20 h, IL-6, IL-8, TNF-α and IL-10 were quantified by ELISA. Although no significant differences were observed in IL-10 secretion after 6 h of treatment, we noticed a significant increase after the long treatment. Both IL-6 and TNF-α decreased in this condition, as well. Eventhough, IL-8 was upmodulated beyond the level induced by LPS in both conditions. Cell viability was verified by MTT assay, showing not just the lack of cytotoxicity but also an increased viability in the amniotic membrane products treated wells. In all cases, ANOVA followed by Tuckey test, $p<0,001$ were performed ($n>2$). To sum up, these derivatives seem to induce a shift in the activation profile of macrophages, from M1 to M2, which might be involved in a faster and better wound healing process.

286. (290) **TYRO3 IS UPREGULATED IN DENDRITIC CELLS AND MACROPHAGES EXPOSED TO EXTRACELLULAR VESICLES (EVS) FROM *ECHINOCOCCUS MULTILOCULARIS***

Juan Manuel Ortiz Wilczyñski[1], María Celeste Nicolao[2], Christian Rodriguez Rodrigues[3], Andrea C. Cumino[2, 3], Eugenio Antonio Carrera Silva[1]
[1]Instituto de Medicina Experimental, CONICET - Academia Nacional de Medicina, Ciudad Autónoma Buenos Aires, Argentina. [2]Laboratorio de Zoonosis Parasitarias, IIPROSAM, CONICET, Universidad Nacional de Mar del Plata (UNMdP), Mar del Plata, Argentina. [3]Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), CONICET, Mar del Plata, Argentina.

The *Echinococcus multilocularis* (*Em*) cestode maintains intense communication with its host employing, among others, EVs as carriers to transport functional proteins, lipids, RNAs and other metabolites conditioning host immune response. Here we explored the influence of EVs on the programing and differentiation of human antigen presenting cells (APCs). Blood CD14+ monocytes were isolated and differentiated toward Macrophages (Mø) and dendritic cells (DCs) under EVs treatment purified from *Em* protoscoleces. The phenotype, activation state and level of TYRO3, AXL and MERTK (TAM) receptors on these Mø and DCs were analyzed by flow cytometry, qPCR, and with functional assays. EVs conditioning treatment of DCs resulted in upregulation of the integrin CD11c, and double positive HLA-DR with costimulatory molecules CD40, CD80, CD86 cells ($p<0.05$), but a strong suppression of $HLA\text{-}DR^+CD40^+$ and $HLA\text{-}DR^+CD86^+$ ($p<0.05$) in terminally differentiated Mø. Furthermore, an upregulation of the three TAM receptors was observed in DCs at the percentage and MFI levels when treated with EVs ($p<0.05$), but only TYRO3 expression was significantly increased in EVs-treated monocytes differentiated toward Mø. An MLR assay was carried out to evaluate EVs-conditioned DC function in the presence or absence of TYRO3 blocking antibody. Surprisingly, $CD4^+$ T cells proliferation was significantly reduced when EVs-conditioned DCs were blocked for TYRO3 compared to control DCs. The EV-treated monocytes did not skew Mø differentiation to a particular polarization state, neither altered metabolic or mTOR related genes. The phagocytosis functional assay of Mø using apoptotic neutrophils was performed in the presence of TYRO3 blocking condition showing a dependency of this receptor for the clearance of apoptotic cells, mainly in EVs-treated Mø ($p<0.05$). Our results show that TYRO3 is the main type 2 stimuli-induced member of the three TAM receptors family and its function is a key factor for appropriated immune response and clearance of apoptotic cells.

287. (353) **MACROPINOCYTOSIS AND PLATELETS ARE INVOLVED IN *CLOSTRIDIOIDES DIFFICILE* UPTAKE BY HUMAN MACROPHAGES**

Angela María Barbero[1,2], Rodrigo Emanuel Hernández Del Pino[1,2], Sabina Palma[1,2], Lucía Romano[1,2], Natalia Menite[1],

Federico Fuentes[3], Paula Barrionuevo[3], Virginia Pasquinelli[3].
1CIBA, UNNOBA, Buenos Aires, Argentina, 2CIT NOBA (UNNOBA, UNSAdA, CONICET) Buenos Aires, Argentina, 3IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

Macrophages (MΦ) constitute the primary barrier against pathogens, engulfing and digesting the microorganisms and thus, protecting the host. In recent years, strong evidence indicates the roles of that platelets (P) in the immune response, including the interaction with MΦ and the modulation of their inflammatory functions. *Clostridioides difficile* infection (CDI) is considered one of the most common healthcare-associated gastrointestinal infections related to antibiotic therapy. Many aspects of the immune response against *C. difficile* have not been addressed yet. Here we explore the interplay between MΦ and P in the endocytosis of *C. difficile*. Peripheral blood, monocyte-derived MΦ and P from healthy donors were cultured in the presence or absence of *C. difficile* (NAP1/BI/027 strain) inactivated by heat treatment (CDH) or with FITC-coupled CDH. Our results showed that human macrophages internalize CDH as measured by flow cytometry and confocal microscopy. By using inhibitors of different endocytic pathways we found that CDH uptake occurs via macropinocytosis. When macropinocytosis was blocked using amiloride, the internalization of CDH was reduced by more than 70% ($p<0.05$). On the other hand, preliminary results showed that both PMA and LiCl (GSK3 kinase inhibitor) increased macropinocytosis levels, suggesting that β-catenin pathway might be involved in CDH uptake. We also observed that P interact with monocytes in peripheral blood and MΦ forming complexes, which are increased in the presence of CDH ($p<0.05$). Moreover, P can directly interact with CDH as revealed by fluorescence microscopy. Finally, P enhanced CDH macropinocytosis by human MΦ. In conclusion, we showed for the first time that *C. difficile* access human MΦ in its vegetative form by macropinocytosis. We also highlight the role of P in the innate immune response during CDI, as they can not only promote *C. difficile* uptake but also interact with monocytes and macrophages.

288. (363) **PROSTAGLANDIN E2 PROMOTES POLARIZATION OF MACROPHAGES TO A RESOLUTIVE PROFILE THAT PERSISTS AFTER LPS STIMULATION**

Gonzalo Cabrerizo, Ana Paletta, Facundo Di Diego García, Jorge Geffner, Ana Ceballos, Federico Remes Lenicov
Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires - CONICET

Prostaglandin E2 (PGE2) is an immune mediator with recognized inflammatory properties, but also required for the initiation of the resolution process. Previously, we showed that PGE2 induced a pro-resolutive profile in macrophages. Alternative activation of macrophages with IL-4 is known to induce pro-resolutive features, although these can be reversed by inflammatory stimuli. Here, we aim to compare the actions of PGE2 with those of IL-4 in the acquisition of a resolutive profile by monocyte-derived macrophages. Human monocytes isolated from peripheral blood were cultured during 7 days in the presence of M-CSF (50 ng/ml). Macrophages were treated at day 5 of culture with IL-4 (20 ng/ml) or with PGE2 (1 μM). Control macrophages were cultured without PGE2 or IL-4. Efferocytosis was quantified by incorporation of CFSE-stained apoptotic Jurkat cells and phenotype was analyzed by flow cytometry. Cytokines were measured by ELISA. PGE2 promoted efferocytosis by an average 15% increase of phagocytic macrophages ($n=10$, $p<0,01$), compared to control macrophages. Conversely, addition of IL-4 decreased phagocytosis by 20% ($n=6$, $p<0,05$). Treatment with PGE2 resulted in macrophages characterized by higher expression of CD14 and MerTK ($n=10$, $p<0.01$). In contrast, IL-4 led to a phenotype characterized by increased expression of CD206, CD209, CD36 and lower CD14 ($n=5-7$, $p<0.05$). Treatment with PGE2, but not IL-4, elicited spontaneous production of VEGF-A ($n=2-4$, $p<0,05$). Notably, LPS stimulation (100 ng/ml, 24 hours) enhanced production of VEGF-A in PGE2-derived macrophages ($n=10$, $p<0.05$), but not in IL-4-treated macrophages. Conversely, production of LPS-induced inflammatory cytokines TNF and IL-6 was enhanced in macrophages treated with IL-4, but it was equal to, or lower, in macrophages treated with PGE2. Our results suggest that treatment with PGE2, unlike treatment with IL-4, promotes a stable pro-resolutive phenotype in macrophages, that is not reversed by inflammatory stimuli.

289. (420) **IMPACT OF CD40L ON THE *EX VIVO* PROLIFERATION AND ALTERNATIVE ACTIVATION OF PERITONEAL CAVITY MACROPHAGES**

Ignacio González Alayón[1], Mariana Suárez Martins[1], Cecilia Casaravilla[1], Conor Finlay[2,3], Judith E. Allen[3], Álvaro Díaz[1]
1 Área Inmunología, Departamento de Biociencias (Facultad de Química) and Cátedra de Inmunología, Instituto de Química Biológica (Facultad de Ciencias), Universidad de la República, Montevideo, Uruguay.
2. Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland.
3 Lydia Becker Institute of Immunology and Inflammation, School of Biological Sciences, University of Manchester, Manchester, United Kingdom.

Macrophage proliferation is important in Th2 inflammation, in which macrophages also acquire M(IL-4) phenotypes in response to the cytokines IL-4 and IL-13. In Th2 inflammation, macrophages can also proliferate in response to M-CSF, which additionally maintains the homeostatic levels of these cells. In contrast to M-CSF, IL-4 does not induce macrophage proliferation *ex vivo*. Since IL-4 acts in synergy with CD40 ligand (CD40L, CD154) for the proliferation of B cells, we explored whether CD40L allows IL-4 to induce macrophage proliferation *ex vivo* or potentiates proliferation induced by M-CSF. We used cultures of mouse peritoneal exudate cells (PEC), in which resident macrophages ($CD11b^{high}$, $F4/80^{high}$ cells) account for approximately half the cells. The expression of CD40 on the surface of resident macrophages was enhanced by IL-4, as determined by flow cytometry. Also, endogenous CD40L was present intracellularly in PEC $CD4^+$ T cells, but its blockade with antibodies did not influence expression of M(IL-4) markers Relm-α and CD206 by macrophages in response to IL-4. CD40L blockade also did not influence M-CSF-driven macrophage proliferation, as determined by BrdU incorporation and Ki67 expression. Stimulation with recombinant soluble CD40L (sCD40L) did not allow IL-4 to induce the proliferation of resident macrophages and instead it inhibited their proliferation driven by M-CSF. Moreover, sCD40L inhibited the expression of M(IL-4) markers Relm-α and ChiL-3, although not that of CD206. All the results mentioned were significant by non-parametric two-way statistics (Mack-Skillings test with post-test described by Conover, and the Benjamini and Hochberg correction for multiple comparisons). Our main conclusion is that, in contrast to our initial hypothesis, CD40L does not promote peritoneal macrophage proliferation *ex vivo*, and it instead appears to negatively regulate M-CSF-driven proliferation and M(IL-4) polarization in these cells.

290. (421) **ROLE OF CD40L IN MACROPHAGE PROLIFERATION ASSOCIATED WITH TYPE 2 CONTEXTS: EVIDENCE OF CONTRASTING IMPACTS ON PERITONEAL MACROPHAGES AND KUPFFER CELLS**

Mariana Suárez-Martins[1], Ignacio González-Alayón[1], Camila Mohuape[1], Anabella A. Barrios[1], Cecilia Casaravilla[1], Conor Finlay[2,3], Pedro Papotto[3], Judith E. Allen[3], Álvaro Díaz[1]
[1] Área Inmunología, Departamento de Biociencias (Facultad de Química) and Cátedra de Inmunología, Instituto de Química Biológica (Facultad de Ciencias), Universidad de la República, Montevideo, Uruguay.
[2.] Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland.
[3] Lydia Becker Institute of Immunology and Inflammation, School of Biological Sciences, Faculty of Biology Medicine and Health, University of Manchester, Manchester, United Kingdom.

The accumulation of macrophages is a central aspect of inflammatory responses. Increased numbers results from the recruitment of monocyte precursors and the proliferation of resident cells. The proliferation of macrophages beyond their homeostatic numbers was initially described in type 2 contexts, in which it is mainly driv-

en by the cytokines IL-4 and IL-13. In these contexts, proliferating macrophages additionally acquire M(IL-4) phenotypes, functionally opposed to classical activation. The CD40-CD40L interaction, has been extensively studied throughout immunology and is critical for classical macrophage activation. However, little is known about the role of this interaction in type 2 macrophage polarization. We studied whether endogenous CD40L influences macrophage proliferation *in vivo* after IL-4 injection and during *Heligmosomoides polygyrus* infection, which induces IL-4 expression by $CD4^+$ T cells systemically. In the peritoneal cavity under basal conditions and during thioglycollate medium-induced inflammation, CD40L blockade had limited and nil effect respectively on macrophage proliferation induced by exogenous IL-4. During *H. polygyrus* infection, CD40L blockade enhanced peritoneal macrophage proliferation as well as their expression of M(IL-4) markers. On the other hand, in the liver compartment, CD40L blockade strongly decreased Kupffer cell proliferation both after IL-4 injection and in the *H. polygyrus* system. In sum, CD40L appears to have contrasting effects on macrophage proliferation in type 2 contexts in the two anatomical sites analyzed. In the peritoneal cavity, CD40L appears to be relevant only in the presence of a specific adaptive response and to act as a negative regulator of type 2-associated macrophage responses. On the other hand, CD40L appears to be necessary for Kupffer cell proliferation in type 2 settings, both in the absence and presence an ongoing adaptive response.

291. (427) **EFFECT OF ENVIRONMENTAL PARTICLES PRODUCED BY FOREST FIRES OCCURRING IN THE DELTA WETLANDS IN FRONT TO ROSARIO CITY ON THE THP1-DERIVED MACROPHAGES RESPONSE**

Diab, Magdalena[1]; Díaz, Ariana[1,2,5]; Gabellini, R[4,5]; Pochettino, A[4,5]; D'Attilio, Luciano[1,2], Bay, María L[1,2]; Bottasso, O[1,2]; Bongiovanni, Bettina[1,2,3,5]

[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER CONICET-UNR), Rosario, ARGENTINA. [2]Facultad de Cs Médicas, [3]Facultad de Cs Bioquímicas y Farmacéuticas, [4]Facultad de Ciencias Exactas, Ingeniería y Agrimensura; [5]Plataforma Ambiental, UNR. Rosario, ARGENTINA.

Wetlands are flood-prone areas, and their soil can remain covered by water for considerable time periods. Their fauna and flora are adapted to these particular the wetlands of the Paraná River Delta have increased, reaching extremely high levels of smoke with toxic particles and gases. These fires affect the biodiversity of the place producing high atmospheric pollution able to cause serious health damage, as it may happen to the Rosario city inhabitants. To improve our knowledge of the role of environmental particles in the innate immune response, we treated macrophages derived from the THP-1 cell line with different concentrations of pollutant particles. The latter were collected for 24 hours with equipment manufactured by Baldor S.A (17.6 lt/min flow) in Rosario. Subsequently, they were placed in RPMI 1640 and sonicated for 10 minutes to suspend the particles in the liquid. Then the particle suspension was added to the macrophage cultures at different doses for 24 hours. After that, the cultures were used to analyze their cellular viability (MTT), IL1β, IL6, and IL10 production (ELISA), and expression of IL1β, NFκB and NFκB inhibitors (α and β) transcripts by qRT-PCR. The particles collected in January (Jan), September (Sept), October (Oct), and December (Dec) 2021 decreased macrophage viability with respect to unexposed cells. Such particles also increased macrophage IL1β production, especially those from Jan and Sept, as was the case of IL-6 (Oct and Dec) and IL-10 (Jan) synthesis. The same was true when assessing IL1β expression mostly in cells treated with Jan, Sept, and Oct particles. NFκB and both NFκB inhibitor transcripts were increased in cells exposed to Jan, Sept, and Oct particles, whereas iNOS expression was inhibited by the whole set of particles. The environmental pollutants obtained during the months of high forest fires are likely to exacerbate the response developed by the macrophages.

292. (459) **PLASMA EXTRACELLULAR VESICLES DAMPEN ACUTE INFLAMMATORY RESPONSES IN NEUTROPHILS STIMULATED WITH BACTERIAL PAMPs**

Martina P. Fabiano, M. Luz Leicaj, Alan M. Adamczyk, Ignacio G. Mazzitelli, Fernando Erra Diaz, Paula S. Perez, Matías Ostrowski

INSTITUTO DE INVESTIGACIONES BIOMÉDICAS EN RETROVIRUS Y SIDA (INBIRS, UBA-CONICET)

Extracellular vesicles are heterogeneous membrane structures that mediate intercellular communication both in physio and pathological conditions. We have previously shown that healthy donors' plasma EVs (pEVs) have anti-inflammatory and pro-resolution effects on monocyte derived macrophages simultaneously treated with a PAMP. Herein we aimed at studying the pEV-mediated modulation of neutrophil activation by diverse PAMPs. We analyzed neutrophils' oxidative burst, degranulation, cytokine production and cell viability. pEVs were purified from healthy donor plasma by size-exclusion chromatography followed by centrifugation, and characterized by western blotting (WB). Neutrophils were isolated from blood from healthy donors by centrifugation on Ficoll-Paque followed by dextran sedimentation and hypotonic lysis of erythrocytes. N-Formyl-Met-Leu-Phe (fMLP)-induced oxidative burst was analyzed by determining dihydrorhodamine 123 (DHR) oxidation; neutrophils' degranulation was generated also with fMLP and assessed by CD11b and CD66b surface staining; cell viability was evaluated with Annexin V and propidium iodide. Measurements were obtained by flow cytometry (FC). Cytokines in cell culture supernatants were evaluated by ELISA. We observed that pEV treatment induced a dose-dependent reduction of both oxidative burst and degranulation of neutrophils following fMLP stimulation. However, IL-8 concentrations were higher in simultaneously LPS and pEVs treated neutrophils. Neutrophils stimulated with LPS 150 ng/mL and pEVs for 18 hs showed similar live cells percentages than only LPS treated ones. In conclusion, pEVs may contribute to controlling inflammation by diminishing neutrophils' respiratory burst and degranulation while contributing to wound healing by promoting the secretion of the angiogenic cytokine IL-8.

293. (460) **CD40L+ EXTRACELLULAR VESICLES FROM EFFECTOR CD4+ T LYMPHOCYTES HAVE A PRO-INFLAMMATORY EFFECT ON MACROPHAGES**

Paula S. Pérez, Alan M. Adamczyk, María Luz Leicaj, Martina P. Fabiano, Matías Ostrowski

INSTITUTO DE INVESTIGACIONES BIOMÉDICAS EN RETROVIRUS Y SIDA (INBIRS, UBA-CONICET)

Extracellular vesicles (EVs) are membrane-surrounded structures released by all cells which mediate intercellular communication. In T lymphocytes, antigen encounter dramatically stimulates secretion of EVs, which in turn interact with diverse cell types to mediate different effector functions, such as promotion of resting T cells proliferation. However, their interaction with innate immune cells has not been thoroughly explored. Macrophages are key players driving inflammation both in acute and chronic conditions. T helper cells (CD4+) play a crucial role in modulating macrophage activation. Thus, we aimed to study the interplay between CD4+ T cell EVs and macrophages. Primary CD4+ T cells cultured in EV-depleted medium were activated with CD3/CD2/CD28 antibodies-coated beads. After 48 h, EVs were purified by differential ultracentrifugation and used to stimulate monocyte derived macrophages (MDMs) for 24 h. EVs secreted by activated CD4+ T cells induced a dose-dependent pro-inflammatory phenotype in exposed MDMs, characterized by high levels of IL-6 and TNF secretion, as measured by ELISA. EV-activated macrophages also presented high expression of PDL-1, analyzed by flow cytometry. Proteomics analysis on CD4+T-cell EVs revealed several proteins that could be potentially involved in the interaction with leukocytes. Among these, CD40L was chosen for further analysis. CD40L is upregulated in CD4+T lymphocytes upon activation and remains at high levels after 48 h. CD40L was shown to be enriched in activated CD4+T cell EVs by western blot. Strikingly, inhibition of CD40L/CD40 interaction using blocking antibodies diminished IL-6 secretion in EV-stimulated MDMs. In conclusion, EVs released by activated CD4+ T cells promote macrophage activation and pro-inflammatory cytokine release. This effect is mediated, at least partially, by CD40L present on EVs. We propose that

this type of intercellular communication is relevant in the coordination of adaptive and innate responses.

294. (474) **INDUCTION OF A REGULATORY ENVIRONMENT DURING INFECTION BY LARVAL *ECHINOCOCCUS GRANULOSUS* CAN BE MIMICKED BY REPEATED INJECTION OF PARTICLES FROM ITS ACELLULAR LARVAL COAT**

Leticia Grezzi[1], Álvaro Díaz[1] and Cecilia Casaravilla[2]
[1] Laboratorio de Inmunología, DepBio, Facultad de Química, Instituto de Higiene, UdelaR, Uruguay
[2] Laboratorio de Inmunología, IQB, Facultad de Ciencias, Instituto de Higiene, UdelaR, Uruguay

The larval stage of the parasite *Echinococcus granulosus* (hydatid) exerts an extreme control of the host immune system. The hydatid is protected by an acellular layer called laminated layer (LL), which is the main parasite structure exposed to the host immune system. This points the LL as a candidate to contribute to the immunsupression imparted by the parasite. We are interested in studying the interaction of this parasite with macrophages (Mϕ). We use a model of secondary infection injecting protoscolex in the peritoneal cavity of C57BL/6 mouse (or saline as control). After chronic infection establishment (6 months), we analyze cells (PEC) by flow cytometry and soluble mediators in the cavity by ELISA. We observed that local Mϕadopt an M2-like phenotype, with high levels of Ym-1 and Relm-α, and an increase in the expression of PD-L1. We also looked to T cell compartment and observed an expansion on Tregs (FoxP3+) and that effector T cells increased the expression of PD-1. The analysis of lavage supernatants by ELISA showed the presence of TGF-β and IL-1Ra (in absence of a myriad of other proinflammatory cytokines studied). These results indicate a local environment under immunosuppression. In parallel, based on the fact that during chronic infection the immune system is continuously exposed to particles shed from LL, we also optimized a model of intraperitoneal injections of LL particles (5 or 10 injections, twice a week; or saline in control groups). After these injections, we observed similar effects to those observed during chronic infection, indicating that LL is strongly contributing to those effects. Finally, we want to evaluate the role these M2-like Mϕ play on the local T cells. Till now, we cultured PECs in absence or presence of anti-CD3 and observed that T CD4+ cells from infected (or LL injected) mice proliferate less than T cells from control animals. In the future, we want to decipher the contribution of the PD-1/PD-L1 axis in the observed effects.

295. (494) **HIGH SALT BUT NOT HYPEROSMOLARITY INDUCES A DELAYED ACTIVATION PATTERN IN HUMAN NEUTROPHILS**

Ignacio Mazzitelli[1], Lucia Bleichmar[1], Fernando Erra Diaz[1], Claudia Melucci[1], Jorge Geffner[1]
[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS).

Elevated concentrations of salt (NaCl) are present in a number of tissues under physiological and pathological conditions. We have previously reported a paradoxical time-dependant effect of high NaCl (50 mM) on neutrophil function: an early paralysis followed by a delayed stimulation of neutrophil responses, such as IL-8 and ROS production. Here, we analyzed whether the effect induced by NaCl on neutrophil function was related to a direct action exerted by NaCl or to osmotic stress. Neutrophils were isolated from human blood by conventional procedures (purity >95%). Increasing osmolarity of the culture medium by the addition of sorbitol or mannitol (100 mM) was completely unable to stimulate neutrophil responses, suggesting that high sodium but not an increased osmolarity mediates the activation on neutrophil responses: production of IL-8 at 8 h of culture was 0±0, 4102±384, 0±0 and 0±0 (pg/ml), for neutrophils cultured in control medium or in the presence of medium supplemented with NaCl (50 mM), manitol (100 mM), or sorbitol (100 mM) ($p<0.001$ for NaCl 50 mM vs other conditions, N=3). Similarly, production of ROS evaluated by DHR oxidation and flow cytometry revealed that high NaCl, but neither sorbitol nor manitol, increased ROS production (not shown). By contrast, the early (< 2 hs) inhibition of IL-8 production mediated by LPS stimulation was inhibited more than 95% (n=6) by NaCl (50 mM), manitol or sorbitol (100 mM). Surprisingly, early ROS production induced by fMLP was unaffected by sorbitol and mannitol, but strongly inhibited by NaCl 50 mM (% inhibition > 50, n=8). Interestingly we also observed that while phagocytosis of unopsonized-C. albicans was significantly inhibited by high salt (>72%, $p<0.01$, n=4), no inhibition was observed when opsonized yeast were used (not shown). Our results shed light about the complex mechanisms through which high salt modulates neutrophil function.

296. (500) ***ECHINOCOCCUS GRANULOSUS* ANTIGEN B MODULATES LPS-DRIVEN DENDRITIC CELL ACTIVATION**

Sofía Lagos[1], Ana Maite Folle[1], Martín Fló[2], Otto Pristch[2], Jeremías Dutto[3], Mariana Maccioni[3], Ana María Ferreira[1].
[1] Laboratorio de Inmunología, Unidad Asociada al Instituto de Química Biológica de Facultad de Ciencias, Universidad de la República. [2] Laboratorio de Inmunovirología, Instituto Pasteur Montevideo. [3] Centro de Investigaciones en Bioquímica Clínica e Inmunología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

The larvae of Echinococcus granulosus s.l. (hydatid) establishes and grows in the liver of the intermediate hosts (mainly domestic ungulates, but also humans). Antigen B (EgAgB) is the major hydatid lipoprotein which size and biochemical composition are similar to that of the plasma HDL_3. Its apolipoproteins are encoded by five genes (EgAgB1-5) that belong to the cestode specific family of hydrophobic ligands binding proteins (HLBP). EgAgB was described as a parasite immunomodulator based on its ability to interfere with dendritic cell (DC) activation in vitro*; however,* these studies used denatured EgAgB preparations. In this work, we found that immunopurified EgAgB (native and the recombinant EgAgB1 protein expressed in insect cells) showed a dual dose-dependent effect on DC activation: per se proinflammatory as well as modulatory on LPS-driven DC activation (inhibiting IL6, IL12 and IFNβ secretion). Since EgAgB could compete with LPS for TLR4 binding, leading to a poor TLR4 activation, we explored TLR4 involvement in EgAgB-induced cytokine secretion using the specific inhibitor TAK-242 and TLR4-/- and TLR2-/- bone marrow derived DC (BMDC). We found that TLR4, but not TLR2, was involved in EgAgB-induced cytokine secretion (IL6, IL12, IL1β). Interestingly, EgAgB did not induce TLR4 dimerization, although it inhibited LPS-induced TLR4 dimerization and CD14 membrane expression. On the other hand, in a mixed lymphocyte reaction assay, we observed that EgAgB could influence T cell activation driven by LPS-stimulated BMDC, increasing IL4 and IL5 meanwhile decreasing TNFα secretion. Overall, results suggest that EgAgB might interfere with TLR4-dependent LPS activation of DC, contributing to skew T cell activation towards a Th2 profile. The molecular interactions underlying EgAgB modulation might include LPS binding and neutralization, resembling the physiological mechanism linked to HDL_3-mediated inactivation of LPS in the liver.

297. (515) **SEMEN EXTRACELLULAR VESICLES INHIBIT ZIKA VIRUS INFECTION ON DENDRITIC CELLS**

Paletta, Ana (1); Di Diego García, Facundo (1); Cabrerizo, Gonzalo (1); Mazzitelli, Bianca (1); Pérez, Paula (1); Geffner, Jorge (1); Remes Lenicov, Federico (1); Ceballos, Ana (1)
1. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires (UBA)-CONICET, Buenos Aires, Argentina.

It has been reported sexual transmission of Zika virus (ZIKV). Infectious virus persists in semen after symptoms onset at higher viral loads. Seminal plasma contains trillions of extracellular vesicles that mediate intercellular signaling. Knowing that semen is not merely a carrier for sexually transmitted diseases; our aim was to evaluate the role of semen extracellular vesicles (SEV) on ZIKV infection of dendritic cells (DCs). SEV were purified from healthy donors using size exclusion chromatography, and then analyzed by western blot, electron microscopy and proteomics. Monocytes were purified from peripheral blood and differentiated to DCs with GM-CSF and IL4 for 5 days. ZIKV infection was measured by UFP, qPCR, flow cytometry,

and epifluorescence microscopy. Phenotype was analyzed by flow cytometry and the cytokine production was measured by ELISA. Initially, DCs (2*10^5) were infected with ZIKV (MOI=0.1) in presence or absence of or SEV (200ug/ml) for 72hs. We first analyzed DCs phenotype and measured cytokine production. Neither SEV nor ZIKV altered IL1β, IL6. IL10 or IL12 production (n=5-8). Expression of CD83, HLA-DR, CD86, DC-SIGN or CD40 was not modified by SEV nor ZIKV. Purified SEV inhibited ZIKV infection ($p<0.001$, n=10). When SEV was added to the culture 1hs after infection there was also a reduction on the inhibition. The binding of ZIKV to cells was not affected by SEV (n=9). Interestingly, the infection of DCs in the presence of SEV increased the expression of IFN-β mRNA ($p<0.05$, n=7), OAS, MX1 and IRF7 ($p<0.05$, n=5). However, inhibiting the interferon response using an IFNar blocking antibody didn´t revert the antiviral effect of SEV (n=3). Proteomic analysis of SEV showed that SEV carried IFITM1 and IFITM3, this observation was confirmed by western blot. SEV inhibit ZIKV infection on DCs. We hypothesize that IFITM 1 and IFITM 3 might be involved since there are previous reports showing that these proteins are capable of inhibit ZIKV infection.

298. (542) **DOES THE LECTIN RECEPTOR CLEC4F HAVE A ROLE IN INTERCELLULAR ADHESION?**

Camila Mouhape[1], Anabella A. Barrios[1], Mariana Suárez-Martíns[1], Cecilia Casaravilla[1], Stephen J. Jenkins[2], Álvaro Díaz[1].
1. Área Inmunología, DepBio, Facultad de Química and Cátedra de Inmunología, IQB, Facultad de Ciencias; Universidad de la República, Uruguay.
2. University of Edinburgh Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, UK

Kupffer cells (KCs) are the sessile macrophages of the liver that are exposed to the vascular space, making contact with liver sinusoidal endothelial cells (LSECs). The analysis of KCs by flow cytometry requires processing of the liver including digestion and differential centrifugation steps, so as to obtain suspensions of non-parenchymal cells including KCs themselves and LSECs. Flow cytometric analysis of such preparations reveals events that share KCs and LSECs markers, suggesting that these two cell types are tightly adhered in vivo and remnants of one cell type contaminate the other after processing (*J Leukoc Biol* 104:579). Clec4F is a trimeric cell surface C-type lectin receptor, which in rodents is expressed only in KCs. Its only documented function so far is a participation in the uptake of de-sialylated platelets by KC. Clec4F gene-deficient (KO) mice have normal numbers of KC by liver histology (*Cell Death Different* 28:3009). However, we observed that after processing the liver for flow-cytometry of the non-parenchymal fraction (using liberase™ and DNAse for digestion), we recover significantly (by non-parametric two-way statistics) higher numbers of KCs negative for the LSEC marker CD31 from Clec4F KO than from WT mice. We made a similar observation for LSECs without KC markers (F4/80, CD11b, CD45). In contrast, with found no differences between Clec4F KO and WT mice in the recovery of the cells that contain both KC and LSECs markers. This suggests that the KC-LSEC adhesion is looser in Clec4F KO than in WT mice. If Clec4F participates directly in KC adhesion to LSECs, then LSECs should have Clec4F ligands. Flow cytometry assays using the soluble recombinant Clec4F lectin domain (expressed as a fusion with the Fc fragment of human IgG1) revealed the presence of Clec4F ligands in LSECs, as well as in KC themselves. We are currently seeking more direct evidence that the Clec4F receptor is involved in the intercellular interaction between KCs and LSECs.

299. (564) **KETAMINE AND RAPAMICYN DAMPEN THE UP-REGULATION OF IDO IN PRO-INFLAMMATORY MACROPHAGES**

Angeles R. Arena1, Cinthia M. Olexen1, Juan M. Ortiz Wilczyñski2, Paula Oneto2, Eugenio A. Carrera Silva2, Federico M. Daray1, Andrea E. Errasti1
1. Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires and 2. IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina

In the last decade, evidence shows that increased pro-inflammatory IL-6, IL-12, IL-1β, TNF-α and IFN-γ cytokines as well as activated circulating monocytes play a significant role in the initiation and maintenance of mood disorder (MD). The increased inflammation alters the tryptophan metabolism toward the kynurenine pathway, which is mediated by the IDO enzyme leading to the production of neurotoxic quinolinic acid (NMDA agonist). Sub-anesthetic doses of the NMDA antagonist, ketamine, has demonstrated a rapid and sustained antidepressant effect acting not only in CNS but also as anti-inflammatory signal in the periphery and increasing neuroprotective kynurenic acid serum level. We previously showed that patients with MD have increased activated monocytes in the blood, and that ketamine skew monocytes to an anti-inflammatory M2 macrophages. Here, we aim to characterize the effect of ketamine and rapamycin on the IDO pathway in polarized macrophages. Circulating monocytes were isolated and differentiated into non-polarized (M0) and polarized M1 (IFN-γ plus LPS), M2a (IL-4) and M2c (IL-10 or dexamethasone) macrophages after 5 days of culture. The expression of IDO, and kynurenic and quinolinic acid pathway enzymes, as well as TNF-α, IL-1β, CD163, and CD206, were evaluated in the presence of ketamine (10 μM) and/or rapamycin (1 nM) by qPCR. Pro-inflammatory M1 macrophages, induced by IFN-γ plus LPS, strongly up-regulates the IDO expression (105 fold, N=12) compared to acutely isolated monocytes and M0, M2a and M2c macrophages. The treatment of pro-inflammatory M1 macrophages with ketamine and rapamycin in combination significantly reduced ($p<0,05$, Mann Whitney test) the induction of IDO as well as IL-1β and TNF-α. Altogether, these results suggest that the treatment with both ketamine and rapamycin dampens the IDO levels of pro-inflammatory M1 macrophages to balance the tryptophan metabolism toward the neuroprotective pathway.

300. (568) **A POTENTIAL METABOLIC AND IMMUNOREGULATORY ROLE OF ANTIGEN B LIPOPROTEIN IN *ECHINOCOCCUS GRANULOSUS* BIOLOGY**

Ana Maite Folle[1], Sofía Lagos[1], Martín Fló[2], Federico Carrión[2], David Santos[3], Josep Julve[3], Otto Pritsch[2], Ana María Ferreira[1]
1. Área Inmunología, Facultad de Química/Facultad de Ciencias, Universidad de la República - Montevideo, Uruguay
2. Laboratorio de Inmunovirología, Instituto Pasteur de Montevideo - Montevideo, Uruguay
3. Laboratorio del Grupo de Bases Metabólicas del Riesgo Cardiovascular, Instituto de Recerca de l'Hospital de la Santa Creu i Sant Pau - Barcelona, España

The parasite *Echinococcus granulosus* causes cystic echinococcosis, a chronic infection that implies a strong control of host immunity. Moreover, its location in a medium rich in nutrients shaped parasite's metabolism, loosing *de novo* fatty acid and cholesterol synthesis pathways, together with the expression of proteins capable of capturing and transporting essential lipids. Antigen B (EgAgB) is a lipoprotein member of the hydrophobic ligand binding protein family (HLBP), specific of cestodes, with important diagnostic value. EgAgB effects were studied on innate immune cells, employing immunopurified EgAgB preparations: the native lipoprotein and the recombinant rEgAgB8/1 (the main apolipoprotein in native EgAgB, expressed in insect cells). This work provides the first evidence of EgAgB ability to exchange lipids with host cells, by discharging cholesterol from human and murine macrophages similarly to HDL. In addition, EgAgB inhibited macrophage activation induced by LPS *in vitro*, decreasing IL1β, IL6, IL12p40 and •NO. *In vivo* EgAgB reduced the LPS-induced IL6 secretion, together with an IL10 potentiation, at 4 h post-injection in the peritoneal cavity of BALB/c mice. Interestingly, EgAgB itself showed a pro-inflammatory effect, leading to peritoneal neutrophil, eosinophil and monocyte recruitment at 4 and/or 24 h post-stimulation. However, myeloid peritoneal cells showed a poorly activated phenotype (MHC-II, CD86, CD40) in comparison with LPS. Moreover, EgAgB decreased *in vivo* LPS-induced activation of resident macrophages (MHC-II for LPM; CD40 and CD86 for SPM), dendritic cells (CD86) and recruited monocytes (CD86). On the other hand, EgAgB and LPS exhibited *in vitro* as well as *in vivo*

mutual interference in cell recognition and/or effects, suggesting the involvement of a common cellular receptor and/or EgAgB ability to scavenge LPS. Taken together, our results support a potential metabolic and immunoregulatory role of EgAgB in *E. granulosus* biology.

301. (616) **IMPACT OF EXTRACELLULAR VESICLES RELEASED BY HUMAN NEUTROPHILS TREATED WITH SHIGA TOXIN ON EPITHELIAL AND ENDOTHELIAL RENAL CELLS.**

Shiromizu, Carolina Maiumi(1), Keitelman, Irene Angélica(1), Gomez, Fernando Daniel(3), Vereertbrugghen, Alexia(1), Vera Aguilar, Douglas(1), Perez Paula S(4), Rosato, Micaela(1), Ramos, María Victoria(1), Jancic, Carolina(1,2), Fuentes, Federico(1), Galletti, Jeremías(1), Amaral, María Marta(3), Palermo, Marina(1), Sabbione, Florencia(1,*), Trevani, Analía(1,2, *).

1 IMEX-CONICET-Academia Nacional de Medicina;
2 Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires;
3 Laboratorio de fisiopatogénesis, Departamento de fisiología, IFIBIO Houssay-CONICET, Facultad de Medicina, Universidad de Buenos Aires;
4 INBIRS, Facultad de Medicina, UBA-CONICET.
** Ambas autoras contribuyeron por igual*

Shiga toxin (Stx) *producing Escherichia coli* (STEC) is a non-invasive pathogen that colonises the intestine where it releases the Stx which can reach the blood stream and lead to the Haemolytic Uremic Syndrome (HUS). In HUS neutrophilia is a typical sign and a poor prognosis factor. Previous studies suggest that Stx is transported to target organs like kidneys, in extracellular vesicles (EV) generated by blood cells. The aim of this study was to determine if neutrophils (N) produce EV in response to Stx (EV-Stx) and their impact on the viability and cytokine production by primary human glomerular endothelial cells (HGEC) and renal epithelial cells (HK-2 cells). Human N (10^6) isolated from peripheral blood were treated with purified Stx2 (100 ng/ml), heat-inactivated Stx2 (StxØ) or vehicle (C) for 4 h and EV released were isolated by differential centrifugation. By both confocal laser scanning microscopy (n=4) and detection of CD63 expression by western blot (n=6), we determined that N release EV in all the conditions studied. By employing the VERO cell line susceptible to Stx, we observed that EV-Stx but not EV-StxØ or EV-C significantly reduced cell viability (n=10; $p<0.05$) demonstrating that the EV-Stx carry Stx. Treatment of EV-Stx with Proteinase K prior to the addition to VERO cells did not reduce the cell mortality (n=5; $p<0.05$), suggesting that the Stx is transported inside the EV. We also co-incubated the EV for 16 h with either HGEC or HK-2 to determine their biological impact. Only EV-Stx promoted HK-2 death ($p<0.05$; n=4) evaluated by LDH release, and reduced IL-6 and IL-8 secretion by both cell types (HGEC: n=5; $p<0.05$; HK: n=4; $p<0.05$) measured by ELISA in culture supernatants. EV-Stx also appeared to stimulate the adhesion of neutrophils to HGEC (n=2). These results show that N can release EV-Stx that might contribute to the Stx-systemic dissemination and to renal tissue injury in the HUS.

302. (651) **EFFECT OF FUNGAL PROTEIN EXTRACTS ON INNATE IMMUNE CELLS**

María Eugenia Díaz[1,2], Sofía Díaz[1], Exequiel Giorgi[1,2], Sofía Genovés[1], Mauricio De Marzi[1,2]

[1] Universidad Nacional de Luján, Departamento de Ciencias Básicas, Buenos Aires, Argentina [2] INEDES (Universidad Nacional de Luján - CONICET), Buenos Aires, Argentina

Fungal bioactive compounds have a great potential to be used in the medical field. In particular, different fungal proteins with immunomodulatory activity have been described. The aim of this work was to obtain enriched protein extracts from fruiting bodies of *Pleurotus ostreatus (Po)* and *Agaricus bisporus (Ab)* in order to evaluate their effect on RAW 264.7 cells. To achieve this goal, fruiting bodies obtained from the local market were lyophilized and homogenized with Tris-Glycine buffer pH 8.4. Homogenates were centrifuged and the supernatants were treated with ice cold ethanol to precipitate soluble proteins. The precipitates were resuspended in phosphate buffer 50 mM, pH 7. Protein concentration was measured by Bradford method and a SDS-PAGE was performed to obtain the protein band profile. An MTT assay was carried out to assess cellular metabolic activity. RAW 264.7 cells (100.000 cells/well) were seeded in 24-well culture plates and allowed to attach for 24 h. Culture medium was replaced with DMEM containing different protein concentration of *Ab* or *Po* extracts, and cells were incubated at 37 °C for an additional 24 h. At the end of incubation, supernatants were collected and stored at -20°C, and cells were washed with PBS and incubated at 37 ºC for 30 min in the presence of MTT. Purple formazan crystals were solubilized with etanol and absorbance was measure. For nitrites quantification, Griess assay was performed using the previously collected supernatants. The results showed that both extracts diminished metabolic activity of RAW 264.7 in a dose-dependent manner. In the case of *Po* extract, IC50 was less than 0,025 mg/ml, while with *Ab* extract the IC50 was 0,078 mg/ml. Regarding nitrites concentration, none of the treatments showed differences with the control. The results obtained showed that the fungal extracts used have an impact on RAW 264.7 cells, but more studies are needed in order to completely understand their effect on these cells.

303. (653) **MURINE PERITONEAL MACROPHAGES SURFACE N-GLYCANS REGULATE RESPIRATORY BURST THROUGH ERK 1/2 AND P38 MAPKs PATHWAY**

Juan Agustín Garay[1], Juan Eduardo Silva[1,2], Brenda Jofré[1,2], María Silvia Di Genaro[1,2], Roberto Carlos Davicino[1,2]

[1]Universidad Nacional de San Luis, Argentina. [2] Instituto Multidisciplinario de Investigaciones Biológicas de San Luis, CONICET, Argentina.

Recent studies have explored the role of surface N-glycans in neutrophil effector functions, however there is little information regarding macrophages (MΦ). Thus, in the present work, murine peritoneal MΦ treated with PNGase F were used in order to study N-glycans role on respiratory burst. MΦ were obtained through peritoneal lavage with saline solution and adjusted at $1x10^6$ cells/ml, then purified by incubation for 2 h at 37°C in DMEM medium supplemented with 10% fetal bovine serum (FBS) in an atmosphere with 5% CO_2. Then, MΦ were incubated with 5 IU of PNGase F in DMEM medium without FBS for 4 h, then, washed and cultured overnight in supplemented DMEM medium. After deglycosylation, NBT assay was performed and Cd^{2+} (NADPH oxidase inhibitor), malonate, or barbital (mitochondrial complex II and I inhibitors, respectively) were used. Flow cytometry analysis was performed with the DHR-PMA assay in presence and absence of ERK1/2, p38, or JNK inhibitors. The expression of CD115, CD11b, F4/80 and Ly6C was also examined. Finally, we evaluated phagocytosis using *Yersinia enterocolitica* (Ye)-GFP assay. We have found that N-deglycosylation increases the respiratory burst according to NBT test ($p<0.05$) and that O_2^- has a phagosomal origin ($p<0.01$). Consistently, the DHR-PMA assay revealed an increased respiratory burst in N-deglycosylated MΦ (MΦd) even in the absence of the PMA stimulator ($p<0.05$). Inhibition of ERK 1/2 and p38 on MΦ induced a respiratory burst level comparable to MΦd, and no significant difference between MΦd and MΦd treated with ERK 1/2 and p38 inhibitors was found. Additionally, no significant differences were observed in Ye-GFP phagocytosis assay. Finally, MΦd showed an increased CD11b as well as a decreased CD115 expression. Our findings suggest a critical role of surface n-glycans in the regulation of macrophage function, however further studies are required to identify the specific glycan motifs involved in this process.

304. (658) **CROSS-REGULATION BETWEEN SERINE PROTEASES AND CASPASE-1 CONTROL PRO-IL-1 BETA PROCESSING BY HUMAN NEUTROPHILS**

Irene A. Keitelman[1], Carolina M. Shiromizu[1], Douglas Vera Aguilar[1], Manuela Pizzano[1], Alexia Vereertbrugghen[1], Jeremías G. Galletti[1], Florencia Sabbione[1] and Analía S. Trevani[1,2]

[1] Instituto de Medicina Experimental (IMEX) – CONICET, Academia Nacional de Medicina
[2] Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires.

Neutrophils (N) contribute to the inflammatory response by releasing Interleukin-1 beta (IL-1β), a cytokine that is synthesized in the cytosol as a precursor (pro-IL-1β) that is usually processed to an active isoform by caspase-1 (C1). We previously determined that human N secrete IL-1β and that both C1 and the neutrophil serine protease (NSP) are necessary for IL-1β secretion. Other studies showed that C1 allows the NSP elastase to exit azurophil granules to the cytosol. To evaluate the contribution of each of these enzymes to pro-IL-1β processing, we stimulated purified human N with LPS and 2 h later with ATP (inflammasome activator). Before or after the addition of ATP, we treated them or not with AEBSF (NSP inhibitor), VX765 (C1/-4 inhibitor) or both inhibitors together at different time points. After 5 h we determined pro-IL-1β processing by evaluating the total level (intra+extra-cellular) of mature IL-1β by ELISA. The addition of VX765 until 10 min post ATP stimulation led to a marked IL-1β processing reduction (60% $p<0.05$ vs. LPS+ATP, n=5) but this effect was lower when it was added at a later time point (30 min, $p<0.05$). Adding a NSP inhibitor at any time point post ATP also reduced pro-IL-1β processing (≅35%, $p<0.05$). Noteworthy, AEBSF alone or together with VX765 reduced pro-IL-1b processing to the same extent when added at 30 min post ATP. To test if NSP could inactivate C1, we employed the FLICA probe to detect active C1 by flow cytometry. We observed a reduction in active C1 in LPS+ATP stimulated N after 10 min of inflammasome activation ($p<0.05$, n=4). However, this reduction was abolished in the presence of AEBSF. Furthermore, experiments with FLICA added with or without AEBSF for 5 min at different time points after ATP, supported that NSP contribute to inactivate C1 (n=4). Altogether, our findings suggest that both C1 and NSP can process pro-IL-1β, however the contribution of C1 to this processing promptly fades after inflammasome activation.

305. (662) **EFFECT OF RIFAMPICIN AND ADRENAL STEROIDS ON HUMAN MACROPHAGES STIMULATED WITH *Mycobacterium tuberculosis***

Díaz Ariana[1,2], Imhoff Matilde[1,2], Massa Estefanía[1,3], Gallucci Georgina[1], Diab Magdalena[1], Bongiovanni Bettina[1,3], Derio Marisa[1], D'Attilio Luciano[1,2], Bay María Luisa[1,2].
[1]IDICER-CONICET-UNR, [2]Facultad de Ciencias Médicas-UNR, [3]Facultad de Ciencias Bioquímicas y Farmacéuticas-UNR.

Tuberculosis (TB) is a major health problem worldwide. The etiologic agent, *Mycobacterium tuberculosis* (Mtb) is transmitted by air and captured by lung macrophages (Mf). Mf activation along with an efficient cellular immune response (IR) are required for Mtb elimination, which at the same time can mediate tissue damage. We early found that newly diagnosed TB patients showed an immune-endocrine imbalance: high plasma levels of pro- and anti-inflammatory mediators and cortisol (Gc), as well as lowered Dehydroepiandrosterone (DHEA) levels. During specific anti-TB treatment, the proinflammatory mediators and DHEA levels reached values like those found in healthy controls. Rifampicin (R) is a potent antimicrobial agent and a major drug in TB treatment. There is evidence that R also modulates the host IR, influencing lymphocyte migration, cytokine production, and phagocytosis. Accordingly, we analyzed the effect of R (15 μg/ml) on the proinflammatory response of Mf stimulated with Mtbi (irradiated Mtb), added or not with DHEA (10^{-7}M) and/or cortisol (10^{-6}M). All Mf cultures (adherent human THP-1 cells, activated with 30 ng/ml of phorbol-12-myristate-13-acetate -PMA- for 24hs) were stimulated and treated for 24 hrs. Culture supernatants of Mtbi-stimulated Mf had increased contents of IL-1β and IL-10, compared to non-stimulated Mf ($p<0.05$). When R was added to Mtbi-cultures, there was a decrease in IL-1β with respect to their single stimulated counterparts ($p<0.05$), as was in Mf stimulated and treated with R+DHEA ($p<0.05$). The addition of Gc to stimulated Mf decreased IL-1β levels (vs. Mtbi $p<0.01$), and even more in cultures treated with R+Gc (vs. Gc+Mtbi, $p<0.01$). Concerning IL-10 levels, they were decreased in cultures stimulated and treated with R+DHEA and R+Gc when compared with single-stimulated cultures ($p<0.05$). Besides its antibacterial role, Rifampicin is likely to also contribute to the anti-inflammatory effect of cortisol.

306. (666) **SKIN RESIDENT CD4$^-$ CD8$^-$ (DOUBLE-NEGATIVE) αβT CELLS PRODUCE IL-17A DURING *NANNIZZIA GYPSEA* DERMATOPHYTIC INFECTION**

Beccacece Ignacio*, Burstein Verónica*, Almeida Mariel, Guasconi Lorena, Mena Cristian, Chiapello Laura
Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.
**These authors contributed equally*

Dermatophytosis is a highly prevalent superficial fungal disease that affects keratinized tissues such as the skin, hair and nails. We have previously reported that IL-17A produced by skin resident γδT and αβT cells is important in the defense against dermatophytes. Furthermore, the single depletion of γδT cells does not lead to increased susceptibility since IL-17A production by epidermal cells is not abolished. In this study we aim to further characterize the phenotype, origin, and function of the βTCR$^+$ IL-17A-producing cells in the skin. IL-17A-GFP-reporter and CD8 KO mice were epicutaneously infected with *Nannizzia gypsea* in the back. To study resident T cells, a daily dose of Fingolimod (FTY-720, 2μg/g) was intraperitoneally injected starting 24 h prior to infection. On day 6 post-infection, back skin was removed and treated with Trypsin/EDTA (2 h/37°C) and epidermal cell suspensions were used for FACS analysis and fungal burden determination. At 6 days post-infection, FACS analysis revealed that IL-17A$^+$ βTCR$^+$ cells were mostly made up of CD4$^-$CD8$^-$ double-negative (DN) cells. This DN population was not affected by FTY-720 treatment suggesting that this subset is maintained in the skin independently of the influx of T lymphocytes from draining lymph nodes. Previous reports have shown that DN T cells can have a thymic origin or derive from peripheral CD8 T cells by down-regulation of the CD8 receptor. After *N. gypsea* infection, CD8 KO mice exhibited a decreased IL-17A production by skin T cells ($P= 0.0358$) when compared to WT infected mice. However, DN cells were still present suggesting that they do not arise from CD8 T cells during dermatophytosis. Taken together, these data show that skin resident IL-17A-producing βTCR$^+$ T cells are mostly double-negative for CD4 and CD8 expression. Despite the increased susceptibility of CD8 KO mice to infection, these DN cells do not derive from CD8 T cells so further studies are needed to determine their origin and function.

307. (669) **CONDITIONAL DEPLETION OF CD11C-EXPRESSING CELLS GREATLY COMPROMISES INNATE RESISTANCE TO SKIN FUNGAL INFECTION**

Beccacece Ignacio*, Almeida Mariel*, Burstein Verónica, Guasconi Lorena, Mena Cristian, Chiapello Laura
Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.
**These authors contributed equally*

The skin is a physical barrier that protects the body against infections owing to several immune cell populations including two major subsets of CD11c$^+$ myeloid cells: Langerhans (LC) and dermal dendritic cells (DC). CARD9 expression (an adaptor molecule downstream pattern recognition receptors) in dendritic cells plays a central role in antifungal immunity, however, the tissue-specific innate immunity remains poorly understood in dermatophytosis. In this study, we aim to investigate the *in vivo* role of skin CD11c$^+$ cells in the susceptibility to experimental dermatophytic infection. C57BL/6 (WT), CD11c-DTR-GFP, Lang-eGFP-DTR or IL-17RA KO mice were epicutaneously infected in the back with *Nannizzia gypsea*. To deplete CD11c$^+$ cells or LC, CD11c-DTR-GFP or Lang-eGFP-DTR mice (respectively), received a single dose of diphtheria-toxin (DT, 2 ng/g) 24 h prior to infection. On 3- and 6-days post-infection (dpi), back skin was treated with Trypsin/EDTA (2 h/37°C) and epidermal cell suspension was used to perform FACS analysis and to determine cytokines (ELISA), fungal burden and CARD9 gene expression by real time PCR. At an early time-point of infection (3 dpi), only CD11c$^+$cell-depleted mice showed a significant increase in fungal burden compared to WT, Lang-eGFP-DTR or IL-17RAKO mice ($P<0.05$). In contrast, LC-depleted mice were not susceptible to infection and IL-17RAKO mice

showed an elevated fungal burden from 6 dpi. The susceptibility of $CD11^+$cell-depleted mice correlated with a reduction in IL-23, TNF, IL-12, IFN-γ, IL-1β and IL-17A. On the other hand, PCR analysis revealed that CARD9 gene expression was exclusively detected in $CD45^+$ cells in the skin. Taking together, these data suggest a critical role of $CD11c^+$ cells, but not of the LC subset, in the innate control of dermatophytosis. Further studies are underway to link the role of dermal DC and CARD9 in early antifungal immunity to dermatophyte infection in the skin.

308. (673) **HUMAN NEUTROPHILS SECRETE IL-1B IN RESPONSE TO SHIGA TOXIN (STX)-PRODUCING ESCHERICHIA COLI IN A MECHANISM THAT DEPENDS ON INFLAMMASOME, CASPASE-1 AND OXYGEN REACTIVE SPECIES**

Florencia Sabbione[1], Carolina Maiumi Shiromizu[1], Irene Angelica Keitelman[1], Douglas Vera Aguilar[1], Alexia Vereertbrugghen[1], Manuela Pizzano[1], María Victoria Ramos[1], Carolina Jancic[1,2], Jeremías Galletti[1], Marina Palermo[1], Analía Trevani[1,2].

1 IMEX-CONICET-Academia Nacional de Medicina; Buenos Aires, Argentina.

2 Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are non-invasive bacteria that colonize the intestine causing diarrhea, hemorrhagic colitis, and Hemolytic Uremic Syndrome. This disease is triggered by Stx, that translocates to the circulation affecting organs like the kidney. Stx translocation is promoted by inflammation. As neutrophils (N) are recruited to the intestine upon STEC infections, they might contribute to the gut inflammatory response by secreting Interleukin-1β (IL-1β). We previously determined that STEC (*E. coli* O157:H7 strain) stimulates IL-1β secretion by human N (HN) by a process that involves N serine proteases (NSP). The aim of this study was to further elucidate the mechanisms that lead to IL-1β secretion. We determined that an isogenic STEC strain that does not produce Stx (ΔSTEC) also stimulates HN IL-1β secretion at a similar level than STEC does. Moreover, IL-1β response induced by ΔSTEC mimicked that induced by STEC; the secretion was higher the lower the multiplicity of infection (MOI) was, and this effect was not due to differences in HN lytic death (n=7; $p<0.05$). Also, when either the ΔSTEC supernatant was replaced by STEC supernatant or when Stx was added to ΔSTEC cultures before HN challenge, the IL-1β secretion by these cells did not differ regarding that induced by ΔSTEC (n=7). Furthermore, IL-1β secretion induced by STEC (MOI: 0.5) was markedly inhibited ($p<0.05$; n=4) by pre-treatment of neutrophils with a NADPH oxidase inhibitor (DPI); a NLRP3 inflammasome inhibitor (MCC950), a caspase-1 inhibitor (Ac-YVAD-CMK) or a pan-caspase inhibitor (ZVAD). Altogether, our results indicated that STEC promotes HN IL-1β secretion independently of its capacity to produce Stx. Our data also suggest that this secretion depends on reactive oxygen species production, and involves the NLRP3 inflammasome, caspase-1 and NSP participation. These molecules might be targets to pharmacologically reduce the inflammatory response that usually triggers the infection.

309. (685) **INVOLVEMENT OF NEUTROPHIL ELASTASE IN EFFECTS INDUCED BY SHIGA TOXIN TYPE 2 *IN VITRO* AND *IN VIVO***

Fernando Nicolás Sosa[1], Alan Mauro Bernal[1], Florencia Sabbione[2], Romina Jimena Fernández-Brando[1], Analia Silvina Trevani[2], Marina Sandra Palermo[1], Maria Victoria Ramos[1].

[1]Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, IMEX-CONICET, Academia Nacional de Medicina.

[2]Laboratorio de Inmunidad Innata, IMEX-CONICET, Academia Nacional de Medicina.

Hemolytic Uremic Syndrome (HUS), a vascular disease that results in renal failure is caused by Shiga toxin (Stx)-producing *E. coli*. Besides Stx, inflammation caused by neutrophils (PMN) is needed for HUS. PMN release Neutrophil Extracellular Traps (NETs), involved in many diseases. We showed Stx2 induces NETosis in PMN and an increase of circulating free DNA in mice. Our aim was to study if neutrophil elastase (NE), an enzyme associated to NETs, is involved in NETosis and inflammation triggered by Stx2 in PMN; and to evaluate if there is an increase in NE in a murine model of HUS as a first approach to studying its involvement in the disease. PMN were incubated with medium (Basal), Stx2 (0.1 μg/mL) alone or with an NE inhibitor, Sivelestat (Siv, 10 μM). After 4 h, DNA and NE activity were measured in supernatants by fluorescence and absorbance respectively. IL-8 secretion, an inflammatory cytokine, was measured by ELISA. For *in vivo* assays, BALB/c mice inoculated with saline (Basal), Stx2 (1 ng/kg) or Stx+DNAse (300 U) were bled at day 3. After incubation with Stx2, we found an increase in DNA levels (μg/mL) (Median(IQR)= Basal:0.12(0.09-0.16); Stx:0.27(0.22-0.43)*; Stx+Siv:0.14(0.13-0.16);*$p<0.05$; n=5), NE (Median(IQR)= Basal:1; Stx:1.74(1.60-2.05)*; Stx+Siv:0.54(0.25-1.11); *$p<0.05$; n=5), and in IL-8 (pg/mL) (Mean±SD= Basal:1; Stx:2.00±0.29*; Stx+Siv:0.75±0.14; *$p<0.05$; n=3), that were in all cases impaired by Siv. Regarding our *in vivo* results, we found elevated levels of DNA (μg/mL) (Median(IQR)= Basal:0.62(0.44-0.77); Stx:1.95(1.12-1.23)*; Stx+DNAse:0.61(0.58-0.65); *$p<0.05$; n=4) and NE (A_{450nm}) (Median(IQR)= Basal:0.84(0.48-0,95); Stx:1.43(1.00-2.96)*; Stx+DNAse:0.61(0.58-0.64); *$p<0.05$; n=4), impaired by DNAse. Our results suggest Siv blocks NETosis and inflammation induced by Stx2. Given this blockage and the DNA and NE elevations *in vivo* after Stx2 inoculation, it would be interesting to evaluate if the drug has any effects on survival and renal damage.

310. (719) **ZIKA VIRUS NS4B-C100S MUTANT PROTEIN WEAKLY BINDS TANK-BINDING KINASE AND ELICITS A STRONG ANTIVIRAL RESPONSE**

Maria Belén Sarratea[1], Daniela Redolfi[1], Laura Iannantuono Lopez[1], Sofía Noli Truant[1], Gabriela Elena Gómez[2], José Maria Delfino[2], Roy Mariuzza[3], Andrés Sánchez Alberti[4], Marisa Mariel Fernández[1], Emilio Luis Malchiodi[1].

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina; [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica-IQUIFIB (UBA-CONICET), Buenos Aires, Argentina; [3]University of Maryland, Institute for Bioscience and Biotechnology Research (IBBR), Rockville, Maryland, USA; [4]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Microbiología, Parasitología e Inmunología-IMPAM (UBA-CONICET), Buenos Aires, Argentina.

Type I interferons (IFN-I) are key mediators in antiviral innate immunity. Nucleic acid sensing in the cytoplasm triggers TANK-binding kinase 1 (TBK1)- interferon regulatory factor 3 (IRF3) signaling axis and activate IFN-I transcription. Our previous reports demonstrated that the Zika virus (ZIKV) non-structural protein NS4B inhibits IFN-β production and directly binds TBK1. An attenuated ZIKV with NS4B C100S mutation showed a potent induction of antiviral immune response in a mouse model. In this work, we evaluated the effect of NS4B C100S in the antiviral cellular response and study how it affects the interaction with host ligands. For this purpose, we performed transfection assays with plasmids encoding recombinant ZIKV NS4B or NS4B-C100S mutant. We found that Hela cells transfected with NS4B-C100S secreted higher levels of IFN-β and IL-6 upon dsRNA analog stimulation (poly(I:C), compared to those transfected with wild type NS4B (ANOVA+Tukey´s, $p<0.05$). TBK1, an essential component in IFN-I production, acts as a ligand of ZIKV NS4B, as demonstrated by immunoprecipitation. Here, we found that TBK1 also immunoprecipitated with ZIKV NS4B-C100S mutant. To further study this interaction, we performed Surface Plasmon Resonance assays (SPR). Micelles of recombinantly expressed ZIKV NS4B-C100S were injected over a surface with immobilized TBK1. SPR showed that NS4B-C100S also interacts with TBK1, but with an equilibrium dissociation constant (KD) of $1.1±0.2 \times 10^{-5}$ M, a 10 times lower affinity than TBK1-NS4Bwt binding (KD= $3.7±0.4 \times 10^{-6}$ M). Our data suggest that the NS4B-C100S mutant is associated with a stronger inflammatory response and reduced capacity to bind TBK1. The results correlate with the higher inflammatory and

antiviral response reported for the mutated virus. Altogether, these results highlight the importance of the conserved C100 residue of ZIKV NS4B for its function and viral kinetic and may provide new insights for the development of antivirals.

311. (722) ***Lacticaseibacillus rhamnosus*** **POSTBIOTIC-INDUCE BENEFICIAL EFFECTS ON EMERGENCY MYELOPOIESIS IN MYELOSUPPRESSED MICE**

Vasile B[1], Gutiérrez F[1], Ivir M[1], Alvarez S[1,2*], Salva S[1*].
[1]Laboratory of Immunobiotechnology, CERELA-CONICET. Tucuman, Argentina.
[2]Institute of Applied Biochemistry, Tucuman University. Tucuman, Argentina.

Currently, there has been a great increase in the number of immunocompromised patients for various reasons. Respiratory infections are the most frequent in patients with immunodeficiencies, especially those undergoing chemotherapy. The availability of safe immunopotentiating agents is necessary to improve immunity against respiratory pathogens. In this work we studied the effect of oral administration of *Lacticaseibacillus rhamnosus* CRL1505 (Lr) and its cell wall (CW) on the emergency myelopoiesis against *Streptococcus pneumoniae* (Sp). Adult Swiss-mice were orally treated with Lr or CW during 16 consecutive days. On day 6, treated and untreated mice received one intraperitoneal dose of cyclophosphamide (Cy-150mg/kg). On day 9, mice were infected with Sp (10^7 UFC/mice). Innate and myelopoietic responses were evaluated after the pneumococcal challenge. The Cy group showed a high susceptibility to pneumococcal infection, an impaired innate immune response in lung and a decrease of LSK cells ($Lin^-Sca1^+cKit^+$), multipotent common myeloid progenitor (CMPs, $Lin^-Sca1^+cKit^+CD34^+$), granulocyte-monocyte progenitors (GMPs, $Lin^-Sca1^-cKit^+CD34^+$), and a low expression of CXCR4 and CD62L $Gr1^+Ly6G^+Ly6C^-$ cells in bone marrow (BM). However, Lr and CW treatments were effective to significantly increase lung neutrophils and macrophages, blood neutrophils and peroxidase+ cells and allow an early myeloid precursor recovery in BM with respect to the Cy group. This, in turn, led to an early Sp lung clearance compared with the Cy group. Besides, the treatment with CW was more effective than Lr to decrease retention signals in the BM cells. In conclusion, the postbiotic obtained from *L. rhamnosus* CRL1505 induced a more efficient local innate immune response. This could be related to the modulation of chemokines responsible for stimulating an adequate emergency myelopoietic response in BM against pathogens.

312. (724) **POSTBIOTIC NASAL PRIMING IMPROVES THE INNATE IMMUNE RESPONSE IN RESPIRATORY MUCOSA-ASSOCIATED LYMPHOID TISSUE IN MALNOURISHED MICE**

Ivir M[1], Vasile B[1], Gutiérrez F[1], Alvarez Villamil E[1], Lorenzo Pisarello MJ[1], Salva S[1*], Alvarez S[1,2*].
[1]Laboratory of Immunobiotechnology, CERELA-CONICET. Tucuman, Argentina.
[2]Institute of Applied Biochemistry, Tucuman University. Tucuman, Argentina.

The nasal priming of malnourished mice with the peptidoglycan (PG) of *Lacticaseibacillus rhamnosus* CRL1505 (Lr) is as effective as viable strain for improving systemic and respiratory immune response against *Streptococcus pneumoniae* (Sp). But the impact of these treatments on mucosa-associated lymphoid tissue is unknown. Hence, the effect of nasal administration of Lr or PG on the innate immune response of nasopharynx-associated lymphoid tissue (NALT) and cervical lymph nodes (CLN) in malnourished mice under repletion treatments was evaluated. Weaned Swiss mice were malnourished with a protein-free diet (PFD) for 21d. Malnourished mice received a balanced conventional diet (BCD) during 7d (BCD group) or BCD with nasal supplementation with Lr (10^8 cells/mouse/d) or PG (8 μg/mouse/d) during the last 2d of treatment (Lr or PG groups). Malnourished control mice (MNC) received PFD while the well-nourished control group (WNC) consumed BCD. On d8, all groups were infected with Sp (10^7 cells/mouse). Before infection, MNC showed a significant decrease of the total cells, T and B lymphocytes in NALT and CLN as well as the macrophages, myeloid and dendritic cells in NALT. In addition, the MNC showed an increase of NALT Gr-1+ cells % and the microbial load in nasal washes. BCD treatment was not able to normalize these parameters. However, the Lr and PG groups improved the total cells, B and T cells counts in NALT and CLN. Challenge with Sp increased the numbers of neutrophils and macrophages and TNF-α, INF-γ, IL-6, IL17 and IL-10 levels in nasal washes. The values were lower in MNC than in WNC. However, unlike the BDC group, Lr and PG groups showed values of NALT phagocytes, and lymphocytes, T CD4+ cells in CLN and NALT similar to WNC mice. Moreover, IL-6, INF-γ and TNF-α levels in nasal washes were higher in Lr or PG groups. NALT is a target for postbiotics administration to improve respiratory immunity in immunocompromised malnourished hosts.

313. (751) **ANALYSING THE ROLE OF WNT SIGNALING IN M1 MACROPHAGE POLARIZATION**

Melisa Rocío Herrera[1,2#], Sofía Vallon Trabelsi, [1,2#], Juan Nahuel Quiroz[1,2], María Belén Brugo[1,2], Ximena Volpini[1,2], Claudia Cristina Motran[1,2]
1. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Argentina.
2. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Argentina.
 # These authors contributed equally to this work

Wnt signaling pathways, in addition to participating in key cellular processes during organogenesis, plays an important regulatory role in infectious and inflammatory processes. The expression of Wnt proteins and the activation of the Wnt/β-catenin and Wnt/Ca++ pathways are induced in macrophages (Mo) infected with *T. cruzi* through TLR signaling. Notably, when both Wnt/β-catenin pathway or the secretion of Wnt proteins are blocked using IWP-L6, the activity of arginase-1 (Arg-1) is inhibited, and Tc-infected Mo adopts an M1-like phenotype. Our hypothesis is that the activation of Wnt signaling pathways in Mo would play a critical role in defining the activation/polarization profile of these cells after activation by TLR ligands. Thus, to investigate the role of Wnt signaling pathways in Mo polarization to M1, J774 cells or bone marrow derived Mo were treated with IWP-L6 or vehicle for 24 h and then stimulated with IFN-γ plus LPS (M1 stimulus). The production of NO, IL-6, IL-12 and IL-1β was evaluated in culture supernatant and the expression of CD86, CD38 and Arg-1 determined by FACS at 48 h post stimulation. In addition, phagocytosis assay of heat-killed *Candida albicans* was assessed using light microscopy. Cells treated with IWP-L6 before the M1-polarizing stimulus showed a lower production of NO ($p<0.001$) and IL-6 ($p<0.05$) than control cells. However, they showed higher phagocytic capacity ($p<0.001$) and higher secretion of IL-12 ($p<0.01$) and IL-1β ($p<0.05$) than vehicle-treated cells. As expected, the M1 stimulus increased the percentage of cells expressing the M1 markers CD86+ CD38+ ($p<0.01$), while did not induce Arg-1 expression. Interestingly, inhibition of Wnt signaling increased the percentage of Arg-1+ CD86- CD38- and Arg-1 + CD86+ CD38+ cell populations. Our results suggest that Wnt signaling participates in M1 polarization induced by classical stimulus by modulating the expression of key molecules that contribute to this phenotype.

314. (762) ***TRYPANOSOMA CRUZI*** **PROMOTES MACROPHAGE DEDIFFERENTIATION AND TRANSITION INTO MYOFIBROBLAST-*LIKE* CELLS**

Ximena Volpini1,2,3, Juan Nahuel Quiroz1,2, María Belén Brugo1,2, Jazmín Lavallen1,2, Melisa Rocío Herrera1,2, Laura Fozzatti1,2, Melina Musri3, Claudia Cristina Motrán1,2.
1, Centro de Investigación en Bioquímica Clínica e Inmunología. CIBICI-CONICET, Córdoba, Argentina. 2, Departameto de Bioquímica Clínica e Inmunología. Facultad de Cs. Químicas, Universidad Nacional de Córdoba (FCQ-UNC), Córdoba, Argentina. 3, Instituto de Investigaciones Médicas Merdeces y Martín Ferreyra (IMMF-UNC/INIMEC-CONICET) Córdoba, Argentina.

Dedifferentiation is the process by which cells return from a partially

or terminally differentiated stage to a lesser stage of differentiation within their original lineage. Instead, in cell transition, these acquire markers and functions of a different cell lineage from their original. Previously, we have reported macrophage (Mo)-*like* F4/80+CD11b+ cells expressing α-SMA, a smooth muscle cell, and myofibroblast marker, in the aortas of *Trypanosoma cruzi*-infected mice. These cells were observed in the media and adventitia layers during the acute and chronic phases of Chagas disease. However, we have not been able to identify the original lineage of these cells. In this work, we aim to study *T. cruzi* ability to induce Mo dedifferentiation and/or transition. Bone marrow-derived Mo (M0, BMDM) from BALB/c mice were treated *in vitro* with LPS+IFN-γ or IL-4 to induce M1 or M2 phenotypes respectively, or infected with trypomastigotes of *T. cruzi* (TC). Also, we used combinations of treatment-*plus*-infection. We found that TC-infection decreases the frequency of F4/80+CD11b+ cells on M0, M1, and M2 BMDM cultures. In M0 F4/80+CD11b+, TC induced significatively the expression of α-SMA. F4/80+CD-11b+α-SMA+ from TC-infected M0 expressed lower levels of CD45, F4/80, and iNOS while expressed higher levels of CD206, and Arginase-1 to those M0-controls. Also, TC induced α-SMA expression and increased Arginase-1 levels on M2, while in M1 induced a significant decrease in CD45, F4/80, CD11b, and iNOS levels. Our results suggest that TC infection promotes Mo dedifferentiation and transition into myofibroblast-*like* with possible pro-regenerative functions. Meanwhile, dedifferentiation may be pathological in an infectious context, understanding dedifferentiation mechanisms provides new insights for designing strategies for regenerative medicine. Currently, we are analyzing possible treatments to promote and modulate Mo-dedifferentiation and their tissular pro-regenerative functions.

315. (820) **EFFECTS OF HYPERGLYCEMIC AND CORTISOL-STRESSFUL CONDITIONS OVER GALECTINS GENE EXPRESSION IN CULTURED THP-1 -DERIVED MACROPHAGES**

Matilde Imhoff[1], Magdalena Diab[1], Fernandez Rocío dV[1], Estefania Massa[1], Georgina Gallucci[1], Ariana Díaz[1], Bettina Bongiovanni[1], Bottasso Oscar A[1], María Luisa Bay[1], D'Attilio Luciano D[1]

[1] IDICER, Facultad de Cs Médicas, CONICET-UNR, Rosario, AR

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), continues to be among the top ten leading causes of mortality worldwide. A quarter of the world's population is infected with Mtb and type 2 diabetes mellitus (TB+T2DM) may increase 3 times the possibility of developing active TB. We demonstrated that TB and TB+T2DM patients showed an immune-endocrine- metabolic (IEM) imbalance: elevated plasma levels of pro and anti-inflammatory cytokines as well as cortisol-Gc, along with elevated circulating levels of galectins (Gal-1 and 3). Moreover, this IEM-imbalanced environment may modulate macrophage functions. To expand this knowledge, we studied the effect of glycemia [D-Glucose-Glc 5mM (physiological dose) or 10, 20, or 40 mM (supraphysiological doses-SPD)] and cortisol-induced stress (Gc-IS. 0.1 or 1μM) over the mRNA expression of Gal-1, Gal-3 and Gal-9, along with pro and anti-inflammatories transcripts (IL-1β, IL-6, and IL-10) in cultured THP-1 derived macrophages stimulated with *Mtb*i (strain H37Rv killed by gamma radiation-*Mtb*). Glc SPD did not affect transcripts expression in all culture conditions, except for Gal-3 and IL-10 in stimulated cultures, which appeared to increase ($p<0.01$; 10 vs 40mM Glc). Mtb significantly increased expression of Gal-1 ($p<0.008$), Gal-3 ($p<0.003$) and Gal-9 ($p<0.05$) respect to unstimulated cultures. Although, both doses of Gc, decreased Mtbi-induced expression of Gal-1 ($p<0.001$), Gal-3 ($p<0.001$), and Gal-9 ($p<0.02$). Cytokine assessment showed a similar expression profile as seen for Gals throughout the different culture conditions. There were positive correlations between Glc-SPD and Gal-3 ($p<0.01$, $r=0.84$) and IL-10 ($p<0.01$, $r=0.8$) in stimulated cultures. Hyperglycemic conditions seem to progressively augment Mtbi-induced-pro and anti-inflammatory cytokines with Gal-3 being only modulated in this regard. In a Gc-IS environment, this response would be negatively affected.

316. (821) **INDUCTION OF NEUTROPHIL-DERIVED REACTIVE OXYGEN SPECIES AND NEUTROPHIL EXTRACELLULAR TRAP GENERATION BY CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTALS**

María Belén Luffi[1], Rodrigo Blas[2], Victoria Borgia[1], Marcos Borgia[1], Iván Borgia[1], Alfredo Borgia[1], María Cristina Pistoresi[3], María Silvia Di Genaro[4,5], Carolina Gorlino[4]

[1]Instituto Reumatología, Mendoza; [2]Medici Consultorios externos, San Luis; [3]Facultad de Ciencias Químicas (FCQ), Universidad Nacional de Córdoba; [4]Instituto Multidisciplinario de Investigaciones Biológicas de San Luis (IMIBIO-SL)-CONICET; [5]Facultad de Química, Bioquímica y Farmacia (FQByF), Universidad Nacional de San Luis

Deposition of calcium pyrophosphate dihydrate (CPP) crystals in joints and soft tissues is the cause of acute or chronic inflammatory responses known as Calcium Pyrophosphate Crystal Deposition Disease (CPPD). Cell activation by CPP crystals is a central feature and pro-inflammatory crystals can interact with all of the major synovial cell types, including neutrophils. Although the neutrophil is a major inflammatory cell type found in inflamed joints of acute CPPD patients, little is known about interactions of neutrophils with CPP crystals. In this study, we aimed at studying whether CPP crystals induce a pro-inflammatory profile in neutrophils, measured by degranulation, induction of reactive oxygen species (ROS) and neutrophil extracellular trap (NET) generation. Neutrophils were isolated from healthy subjects and incubated with synthesized CPP crystals (CPP) or with synovial fluid from CPPD patients (CPPD-SF). Degranulation (measured by the expression of CD66b), ROS production (determined by DHR-123 assay) and NET formation (detected by Sytox Green fluorescence) was studied by flow cytometry in basal neutrophils (control), neutrophils stimulated with synthesized CPP crystals (CPP) or with CPPD-SF. P value is the result of one-way ANOVA (Kruskal-Wallis test) followed by Dunn's post-hoc test or Mann-Whitney test, when appropriate. We found that the presence of CPP crystals in SF from CPPD patients induced extracellular DNA exposure in neutrophils (%SYTOX-Green positive cells, control vs CPPD-SF; $p=0.03$) as well as an up-regulation of CD66b expression ($p=0.03$). Moreover, CPPD-SF samples significantly increased respiratory burst in neutrophils (control vs CPPD-SF, $p=0.04$). In conclusion, these data suggest that the presence of CPP crystals in SF from CPPD patients could have a role in the amplification of the inflammatory response by stimulating up-regulation of CD66b, NET release and ROS production in neutrophils.

317. (823) ***Lsp1*$^{-/-}$ DENDRITIC CELLS EXHIBIT DELAYED ANTIGEN DEGRADATION DUE TO AN IMPAIRED UPTAKE**

ND Dho[1], MM Pascual[1], MC Pistoresi[1], BA Maletto[1], MI Crespo[1], G Morón[1].

1. CIBICI–CONICET, Departamento de Bioquímica Clínica, FCQ-UNC, Argentina.

Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes and endothelial cells. LSP1 is an important regulator of actin cytoskeleton remodelling. We have previously shown that *Lsp1*$^{-/-}$ dendritic cells (DCs) have a defective antigen presentation to CD4+ T cells compared to DCs from wild type (WT) mice and that MHC class II dynamics is similar between *Lsp1*$^{+/+}$ and *Lsp1*$^{-/-}$ DCs. We thus studied whether this defective antigen presentation in *Lsp1*$^{-/-}$ DCs is due to either an alteration in the uptake or in the processing of Ag, using DCs *in vitro* derived from bone marrow precursors with Flt3-L. For antigen degradation, DCs were co-cultured with OVA-DQ (which fluoresces after being degraded by proteases) for 15min, 1, 2, 3 and 4h. After these times DCs were fixed, permeabilizated and stained with anti-CD107a, a marker of lysosomes, and analyzed by in cell imaging system (INCell Analyzer 2500HS). We found that *Lsp1*$^{-/-}$ DCs showed significant lower levels of OVA-DQ degradation ($p<0.01$) at 1h and higher levels at 2h ($p<0.0001$) to then been similar at 3h and 4h than *Lsp1*$^{+/+}$ DCs. Regarding to CD107a, we observed *Lsp1*$^{-/-}$ DCs a significant lower amount at 15 min ($p<0.0001$) than WT DCs, which then equals at 1h. To assess antigen uptake, DCs were co-cultured with OVA-AF647 during 30, 60 or 90 minutes and then analyzed by flow cytometry. We observed that at every

time point, *Lsp1*$^{-/-}$ DC population contained a lower proportion of cells with OVA-AF647 ($p<0.001$, $p<0.0001$ and $p<0.0001$ respectively) than *Lsp1*$^{+/+}$ ones. After 30 minutes, *Lsp1*$^{-/-}$ DCs showed a lower mean fluorescence intensity ($p<0.001$) to then reach similar values than *Lsp1*$^{+/+}$ DCs. These results suggest that altered antigen presentation in *Lsp1*$^{-/-}$ DCs could be, at least in part, a consequence of an impaired antigen capture responsible of a delayed degradation.

318. (838) **CONTRIBUTION OF FUNGAL VIRULENCE FACTORS AND FUNGAL RECOGNITION RECEPTORS IN THE REGULATION OF THE EXPRESSION OF HUMAN BETA DEFENSINS 1 AND 3**

Sofia Carla Angiolini, Emilse Rodriguez, María Soledad Miró, Paula Alejandra Icely and Claudia Elena Sotomayor
Laboratory of Innate Immunity to Fungal Pathogen. Department of Clinical Biochemistry, CIBICI-CONICET, Faculty of Chemical Sciences, National University of Córdoba.

Acute vulvovaginal candidiasis (AVVC) is an inflammatory disease that affects up to 75% of women of childbearing age once in their lifetime, but 9% of them present a recurrent form of mycosis (RVVC) (>=8/4 episodes per year). *Candida albicans (Ca)* is the most prevalent agent, its main virulence factors are hyphal formation, adherence, hydrolytic enzyme generation and biofilm forming capacity (BFC). Human β-defensins 1 and 3 (hBDs) are cationic peptides with antimicrobial and chemoattractant activity. Our aim was to characterize the virulence pattern of *Ca* clinical isolates from AVVC and RVVC patients and the role of *Ca* virulence factors and innate recognition in the regulation of hBDs. BFC, lipase activity (LIP) and aspartylproteinases activity (SAP) of strains isolated from patients (CVVR=20 and CVVA=20), collection strains (*SC5314* and *ATCC36801*) and β-glucan mutation (*FKS1*) were evaluated. *Ca*, heat-killed *Candida* (HKC) strains or fungal PAMPs agonists (LPS, Pam3CSK4, Zymosan,Curland) were added to HeLa cell cultures and modulation of hBDs 1 and 3 (2 or 4h) was determined by qPCR and protein expression (INCell Analyzer2500 HS). CVVR strains showed lower CBF than CVVA and *Ca-SC5314* ($p<0.05$), while SAP activity was higher than in CVVA isolates ($p<0.05$). Clinical strains were classified according to their virulence profile and used to stimulate HeLa cells. At different study time, particular transcription profile was observed: at 2h, HKC up-regulate both BDs($p<0.05$); at 4h, *Ca FKS1* increased hBD1,3 mRNA, meanwhile HKC and *Ca SC5314* up-regulate hBD1 and CVVR isolates selectively increased hBD3. Protein expression of hBDs showed different patterns against evaluated stimuli. Knowledge of the virulence attributes of *Ca* and the role of RRPs exhibited during CVVR in the modulation of BDs is relevant for a better understanding of the pathogenesis of the disease and evaluating their potential use as therapeutic agents.

319. (850) **DYSREGULATION OF THE NK CELL COMPARTMENT IN LUNGS OF DP16(1)/YEY MOUSE MODEL OF DOWN SYNDROME: IMPACT OF DOUBLE-STRANDED RNA MIMETIC STIMULATION**

Dutto J, Araya P. Boffelli L, Espinosa J, Maccioni M

Down syndrome (DS) is caused by an extra copy of chromosome (chr) 21. People with DS may experience hyperinflammation during viral infection, which has been associated to the presence of four IFNR genes (Ifnar1, Ifnar2, Ifngr2, Il10rb) in chr21. Here, we use a mouse model of DS, Dp16(1)/Yey (DP16), to analyze the impact of double-stranded RNA mimetics on the innate immune response. DP16 mice have a segmental duplication of a region on murine chr16, syntenic to the portion of chr21 that includes the IFNR genes. DP16 and control mice (n=9) were i.p. injected with the TLR3 ligand poly IC and 15hs later, sacrificed. A 29-parameter spectral flow cytometry was used to characterize innate immune cells within spleen, lung, inguinal lymph nodes (iLN) and mesenteric lymph nodes (mLN). At steady state, the main changes observed were reduced frequencies of cDC1, cDC2, and tDC in spleen ($p<0.001$; $p<0,01$; $p<0,05$ respectively) and augmented percentage of pDC in mLN in DP16 mice vs control ($p<0,05$). Upon in vivo stimulation, the frequencies of each DC subset as well as the expression of the different activation markers analyzed showed a similar pattern in both strains. Interestingly, the major difference observed between both strains at steady state was in the NK cell compartment. Spleens and lungs of DP16 mice showed a reduced frequency of total NK cells ($p<0.05$) but a higher frequency of mature CD11b+ Cx3cr1+ NK cells ($p<0.05$ and $p<0.01$ respectively). Upon stimulation, NK cells of DP16 mice respond better, expressing higher levels of the activation markers such as CD69. Normalization of Ifnr copy number through crossing of the Dp16 with a quadruple knock out of the four Ifnrs showed the same changes, indicating that the alteration in the NK compartment observed in spleen and lung cannot be explained by the extra copy of these genes. These results suggest that DP16 mice show marked changes in the NK cell population that could be related to an altered response to infections in DP16 mice.

320. (857) **TOWARDS AN INTEGRATIVE BIOINFORMATIC AND BIBLIOMETRIC ANALYSIS OF C23/NCL POST-TRANSLATIONAL MODIFICATIONS DIVERSITY. DATA CURATION BY IMPLICATIONS ON MOLECULAR SIZE, FOLDING, TRAFFICKING, INTERACTIONS, FUNCTIONS AND UNCONVENTIONAL SECRETION**

Cristian J. A. Asensio [1]
1. Centro de Investigación Veterinaria de Tandil (CIVETAN)-CONICET. Facultad de Ciencias Veterinarias, UNCPBA. Campus Universitario, Tandil, Argentina.

C23/NCL has multiple domains, PTMs, polyanionic stretches, repeats, functions and subcellular locations and without signal peptide is enriched on cancer cells surface acting as co-receptor, alarmin, etc. Its study is also difficulted by lack of full-length C23 expression in bacteria. C23 integrates multiple aspects of DNA and RNA metabolism and cell cycle/metabolism and senses environmental cues in cell surface, cytosol and nucleus. Its diverse PTMs coordinate interactions with proteins, nucleic acids, carbohydrates, cations, LPS and lipids as well as the trafficking from nucleolus to nucleoplasm, cytosol, microtubules, vesicles, membrane and back again. It is unconventionally secreted, clustering on membranes with receptors, extracellular factors, matrix, cations and pathogens. Most steps on its trafficking, interaction partners, or if the diverse PTMs are hierarchical and/or alternative are unclear. Understanding mechanisms should help testing anti-C23 drugs to prevent viruses/bacteria entry, to target cancer and for diagnostics. Articles have dispersed on different roles and cells, so bibliometric/bioinformatic approaches might help better in making sense on PTMs diversity. Thus, we aimed to recollect C23 PTMs from several databases, curating data by impacts on subcellular location, functions/interactomes, frequency, etc. We detected ~180 PTMs (some alternative) involving phosphoryl, acetyl, succinyl, ubiquitin, sumo, glycosyl, NO and methyl groups plus cleavage. Enzyme crosstalk data and 7 known PTMs were absent. We conclude that a curated PTM database might help in experiment and drug design and in knowledge gap identification. Studies are needed on: a) expansion of sequence/folding diversity by cleavage, isomerization, oligomerization and charge density, b) finding PTMs in cytosolic C23 as markers of shuttling to or from membranes, c) interaction motif identification, d) modeling impacts on folding, e) searching any PTM-code and epigenetic/signaling roles

321. (863) **ROLE OF POLLUTION IN ASTHMA EXACERBATION. DEVELOPMENT OF AN IN VITRO MODEL TO ASSESS EXPOSURE TO PARTICULATE MATTER**

Clara Claus[1], Julieta B. Grosso[1], Ledit Ardusso[2] y Silvana V. Spinelli[1].
1 Instituto de Inmunología Clínica y experimental de Rosario (IDICER CONICET-UNR), 2 Hospital provincial centenario. Rosario-Argentina. claritaclaus97@gmail.com

Pollution represents a high risk to human health, causing around 3 million deaths each year. Particulate matter (PM) exposure associates with exacerbation of respiratory diseases, including asthma. In this work, we developed an in vitro model to assess its effects on macrophage function in the context of an allergic response to dust mites, a common allergen. To emulate an allergic response, THP-1

cells were exposed to extracts of *D.pteronyssinus* (HDM) at two concentrations (HDM 50 and 5 AU), with PM produced by diesel engine exhaust at different concentrations (PM 50 and 5 µg/ml) and with a combination of both treatments. Cells and culture supernatants were collected after 24 hours for quantification of transcripts for cytokines (IL-1, IL-8, IL-10 and IL-23), angiogenin and small non-coding RNAs by RT-qPCR. Our results showed that PM strongly activates the inflammatory response, with a significant increase in transcript levels of IL-1, IL-8, IL-10 and IL-23. (IL-1: p=0.0002 (ANOVA), IL-8: p=0.0009 (ANOVA)). The most significant differences were observed in combined treatments (H50-P50), with increased levels for all cytokines (IL-1: p<0.0001 (ANOVA). IL-8: p<0.0001 (ANOVA). IL-10: p=0.0078 (ANOVA). IL-23: p<0.05 (T-Test)). We also determined the production and secretion of small RNAs, specifically tRNA-derived fragments called tiRNA as potential new biomarkers of severe asthma. Levels of $tiRNA^{GLU}$ and $tiRNA^{GLY}$, two tiRNAs abundant in biofluids and present in pulmonary secretions, were quantified in both THP1 cells and culture supernatants. Our results show that PM50 alone and combined with HDM induce the secretion of these tiRNAs and expression of angiogenin, the enzyme responsible for their production (ANG: PM50 (p=0.0014, ANOVA), H50-P50 (p= 0.003, T-Test), $tiRNA^{GLU}$: PM50 (p<0.0001, ANOVA), H50-P50 (p=0.0074), $tiRNA^{GLY}$: PM50 (p=0.0006, ANOVA), H50-P50 (p=0.0018, ANOVA). In conclusion, in this work we demonstrated that PM exacerbates the response of macrophages to HDM. In addition, we showed that PM is involved in regulatory pathways mediated by non-coding RNAs.

322. (889) **CLEC4F PARTICIPATES IN THE UPTAKE OF CIRCULATING PARASITE MUCINS BY KUPFFER CELLS IN CYSTIC ECHINOCOCCOSIS**

Anabella A. Barrios[1], Camila Mouhape[1], Juliane Nell[2], Leonard Schreiber[2], Mariana Suárez[1], Gustavo Mourglia[1], Thomas Barth[2], Cecilia Casaravilla[1], Stephen J. Jenkins[3] & Álvaro Díaz[1]

1Área Inmunología, Departamento de Biociencias (Facultad de Química) and Cátedra de Inmunología, Instituto de Química Biológica (Facultad de Ciencias), Universidad de la República, Montevideo, Uruguay, 2Institute of Pathology, University Ulm, Ulm, Germany, 3University of Edinburgh Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.

Cystic echinococcosis is caused by the larval stages (hydatids) of cestode parasites belonging to the species cluster *Echinococcus granulosus* sensu lato. Hydatids are bladder-like structures that attain large sizes within various internal organs of livestock ungulates and humans. Hydatids are protected by the massive acellular laminated layer (LL), composed mainly by mucins. Parasite growth requires LL turnover, and abundant LL-derived particles are found at infection sites and draining lymph nodes in infected humans. These observations raise the question of whether LL materials circulate systemically, and if so how they are dealt with by the hosts. Clec4F is a C-type lectin, which in rodents is expressed only in Kupffer cells (KC), the liver macrophages exposed to the vascular space. As recombinant Clec4F bound the LL mucins *in vitro*, we investigated whether Clec4F participates in the uptake of LL mucins by KC *in vivo*. LL mucin preparations, either soluble (injected i.v.) or particulated (injected i.v.) were taken up selectively by KC, as determined by flow cytometry analysis of liver non-parenchymal cells. This uptake was largely dependent on the LL mucin glycans and on Clec4F, as determined using periodate-oxidized mucins and comparison of WT type and $Clec4f^{-/-}$ mice. In mice infected with *E. granulosus sensu lato*, LL mucins were detected at the site of infection (peritoneal cavity) and in the liver KC, but not in other organs/cell types, as determined by immunohistochemistry. Uptake by KC was dependent on Clec4f, on the basis of flow cytometric analysis of WT type and $Clec4f^{-/-}$ mice. It seems likely the composition of the LL was adjusted through evolution so that LL debris were taken up by the major phagocytes of the rodent liver, which is the ancestral infection site for *Echinococcus* larvae. It is also probable that KC act as a sink for LL materials even when the parasite grows in sites other than the liver, as it happens in natural *E. granulosus* infections.

323. (893) **MODULATION OF PROINFLAMMATORY MOLECULES PRODUCTION BY AUTOPHAGY PHARMACOLOGICAL INHIBITORS IN LPS-ACTIVATED MICROGLIAL CELLS**

Clarisa Mazone-Rodríguez[1], Cecilia María Rodríguez[2] and Pablo Iribarren[1].

[1]CIBICI-CONICET, Fac. de Ciencias. Químicas, UNC. Córdoba, Argentina.

[2]Laboratorio de Biología Molecular y Citometría de Flujo del servicio de Oncología y Hematología, Hospital Nacional de Clínicas, Fac. de Medicina, UNC.

E-mail: cmazone@fcq.unc.edu.ar

Objective: Microglial cells are phagocytes in the central nervous system (CNS) and become activated in pathological conditions, resulting in microgliosis, manifested by increased cell numbers and inflammation in the affected regions. Lipopolysaccharide (LPS) is able to activate and promote pro-inflammatory responses in microglial cells. For instance, stimulation of Toll-like receptor 4 (TLR4) induces the production of nitric oxide by these cells, which could be neurotoxic. We previously reported that TLR2 stimulation by peptidoglycan (PGN) from *Staphylococcus aureus*, induced microglial cell activation followed by autophagy induction. In addition, our findings suggest that activation of autophagy in microglial cells might modulate inflammatory responses in these cells. Therefore, our aim is to evaluate the modulatory effect of autophagy inhibitors in LPS-activated microglial cells. Methods: The murine microglial cell line BV2 was stimulated with LPS at different time points after pre-incubation in the presence or the absence of autophagy inhibitors (3-MA and spautin-1) or a general phosphatidyl-inositol-3 kinase (PI3K) inhibitor (LY294002). After treatment, microglial cells were processed to evaluate: 1) cytokine production by ELISA; 2) nitric oxide (NO) production by Griess reaction. All experiments were performed 3 times and $p < 0.05$ was considered to be statistically significant. Results: We observed that activation of microglial cells with LPS induced increase production of TNFα and NO ($p < 0.001$). Interestingly, treatment with autophagy inhibitors or a general PI3K inhibitor prevented the increased production of NO ($p < 0.001$). Additional experiments are currently performed to evaluate the effects of these inhibitors on TNFα production in microglial cells. Conclusions: These preliminary results suggest that both autophagy and general PI3K inhibition may selectively modulate the production of NO and other proinflammatory molecules in LPS-activated microglial cells

324. (903) **NEW INSIGHTS IN THE REGULATION OF THE RECRUITMENT OF ΓΔ T LYMPHOCYTES BY HUMAN GLOMERULAR ENDOTHELIAL CELLS EXPOSED TO SHIGA TOXIN TYPE 2**

David Antonio Rosso[1], Fernando Daniel Gómez[2], Nadia Melillo[1], Micaela Rosato[1], Cristina Ibarra[2], María Marta Amaral[2], Carolina Cristina Jancic[1,3]

[1]Instituto de Medicina Experimental – CONICET – Academia Nacional de Medicina. Buenos Aires, Argentina, [2]Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay – CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. [3]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina

Hemolytic uremic syndrome (HUS) is a late acute onset of symptoms that can occur after an intestinal infection with Shiga toxin (Stx)-producing *Escherichia coli* (STEC). STEC strains expressing Stx2a are the most common etiologic pathogen responsible for severe cases of HUS in Argentina. The immune system participates during the development of HUS by promoting inflammation and exacerbating the renal tissue damage, initially caused by the Stx. γδ T cells are a subset of T lymphocytes that act as early sensors of cellular stress and infection. They infiltrate the tissues and, in kidney injury, γδ T cells can potentially be recruited as they express the fractalkine receptor (CX3CR1), which is involved in the homing of NK cells and monocytes in the kidney. Previously, we demonstrated that conditioned medium derived from Stx2a-treated human

glomerular endothelial cells (HGEC), activated γδ T lymphocytes and induced a Th1-like profile. In this work, our aim was to evaluate whether those conditioned media could modulate γδ T cell motility. To do this, we analyzed cell migration using the Transwell system and F-actin polymerization in γδ T cells, by flow cytometry. For this, the cells were exposed to conditioned media derived from HGEC treated with Stx2a (0.01 ng/mL, 24 h) or untreated ones. As results, we found that Stx2a-treated HGEC conditioned medium increased γδ T cell migration compared with culture medium alone (p<0.01) or conditioned medium from HGEC not exposed to Stx2a (p<0.05). To approach to the role of fractalkine (CX_3CL1) during this process, we first analyzed CX_3CR1expression in γδ T cells, by flow cytometry. We found that 57±18% of γδ T cells were CX_3CR1+, and when we studied γδ T cell migration to CX_3CL1, we observed it was higher compared with medium alone (p<0.05), as was F-actin polymerization. Altogether, our findings suggest that CX_3CL1 may be involved in the modulation of γδ T cell migration triggered by chemokines secreted by Stx2a-treated HGEC.

325. (920) **IMMUNOMODULATORY EFFECT OF AMNIOTIC MEMBRANE IN HERPES SIMPLEX TYPE 1 (HSV-1) – INFECTED CORNEAL CELLS**

Gisela Rodas Rojas [1], Matías Rotela [1], María Ximena Guerbi [1,2], Griselda Moreno [3], Alejandro Berra [1,4], Flavia M. Michelini[1,4]

1, CEMET-HEC, F.Varela, Argentina; 2, CIC; 3, IIFP, CONICET, UNLP, La Plata, Argentina; 4, CONICET

Herpes Simplex type 1 (HSV-1) has ocular tropism. HSV-1 infection in the cornea triggers an inflammatory response to eliminate the virus. This can cause serious damage, leading to Stromal Herpetic Keratitis (HSK), an immunopathology that impairs vision. Acyclovir, the antiviral used to treat these infections, blocks viral dissemination but does not impede the inflammatory response and the immunopathology. And the use of the potent antiinflammatory glucocorticoids has undesirable side effects. Amniotic membrane derivatives (hAM) have antiinflammatory, immunomodulatory, and antimicrobial activity. They are extensively used in tissue regeneration and have become an efficient therapeutic alternative in corneal pathologies.
In this work we studied a potential antiviral activity of rehydrated hAM patches supernatants against HSV-1 in the corneal cells HCLE, through virus yield reduction assays, and we investigated a modulation activity of hAM on inflammatory mediators in HCLE cells infected with HSV-1, through the quantification of the secreted cytokines with enzyme linked immunoassays. ANOVA followed by Tuckey tests were performed (n>2). hAM did not display inhibitory activity against HSV-1 replication, neither when cells were treated 24 h before infection, nor when hAM was added to cells immediately after infection. However, the presence of hAM induced a modulation of cytokine production in infected cells: pretreatment with hAM reduced IL-6 and IL-8 secretion in infected cells, while these cytokines production augment in infected cells treated with hAM after infection. hAM does not exhibit antiviral activity against HSV-1, but it modulates the innate immune response of the epithelial cells to infection.

326. (922) **UMBILICAL CORD PRODUCTS AS A POTENTIAL COMPLEX WOUND DRESSING**

Matías Rotela[1], Gisela Rodas Rojas[1], Flavia Michelini[1,3], Griselda Moreno[3,5] ,Alejandro Berra[1,2,3], Ximena Guerbi[1,4].

1CEMET-HEC, F.Varela, Argentina, 2AMNIOS-BMA, Buenos Aires, Argentina, 3CONICET, 4CIC, 5, IIFP, CONICET, UNLP.

Amniotic membrane derivatives have shown to be safe and efficient biological treatments for damaged soft tissues. The umbilical cord is another promising placental tissue, dismissed as pathogenic waste, with which we have been developing new lyophilized and sterilized wound dressings. Donated umbilical cords are homogenized, lyophilized in Petri dishes and sterilized by gamma radiation. The patches thus obtained were rehydrated in RPMI medium, centrifuged and aliquoted supernatants were characterized biochemically through quantification of total protein content by Bradford assay and cytokines were measured by ELISA. Afterwards, these supernatants were analyzed functionally in a monocytic cell line, THP-1. Following a 72 h PMA differentiation process to macrophages, we evaluated the modulation of LPS induced cytokine secretion, by the umbilical cord products, after 24 hs of treatment. IL- 6, IL-8 and IL-10 were quantified by ELISA, showing a decrease in the mean values of proinflammatory cytokines and suggesting an increase of antiinflammatory IL-10. We have performed similar assays using amniotic membrane derivatives, which highlights its immunomodulatory effect, inducing M1-macrophages re-polarization to an M2 profile.
Cell viability was verified by MTT assay, showing that cell viability was preserved under treatment conditions. In all cases, ANOVA followed by Tuckey test, were performed (n>2).

METABOLISM AND NUTRITION I

Wednesday, November 16, 14-15:30 hr
Chairs: Ana María Puyó - Marisa Gabriela Repetto - Carolina Caniffi

327. (18) **NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2) MEDIATES HEPATIC OXIDATIVE IMBALANCE ASSOCIATES TO NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PROGRESSION IN TUMOR NECROSIS FACTOR-ALPHA RECEPTOR 1 (TNFR1) KNOCKOUT MICE**

Marinochi María Virginia[1], Sedlmeier María Guillermina[1], Lambertucci Flavia[2], Catania Viviana A[1], Ronco, María Teresa[1], Francés Daniel[1]

[1]Instituto de Fisiología Experimental (IFISE-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Suipacha 570, Rosario, Argentina, [2]Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, Inserm U1138, Institut Universitaire de France, Paris, France.

Previously, we demonstrated that disruption of TNFR1 signalling pathway increased plasmatic interleukin-1β levels, liver inflammation and enhanced the progression of NAFLD to steatohepatitis (NASH) in a High Fat Diet (HFD) murine model. In this regard, reactive oxygen species are involved in liver inflammation and HFD-derived hepatic injury. The aim of this work was to evaluate the effect of TNFR1 disruption signalling on the antioxidant response mediated by NRF2 pathway in our mouse model of obesity and insulin resistance. C57BL/6J wild type (WT) and C57BL/6-Tnfrsf1atm1Imx/J knockout (TNFR1 KO) mice (n=6) were fed with regular chow diet (CHOW) or a 40% high-fat diet for 16 weeks (HFD). Hepatic lipid peroxidation levels were increased in TNFR1 KO mice (TBARS; CHOW WT: 101.42±5.27; HFD WT: 118.29±3.64*; CHOW KO: 130.22±5.90*; HFD KO: 127.63±3.76 *; % of CHOW WT, *p<0.05 vs CHOW WT). In addition, NADPH oxidase activity was evaluated in liver homogenate by colorimetric assay (CHOW WT: 101.28±15.30; HFD WT: 117.64±4.90*; CHOW KO: 133.78±23.85; HFD KO: 153.51±5.06#; *p<0.05 vs CHOW WT; #p<0.05 vs HFD WT). In line with this, NRF2 nuclear protein expression determined by Western blot showed a decreased in HFD WT (-48%; p<0.05) and HFD KO (-66%, p<0.05) when compared to paired CHOW fed groups. Consistent with this, Keap 1 cytosolic expression was increased in HFD WT (+37%) and HFD KO (+62%). On the other hand, the autophagy regulatory protein p62 mediated the activation of NRF2 through a non-canonical activation pathway. Cytosolic expression of p62 showed a statistically significant decreased only in HFD KO mice when compared to CHOW KO (-35% p<0.05). Based on these results, we suggest that the disruption of TNFR1 signalling pathway decreases NRF2 nuclear translocation enhancing hepatocyte oxidative stress induced by HFD.

328. (34) **SUPPLEMENTATION WITH DIFFERENT SOURCES OF OMEGA 3 IN DIET WITH OLIVE OIL. STUDY ON SERUM AND THYMUS OF GROWING RAT**

Anabel Impa Condori[1], María Fernanda Godoy [1,2], María Cecilia Mambrin[1], Inés Fernandez[1], María Silvia Giacomino[3], Nestor Pellegrino[3], Slobodianik NH[1], Feliu MS[1].

[1] Universidad de Buenos Aires, Facultad de Farmacia y Bio-

química, Cátedra de Nutrición, [2] INTA, Instituto de Tecnología de Alimentos, [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Bromatología. Argentina.

Dietary lipids have a important role in nutrition and must be ingested in an appropriate proportion. Objective: to analyze the effect of diet containing olive oil, supplemented with different sources of omega 3, on serum and thymus's fatty acid profiles of growing rats. Methods: Weanling Wistar rats received during 10 days a normocaloric diet, fat was provided by olive oil (O group). The other groups received the same diet supplemented with 24mg/day of chía oil (OCh) or fish oil (OF). Control group (C) received AIN´93 diet. Serum fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA test. Results(%Area): SERUM: OLEIC C: 10.11±1.84^{a}, O:22.93±4.66^{b}, OCh:20.98±1.83^{b}, OF:18.31±2.22^{b}; LINOLEIC (LA) C:20.52±3.37^{a}, O:11.74±2.30^{b}, OCh:13.82±0.49^{b}, OF:11.40±2.07^{b}; LINOLENIC (ALA) C:0.93±0.27^{a}, O:0.30±0.06^{b}, OCh:0.40±0.05^{b}, OF:0.32±0.08^{b}; EPA C: 0.92±0.30^{a}, O:0.71±0.06^{b}, OCh:1.06±0.16^{a}, OF:1.63±0.49^{c}; DHA C:1.60±0.55^{a}, O:1.61±0.47^{a}, OCh:2.52±0.44^{b}, OF:4.41±1.51^{c}. THYMUS: OLEIC C:18.90±3.53^{a}, O:24.23±6.54^{b}, OCh:25.31±6.96^{b}, OF:24.40±5.04^{b}; LINOLEIC C:11.05±2.34^{a}, O:5.70±0.63^{b}, OCh: 6.14±0.31^{b}, OF:6.53±0.61^{b}; LINOLENIC(ALA) C:0.55±0.13^{a}, O:0.27±0.02^{b}, OCh:0.49±0.13^{a}, OF:0.30±0.07^{b}; EPA C:0.39±0.18, O:0.43±0.23, OCh:0.41±0.19, OF:0.44±0.19; DHA C:0.54±0.18^{ab}, O:0.35±0.11^{a}, OCh:0.42±0.16^{ab}, OF:0.73±0.17^{b}. Media with no letter (a,b,c) in common, were different (p<0.01). In sera, O, OCh and OF showed lower ALA and LA and higher oleic levels, compared to C. OF presented high levels of EPA and DHA; OCh increased the levels of the omega 3 family with respect to O. In thymus, O, OCh and OF groups showed higher oleic levels and lower levels of LA than C. ALA showed no differences between C and OCh. DHA only increased in the OF. Conclusions: The olive oil exacerbated omega-9 family with diminution of essential fatty acids. The response to supplementation depends on the oil used and its composition impacts on serum and thymus. UBACyT:-20020190100093BA

329. (40) **EFFECT OF LIGARIA CUNEIFOLIA (*Lc*) - PROTOANTHOCYANIDINE ENRICHED FRACTION ON HEPATIC HISTOLOGY IN HIGH FAT DIET (HFD) FED RATS**

Juan Francisco Alonso [1], Carolina Galiasso [1], Sebastián Galliano [1], Diego Crosetti [1], Leda Urli [1], Romina Serena [2] , Daniel Francés [3], Maria Teresa Ronco [3], Marcelo Wagner [4], Cristina Carnovale [3], Alejandra Luquita[1].

[1]Cátedra Biofísica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, CIURN. [2]Biología - Facultad de Ciencias Médicas, Universidad Nacional de Rosario. [3]Cátedra de Fisiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario- IFISE-CONICET. [4]Cátedra de Fármaco-botánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Previously we demonstrated that fraction enriched in Proanthocyanidin obtained from *Ligaria cuneifolia* (P*Lc*) decreased blood viscosity by lowering cholesterol (Co) and plasma triglycerides levels (TG) in rats fed with a high-fat diet (HFD). However, the effect of P*Lc* treatment on liver histology after a HFD had not been studied. The aim of this work was to evaluate the effect of P*Lc* treatment during 7 and 10 days using NASH score and PAS histochemical assay to determinate glucogen content in liver of HFD feds rats. Adult male Wistar rats (aged 70 days, n=18) were fed with standard chow diet added with 40% bovine meat juice during 28 days (HFD) and were administered i.p. each 24hr during 7 and 10 days with either saline vehicle (HFD) or P*Lc* 3mg /100g b.w. (HFD-P*Lc*7 and HFD-P*Lc*10). At the end of PLc-treatment, animals were anesthetized i.p. with Ketamine/Xylazine (100mg/kg/3mg/kg), blood samples were obtained by cardiac puncture and samples of liver tissue were fixed in 4% buffered formalin for paraffin preparation for Heamtoxilin&eosine (H&E) tinction. Another set of samples was processed for PAS (Periodic Acid-Schiff) histochemistry. To assess the diet-induced model, plasmatic glucose levels were determined (Accu-Check® Glucometer, Roche) Results are expressed as medium ± SEM. Glu (mg/dl): HFD: 264.6 ± 16.02, P*Lc*7: 134± 4.03*, P*Lc*10: 131.6± 2.80* (*p<0.01 vs. HFD, Student's t Test for unpaired data). Liver: Score NASH (steatosis grade, % of parenchyma involved by steatosis) HFD: 30-60%; T7:5-30; T10:<5%. H&E histological study showed that P*Lc*-treatment was able to reduce NASH score both at 7 and 10 days when compared to HFD group. PAS histochemical study, also showed a significatively lower cytoplasmatic glycogen depots are in hepatocytes after 7 and 10 days P*Lc*-treatment. These data suggest that P*Lc*-treatment for 7 and 10 days could modulate hepatic lipid metabolism as showed by a lower NASH score and lesser glycogen deposition after a HFD feed model.

330. (73) **ENERGY EXPENDITURE IN ELDERLY: DIFFERENCES AMONG DOUBLY LABELED WATER REFERENCE METHOD AND PREDICTION EQUATIONS**

Cristian Damián Nápoli[1], Silvina Mariela Vidueiros[1], Amalia Paganini[2], Inés Fernandez[1], Gabriel Tarducci[2], Anabel Pallaro[1]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Nutrición. [2]Universidad Nacional de La Plata, Facultad de Humanidades y Ciencias de la Educación, Cátedra de Actividad Física para la Salud, IdHICS CONICET

Doubly labeled water (DLW) and deuterium dilution technique (DDT) are gold standard methods to measure energy expenditure (EE) and body composition, respectively. The aim of this work was to study EE by DLW, accelerometry and FAO/WHO/UNU/2001 equations, as well as body composition by DDT in a group of elderly. A descriptive study was conducted in 64 women (74.9±9.9y) and 23 men (75.1±6.7y) who attended community centers or nursing homes previous signature of informed consent. The protocol was approved by the Ethics and Research Committee of the HIGA San Roque. Body weight (BW, kg) and height (H, m) were determined to calculate Body Mass Index (BMI=BW/H^2, kg/m^2). Fat-free mass (FFM) was determined by deuterium dilution technique after an orally dose of deuterated water and fat mass (FM) was obtained as FM=BW-FFM and expressed as percentage. EE was determined by DLW (EE_{DLW}) in 19 subjects after an orally dose of doubly labeled water and expressed as Kcal/d. EE was also measured by accelerometry (ActivPAL) for 5-7 days ($EE_{ActivPAL}$, expressed in total Mets and Kcal/d). Physical activity level (PAL) was calculated as total Mets/total hours. EE was also estimated by FAO/WHO/UNU/2001 equation using PAL 1.55 (EE_{FAOeq1}) or PAL obtained by ActivPAL (EE_{FAOeq2}) and expressed in Kcal/d. Results were expressed as mean ± SD. Statistical analysis was performed by ANOVA. 39% were overweight and 32% were obese. % FM was 32.6±4.2 in men and 41.8±5.5 in women (p<0.0001). PAL determined by ActivPAL was 1.38±0.08. EE_{DLW} (1792±531), $EE_{ActivPAL}$ (1747±345) and EE_{FAOeq2} (1752±205) were not statistically different and lower than EE_{FAOeq1} (1968±230) (p<0.01). It was observed high obesity prevalence in these older subjects. This preliminary study showed that accelerometry was useful to determine specific PAL for elderly and consequently the EE estimated by FAO equation, being EE like that determined by DLW as gold standard method. Supported 20720170100008BA.

331. (136) **EFFECTS OF CANNABIS OIL ON BLOOD PRESSURE AND CARDIOVASCULAR METABOLIC DISORDERS IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME**

Valentina Degrave[1], Michelle Vega Joubert[1], Darío Andrinolo[2], María Eugenia D'Alessandro[1], María Eugenia Oliva[1].

[1]Laboratorio de Estudio de Enfermedades Metabólicas Relacionadas con la Nutrición-FBCB-UNL - CONICET. [2] Centro de Investigaciones del Medio Ambiente (CIM) / CONICET - UNLP

Metabolic Syndrome (MS) is a cluster of interrelated metabolic disorders which predispose to the development of type 2 diabetes and cardiovascular disease. Interest in the beneficial effects of Cannabis sativa L. has increased in recent years and cardiovascular effects are not well known. Objectives: The aim of this study was to evaluate the effects of cannabis oil on blood pressure, lipid content and oxidative stress in heart of rats fed a sucrose-rich diet (SRD). **Ma**terials and methods: Male Wistar rats were fed with the following diets

for 21 days: Reference Diet (RD): standard commercial laboratory diet, Sucrose rich diet (SRD) and SRD+Cannabis oil (SRD+Ca): the oral administration of 1 mg/kg of body weight of cannabis oil daily. The cannabis oil presented a ratio of total cannabinoids THC:CBD of 1:2. We analized: a) Serum: triglycerides, cholesterol, glucose, non-enzymatic antioxidant capacity (FRAP) and nitric oxide (NO) and nitric oxide synthase (NOs). b) Heart: triglyceride content, NO, NOs, FRAP, substances reactive to thiobarbituric acid (TBARS), reactive oxygen species (ROS), Glutathione (GSH), enzymes Catalase (CAT) and Glutathione reductase (GR) activities. Results: In the SRD+Ca vs SRD group, serum triglyceride and cholesterol levels decreased, reaching similar values to the RD group, without changes in glucose levels. In addition, NO and NOs levels were decreased ($P<0.05$) and FRAP was increased ($P<0.05$), reaching reference values. In heart, triglyceride content and NO and NOs were decreased ($P<0.05$). TBARS and ROS levels were reduced ($P<0.05$). In addition, FRAP, GHS, CAT and GR were increased ($P<0.05$), although the values of the latter were still lower than RD group. Conclusion: This work show that cannabis oil improves blood pressure, lipid content and cardiac oxidative stress in SRD-fed insulin-resistant dyslipidemic rats. Therefore, it represents a potential treatment for cardiovascular complication present in the MS.

332. (138) **SALVIA HISPANICA L. (CHIA) SEED IMPROVES LIVER INFLAMMATION IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME**

Michelle Vega Joubert[1], Paola Ingaramo[2], María Eugenia Oliva[1], María Eugenia D'Alessandro[1].
[1]Laboratorio de Estudio de Enfermedades Metabólicas Relacionadas con la Nutrición-FBCB-UNL - CONICET. [2] Instituto de Salud y Ambiente del Litoral (ISAL), FBCB-UNL-CONICET.

Non-alcoholic fatty liver disease (NAFLD) is recognized as the liver disease component of Metabolic Syndrome (MS). Inflammation clearly play a pivotal role in the progression of this disease process. Objectives: The aim of the present study was to analyze liver inflammation in an experimental model of MS induced by chronic administration of a sucrose-rich diet (SRD) and to evaluate the effects of chia seed as therapeutic strategy. Materials and methods: Male Wistar rats were fed with a reference diet (RD) -6 months- or a SRD -3 months-. Then, the latter group was randomly divided into two subgroups. One subgroup continued receiving the SRD for up to 6 months and the other was fed with a SRD where whole chia seed was incorporated as a source of dietary fat for the next 3 months (SRD+CHIA). We analized: in serum: triglycerides, cholesterol, glucose and insulin, interleukin-6 (IL-6) and TNFα. In liver: NAS (NAFLD activity score), IL-1β, IL-10, nuclear factor-kB (NFkB), PAI-1, F4-80 expression, MPO activity and Nuclear erythroid 2-related factor 2 (NrF2). Results: The results showed that rats fed with a SRD developed dyslipidemia, hyperglycemia and inflammation. Hepatic NAS, IL-1β, NFκB p65, PAI-1, F4-80 expression, MPO activity were significantly increased and IL-10 expression was decreased. This was acompanied for an increased plasma IL-6 and TNF-α levels. In addition, we observed a significant decrease in liver Nrf2. The administration of chia seed for the last 3 months reversed dyslipidemia, hyperglycemia and inflammation. In the liver: NAS, IL-1β, IL-10, PAI-1, F4-80, NFκB, NrF2p65 and MPO activity were normalized. Conclusion: This study showed new aspects on liver inflammation in dyslipidemic insulin resistant rats chronically fed with a sucrose-rich diet. In addition, we demonstrated new properties and molecular mechanisms associated with beneficial effects on inflammation of chia seed as a therapeutic strategy.

333. (171) **FRUCTOSE-INDUCED MATERNAL METABOLIC SYNDROME LEADS TO CEREBRAL OXIDATIVE STRESS, NEUROINFLAMMATION AND NEUROBEHAVIORAL IMPAIRMENTS IN OFFSPRING**

Prado Spalm, FH*; Cuervo Sánchez, ML*; Furland, NE; Vallés, AS.
*INIBIBB, CONICET-UNS, Laboratorio de Nutrición y Neurodesarrollo, Bahía Blanca, Buenos Aires, Argentina. * Authors equally contributed to this work. Mail: svalles@criba.edu.ar*

Since fructose (F) consumption has been linked to the aetiology of metabolic syndrome (MetS), we studied the implications of long-term effects of F consumption by dams on rat offspring neurodevelopment and neurobehavior. Two-month-old Wistar rats were fed standard chow and drank water (control, C) or 20% F (ad libitum) for 10 weeks before mating and throughout pregnancy and lactation. In dams, macronutrient and caloric intake were calculated and blood samples were collected for biochemical analysis. At postnatal day 1 (PN1) oxidative and inflammatory brain status were evaluated in male (♂) and female (♀) pups. The remaining litters (reduced to 8 pups with equal sex ratios) were reassigned to a nursing dam from the C group (NC) or from the F group (NF). Offspring were evaluated for sensorimotor reflexes, reciprocal social interaction, memory retention, novel object recognition and nociception. A p value <0.05 was regarded as statistically significant. F consumption in dams induced MetS, increased caloric and carbohydrate intake and reduced protein and fat consumption. Weight, waist circumference and fat % were also higher in F dams. At PN1, ♀ from the F group showed enhanced lipid peroxidation (TBARS) levels, elevated catalase activity and increased superoxide dismutase protein expression in the whole brain. Neuroinflammation was corroborated by increased protein expression of cyclooxygenase 2. The acquisition of developing milestones was delayed in ♀ from F-NC and F-NF groups. In comparison with C-NC litter, aggressive social behavior increased while memory retention and nociception were reduced in offspring from F-NC. The ability of recognizing a previously presented stimulus was affected only in ♂ from the F-NC group. These findings suggest that F-induced maternal MetS results in offspring neurodevelopmental delay and neurobehavioral changes probably associated with early brain oxidative stress and inflammation. Interestingly, offspring gender differences were noted.

334. (182) **HIGH FAT DIET INDUCES COENZYME Q10 DEPLETION AND DYSREGULATION OF MITOCHONDRIAL COMPLEXES IN RAT LIVER CELLS**

Francisco Baez[1], Damian Soria[2], Mario Contin[1,2], Laura Valdez[1,2], Carolina Caniffi[1,2], Valeria Tripodi[1,2]
[1]CONICET, [2]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

High-fat diet induces metabolic syndrome, oxidative stress, proinflammatory and prothrombotic state, hypertension, central obesity, and cardiovascular disease. Coenzyme Q (CoQ), is an endogenous potent antioxidant located in the mitochondria with a key role in the ATP production in the electron transport chain (ETC). Our aim is to assess the status of CoQ along with oxidized and reduced glutathione (GSSG and GSH), 2-thiobarbituric acid reactive substances (TBARS), mitochondrial complex activity (I-III and II-III), and serum lipidic profile in high-fat diet (HFD) fed rats. Wistar rats (n=20) were equally and randomly divided at weaning (21 days), into two groups that received HFD or control diet (CD) until 14 weeks of age. The diets used differ only in fat content (60% of total calories came from fat in HFD and 16% in CD). After sacrifice, plasmatic CoQ and TBARS and CoQ, GSSG and GSH in liver homogenate were chromatographycally determined together with the activity of liver mitochondrial complexes by spectrophotometry. In liver homogenate, CoQ and GSH were highly reduced in HFD respect to CD (CoQ: 0.7±0.2 vs 1.4±0.7uM and GSH: 38±3 vs 54±3μmol/mg protein, respectively, $p<0.05$, mean±SEM). In mitochondria, complex activities were higher for both complexes in HFD respect to CD (I-III: 134±10 vs 102±13 and II-III: 24±2 vs 19±2nmol/min/mg protein, respectively, $p<0.05$, mean±SEM). Lipidic profile was consistent with an obesity model. Plasmatic CoQ and TBARS results reveal no changes between diets. HFD promotes a prooxidant imbalance with significative CoQ depletion in liver and a probable vicious circle in which higher ETC activity increase mitochondrial reactive oxygen species generation and thus a higher antioxidant consumption.

335. (204) **EPICARDIAL ADIPOSE TISSUE CERAMIDES AS MARKERS OF TISSUE METABOLISM IN CORONARY DIABETIC PATIENTS**

Magalí Barchuk[1,2], Jeanniel Campos[1], Daniel Yñón[3], Patricia

Ancel[4], Ljubica Svilar[4], Juan Patricio Nogueira[5], Anne Dutour[4], Jean Charles Martin[4], Bénédicte Gaborit[4], Miguel Rubio[3], Laura Schreier[1], Gabriela Berg[1,2].
1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires.
2. Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Argentina
3. Universidad de Buenos Aires, Hospital de Clínicas "José de San Martín", División de Cirugía Cardiovascular, Argentina.
4. Aix-Marseille University, INSERM, INRAE, C2VN, France
5. Servicio de Docencia e Investigación, Hospital Central de Formosa, Facultad de Ciencias de la Salud, Universidad Nacional de Formosa, Argentina.

Ceramides(Cer) emerged as new markers for CVD risk, altered in plasma before the onset of coronary artery disease(CAD). Circulating Cer18:1/C16:0, 18:1/C18:0 and 18:1/C24:1 to Cer18:1/24:0 indexes are proposed to be indicative of CAD. Epicardial adipose tissue(EAT) is a visceral adipose tissue, surrounding myocardium and coronary arteries, which volume is considered a risk factor for CVD. Studies from our laboratory demonstrated an increase in Lipoprotein Lipase(LPL) activity in EAT from CAD and Diabetes Mellitus 2(DM2) patients, suggesting that LPL would be involved in EAT expansion. We also reported an increase in EAT Cer from CAD patients, directly associated with LPL activity. Our aim was to evaluate EAT Cer indexes in CAD patients with and without DM2, and their association with LPL activity and markers of EAT lipid metabolism. Methods:we studied patients undergoing coronary by-pass graft(CAD,n=24), with and without DM2(DM2,n=7; No-DM2,n=17), and patients without CAD(noCAD,n=13). In EAT, LPL activity, Angiopoietin-like protein 4, Glycosylphosphatidylinositol anchored high density lipoprotein binding protein-1(GPIHBP1), Peroxisome Proliferator Activated Receptor γ(PPARγ), VLDL-Receptor(R) and Fatty Acid Binding Protein-4 expression were assessed. Tissue lipidome was evaluated by UHPLC-MS. Results:CAD-DM2 patients presented an atherogenic lipoprotein profile compared to the other groups. CAD-No DM2 and CAD-DM2 presented a higher Cer18:1/C24:1 to Cer18:1/24:0 index than noCAD(p=0.017 and p=0.003 respectively), with no differences between them. This index was directly associated with LPL activity(r=0.373,p=0.042), PPARγ(r=0.637,p=0.009), GPIHBP1(r=0.497,p=0.18) and VLDL-R(r=0.441,p=0.031). No differences were found among groups neither in Cer18:1/C16:0 nor Cer18:1/C18:0 to Cer18:1/24:0 indexes. Conclusion:This is the first time that a relation between LPL and Cer is reported in AT. EAT Cer indexes could be suggested as markers of the tissue metabolism in CAD and DM2.

336. (207) **NITRO OLEIC ACID MODULLATES METALLOPROTEINASES ACTIVITY IN EPICARDIAL ADIPOSE TISSUE SECRETOME: A PRELIMINARY STUDY**

Jeanniel Campos[1], Andres Trostchansky[2], Julio Baldi[3], Miguel Rubio[3], María del Carmen Fernández Tomé[4,5], Laura Schreier[1], Homero Rubbo[2], Magali Barchuk[1,6], Gabriela Berg[1,6].
1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires.
2. Departamento de Bioquímica, Facultad de Medicina and Centro de Investigaciones Biomédicas, Universidad de la República, Montevideo 11800, Uruguay.
3. Universidad de Buenos Aires, Hospital de Clínicas "José de San Martín", División de Cirugía Cardiovascular, Argentina.
4. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Buenos Aires, Argentina.
5. Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Química y Fisicoquímica Biológicas Prof. Dr. Alejandro C. Paladini (IQUIFIB)-Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina
6. Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Argentina

In the last decades, modified fatty acids have emerged as novel either protective or harmful molecules for tissues metabolism. Among these, nitro-oleic acid (NO2AO) is considered an anti-oxidant and anti-inflammatory molecule, also exerting many other protective activities in different tissues. Epicardial adipose tissue (EAT) is a visceral adipose tissue, which surrounds the myocardium and coronary arteries, considered a risk factor for Cardiovascular Disease (CVD). Results from our laboratory showed increased metalloproteinase (MMP) 2 and 9 activity in EAT from coronary patients, although they have not been studied in EAT secretome. Our aim was to evaluate MMP2 and MMP9 activities in EAT and subcutaneous AT (SAT) secretome from coronary patients, and their modulation by NO2AO at different times. Materials and Methods: EAT and SAT biopsies from patients undergoing by-pass graft were incubated in DMEM or DMEM+NO2AO 10 uM for 30 minutes or 3 hours (n=3 for each condition). After incubation, AT secretomes were stored at -70ºC until analysis. MMP2 and MMP9 activities were evaluated in EAT and SAT explants culture media, with and without NO2AO, obtained at both times by gelatinolytic zymography. Results: In EAT culture media, MMP2 activity tended to be lower in DMEM+NO2AO compared to DMEM after 3 hours of incubation (DMEM+NO2AO/DMEM ratio=0.8), while MMP9 activity tended to increase in DMEM+NO2AO at both times (DMEM+NO2AO/DMEM ratio=1.6 at both times). In SAT DMEM and DMEM+NO2AO, no differences were observed neither in MMP2 nor in MM9 activities at both times. Conclusion: In this preliminary report, it is the first time EAT secretome is evaluated after NO2AO treatment. EAT would secrete MMPs to its surrounding media, which could impact on myocardium and coronary arteries, and this could be differentially modulated by NOA. Further studies are required to elucidate NO2AO effect in EAT.

337. (215) **COMPARISON BETWEEN THE INTAKE OF WHOLE OAT MALTED FLOUR AND WHEAT FLOUR ON CHOLESTEROL LEVELS AND MOISTURE CONTENT IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RATS**

Adriana Weisstaub[1], Claudia Larregain[2], Ethel Coscarello[2], Magalí Barchuk[3], Gabriela Berg[3,4], Ángela Zuleta A[1].
1 Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 2 Facultad de Agronomía y Ciencias Agroalimentarias, Universidad de Morón, 3 INFIBIOC, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 4 CONICET.

The whole grain oat can be malted obtaining a flour with higher amount of soluble fibers like betaglucans, improving functional properties. The aim of this study was the comparison between the intake of malted flour oat (MFO) and wheat flour (WF) and its influence on cholesterol levels and volume stool during 60 days, in a rat model. A total of 24 male Wistar rats recently weaned (8/group) were fed with a control diet prepared according to the American Institute of Nutrition Diet (C), and semisynthetic diets prepared with WF and MFO. During the experience, feces were collected every 15 days until day 45 to determine average moisture content (AMC). At the end of the study rats were anesthetized and the cecum was excised, split open, and the pH of the cecal content and cecum weight were measured. Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) were measured in serum. Non-HDL cholesterol (Non-HDL-c) was calculated as a marker of atherogenic lipoproteins and TC/HDLc ratio as a risk index. The results showed that cecal content of MFO presented a lower pH than C without significant differences with WF (6.98±0.09 vs 7.42±0.25 vs 7.15±0.29; *p= 0.0054*). MFO showed higher values of cecum weight than C and WF (1.79±0.20 vs 1.19±0.24 vs 1.33±0.26 g; *p< 0.0001*). TC values were lower in MFO and C comparing with WF group (92.0±11.4 vs 62.3±8.8 vs118.2±17.6 mg/dl; *p< 0.0001*). HDLc levels were higher in MFO than in C and WF groups (58.8±6.6 vs 45.6±8.5 vs41.1±11.4 mg/dl; *p=0.0030)*. Non-HDLc levels were lower in MFO and C than WF group (33.3±7.6 vs 16.7±2.4 vs 77.9±9.8 mg/dl; *p<0.0001)* as well as TC/HDLc ratio (1.6±0.1 vs 1.4±0.1 vs 3.2±1; *p=0.0002*). AMC

was higher in MFO than C and WF groups (17.4±6.4 vs 14.0±6.7 vs 12.6±2.0 %; *p=0.0047*). Whole oat malted flour showed a prebiotic effect improving lipid profile and moisture content, compared to wheat flour, considering the design of healthier beaked goods.. Financed by UBACyT Nº 20020170100148BA.

338. (217) **ANGIOPOIETIN-LIKE PROTEIN 3 IN INSULIN-RESISTANCE: A DIRECT MODULATOR IN LIVER-ADIPOSE TISSUE CROSS TALK?**

Fariña Gregorio[1], Campos Jeanniel[1], Olano Carolina[1], Barchuk Magali[1,2], Macri Vanesa[3], Schreier Laura[1], Zago Valeria[1,2], Berg Gabriela[1,2].

1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires.

2. Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Argentina

3. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Bioquímica General y Bucal, Buenos Aires, Argentina

Atherogenic dislipemia is a risk factor for different diseases, such as cardiovascular disease (CVD), diabetes and nonalcoholic fatty liver disease (NAFLD). Angiopoietin-like proteins (ANGPTLs) family, especially ANGPTL3, 4 and 8, which regulate lipoprotein lipase (LPL) activity, play pivotal roles in triglyceride (TG) rich lipoprotein metabolism. Besides, little is known about the impact of ANGPTL3 on hepatic lipase (HL), responsible for LDL and HDL catabolism. Although there is some data on the relationship between ANGPTL3 and NAFLD, their results are inconsistent. Our aim was to evaluate HL and LPL activities and their regulation by ANGPTL3 in an insulin-resistance (IR) and NAFLD animal model. Methods: We studied 22 male Wistar rats, fed a high-fat and high sucrose diet (HFHS, n=11) or control diet (C, n=11) during 14 weeks. At euthanasia, blood, liver, and adipose tissue (AT) were obtained. In serum lipoprotein profile, glucose, and free fatty acids were assessed, and lipoproteins were isolated by ultracentrifugation and characterized. In liver, intracellular fat, HL activity, and ANGPTL-3 levels were evaluated. In AT, LPL activity was measured. Results: HFHS group presented higher IR markers than C, as well as increased liver fat content (p= 0.03). In liver, no differences were observed neither in HL activity nor in ANGPTL-3 levels. LPL activity in AT tended to be lower in HFHS compared to C (p=0.06), inversely associated with TG (r=-0.673,p=0.03) and with VLDL-TG content (r=-0.703,p<0.05). Conclusion: Even though it is reported that ANGPTL3 inhibits LPL, in this model, the lack of differences in liver ANGPTL3 expression, despite the decrease in AT LPL activity would indicate that other regulators could be involved in liver-AT cross talk. Further studies would be necessary to elucidate ANGPTL3 role in NAFLD.

339. (219) **EVALUATION OF SODIUM INTAKE IN OLDER ADULTS: AN UNDERUSED TOOL**

Cueto María del Rosario[1], Nápoli Cristian[1], Pennisi Mariana[1], Fernandez Inés[1], Possidoni Cristina[2], Tarducci Gabriel[3], Vidueiros Silvina Mariela[1], Pallaro Anabel[1]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Nutrición. [2]Hospital Sagrado Corazón de Jesús, Basavilbaso, Entre Ríos. [3]Universidad Nacional de La Plata, Facultad de Humanidades y Ciencias de la Educación, Cátedra de Actividad Física para la Salud, IdHICS CONICET.

Elevated sodium intake (NaI) is a risk factor related to NCDs such as hypertension and cardiovascular diseases, being a huge public health problem. In Argentina, NaI data is very scarce particularly in older adults. In this work, the aim was to assess the NaI in a group of older adults. A descriptive study was conducted in 19 women (W) (73±10y) and 11 men (M) (76±10y) who had signed an informed consent. These subjects had either attended a Primary Health Care Center in Entre Ríos province, reside at nursing homes for elderly in Buenos Aires province or took part as participants in a research scholarship. Body weight (BW, kg) and height (H, m) were determined to calculate Body Mass Index (BMI= BW/H^2, kg/m^2). Sodium, potassium (direct ISE) and creatinine excretion (Jaffé method) were determined in spot urine and the 24h urinary sodium excretion (24-hUNa) was estimated using the INTERSALT (I) and Tanaka (T) prediction equations. In a subsample (n=11), 24h-UNa was determined in 24-h urine collections. The results showed that 68% of W and 45% of M were overweight or obese. 24-hUNa estimated by both equations pointed out an increased sodium dietary intake (>2g/d) in 50% of the subjects up to 93%. In addition, Na/K ratio was increased in 83% of the subjects (2,7±2,1; cutoff 1:1). Moreover, 24-hUNa estimated by I in W was significantly different compared to T (1,7±0,8 vs 3,4±1,1; p<0.0001) but no difference was observed in M (3,6±1,0 vs 3,4±1,0). In the subsample, the NaI determined in 24-h urine collections was associated to 24-hUNa estimated by I (r=0,8761; p=0,0004) and no differences were observed between methods (B&A test, r= -0,002; p<0,995). Regardless of the used prediction equation, an excess in sodium intake was observed in this group of older adults. INTERSALT was a good predictor of 24-hNa while Tanaka predicted higher, mainly in W. The assessment of this underused risk factor would contribute as a tool for better diagnosis of NCDs. Supported 20720170100008BA.

340. (272) **TOTAL OXIDANT STATUS IN CELIAC DISEASE AND DIET**

Motta Estela[1], Castelli Carla[1], Franco Micaela[1,3], Chisari Andrea[1,2]

[1] Departamento de Química y Bioquímica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata.

[2] CONICET. Buenos Aires. Argentina.

[3] Sector Inmunología Clínica, Laboratorio Central Hospital Oscar Alende, Mar del Plata, Argentina

Celiac disease (CD) is a chronic small intestinal immune-mediated enteropathy induced by dietary gluten. The only treatment is a gluten-free diet (GFD). The gliadin sequence contains regions which play a special role in CD pathogenesis and trigger oxidative stress. Various dietary components have the potential to modulate predisposition to intestinal chronic inflammatory conditions and have a role in nutritional therapy of celiac disease. Objective: to determine the total oxidant status in celiac and non-celiac people. Materials and Methods: Adults diagnosed with biopsy-confirmed CD (7 females) and non-CD (3 females and 7 males) participated in this study followed by completion of one food questionnaire and had blood drawn for total oxidant status (TOS), hemoglobin and anti-transglutaminase antibodies. The estimations of the total antioxidant capacity (TAC) were carried out with the food intake data and the tabulated TAC values (umol TE / g of food) in previous publications. Results: TOS was measured using Erel's method (2005), the results were in non-celiac adults and celiac patients between undetectable and 6.6 uM (mean: 2.07 uM) and between undetectable and 2.84 uM (mean: 1.73 uM) respectively. Non statistically significant differences were found between the two groups (p = 0.3415, > 0.05). In celiac patients, serum anti transglutaminase IgA results were negative and Ig A levels were within the normal range (40-295 mg%). Hemoglobin content in celiac patients were in the normal range (12.2-18.1 g%). Eighty-six percentage of celiac patients adhere well to a GFD. An adequate consumption of antioxidants from diet (>10000 trolox, umol TE / day) was determined. Conclusions: No oxidative increase was observed in celiac vs non celiac adults. An adequate antioxidant intake from food in both groups was observed. Biomarkers studies of antioxidant status are necessary to calculate the oxidative stress index (OSI)

341. (311) **HYPERLIPEMIC DIET WITHOUT CARBOHYDRATE SUPPLEMENTATION: BIOCHEMICAL ASSESSMENT AND EFFECT ON LIVER TISSUE IN ADULT NEWZEALAND RABBITS**

María Virginia Avena[1,2], Abi Karenina Funes[2], Rocío Miranda Heredia[1], María Lucía Elias[1]. Pérez, Franco Manuel Pérez[1], Jesica Anabella Mussi[1], Miguel Walter Fornés[2], Tania Emilce Estefanía Sáez Lancellotti[2], Paola Vanina Boarelli[1]

1 Laboratorio de Enfermedades Metabólicas (LEM) – UMa-

za, 2 Laboratorio de Investigaciones Andrológicas de Mendoza (LIAM) – IHEM -CONICET

Acquired dyslipidemias are associated with various metabolic disorders such as obesity and metabolic syndrome (MS). Our objective was to develop an experimental model (adult male New Zealand rabbits) with dyslipidemia induced by a lipid diet without added carbohydrates, and thus develop biochemical and tissue parameters without generating MS. Nine animals (N:9) were fed for 3 months with standard rabbit chow (SRC). Dietary supplementation was performed during three consecutive trimesters (I, II, III). I: N:5 (Fg) were fed with SRC+14% of bovine fat (F) and N:2 (Og) were fed with SRC+14% of olive oil (OO). N:2 were fed only SRC (SRCg). II: N:3 from Fg consumed 7% of F (HF sg). III: HFsg consumed 7% of F+7% of OO (HFOsg). Weight (W), glucose (G), insulin, liver enzymes (LE) and serum lipid [cholesterol (C), HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglycerides (TG)] were measured at each thirty days. In the W was not observed significant differences with the mean age. Insulin and LE were not conclusive, G and TG, there were not significant differences. However, C was increased in Fg (57.3±10.5 mg/dL) *vs* SRCg (39.1±4.9 mg/dL), with a decrease of HDL (5.2±1.7*vs*.3.9±0.5: mg/dL) and an increase in LDL (21.3±5.6 *vs.* 36.1±11.4; mg/dL). The Og did not present a significant increase in HDL compared to Fg. The HFOsg presented an increase in HDL (7.9±0.4 mg/dL) with OO added to the diet, but without modifying C (HFsg *vs* HFOsg: 44.9±7.2 *vs.* 46.9±15.2; mg/dL). During I and II, Fg and HFsg did not experience significant changes in C and HDL. Only in III, an increase in HDL could be observed in HFOsg. The liver tissue showed low microvesicular steatosis in Fg and HFg. No significant signs of inflammation or fibrosis were demonstrated. An increment of C by Filipin III was observed in Fg, consistent with the decrease in immunostaining by IFI and WB of $SREBP_2$. These preliminary results allow study the relationship between biochemical and tissue results.

342. (372) **OXIDATIVE STRESS BIOMARKERS AND PATHWAYS OF HEPATIC LIPID METABOLISM IN DAIRY CATTLE SUPPLEMENTED WITH MINERALS AND VITAMINS DURING THE TRANSITION PERIOD**

Emmanuel Angeli[1,2]; Daiana Barcarolo[1]; Joaquín Canavecio[1,2]; Florencia Rey[1,2]; Valentina Matiller[1,2]; Hugo Héctor Ortega[1,2]; Gustavo Juan Hein[1,3]

[1]Laboratorio de Biología Celular y Molecular Aplicada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina.[2]Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. [3]Centro Universitario Gálvez (CUG-UNL), Gálvez, Santa Fe, Argentina

The transition period is the most critical stage in the lactation of a dairy cow, characterized by lipid mobilization and a great metabolic demand that could increase oxidative stress (OS). Therefore, this study aimed to evaluate some OS parameters and pathways of hepatic lipid metabolism in cows supplemented with vitamins and minerals during this period. The supplemented group (SG; n = 11) received a subcutaneous dose of 5 ml of the vitamin supplement ADAPTADOR® Vit and 5 ml of the mineral supplement ADAPTADOR® Min (Biogenesis Bagó, Bs. As.; vitamin A palmitate 3.5% and vitamin E acetate 5%, copper edetate 1%, zinc edetate 4%, manganese edetate 1% and sodium selenite 0.5%) on -60, -30 and 7 days relative to calving. The control group (CG; n = 11) received two injections of 5 ml of 0.9% sodium chloride solution. Blood, urine and liver biopsies were sampled at -21, 7 and 21 days relative to calving, and malondialdehyde (MDA) was evaluated by mass spectrometry as an OS biomarker. In addition, serum concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate acid (BHBA) and the total antioxidant status (TAS) were determined by using commercial colorimetric assay kits. Finally, triacylglycerol content was measured spectrophotometrically, and protein expressions of carnitine palmitoyltransferase-1 (CPT1), acyl-CoA oxidase-1 (ACOX1); diacylglycerol acyltransferase-1 (DGAT1) and nuclear receptor PPAR-alpha were evaluated in liver biopsies by Western blot. No differences in NEFA and BHBA concentrations were observed ($p > 0.05$). However, a lesser hepatic triacylglycerol content was registered in cows of the SG ($p < 0.05$). Regarding liver enzymes, a greater protein expression of CPT-1 was observed in the SG ($p < 0.05$) without differences in the other enzymes evaluated ($p > 0.05$). Similar MDA concentrations and TAS were observed ($p > 0.05$). These results suggest that mineral and vitamin supplementation could influence liver physiology by improving the adaptation of the dairy cow during the transition.

343. (377) **MATERNAL INTAKE OF HIGH-FAT DIET DURING PREGNANCY AND LACTATION: SEX DIFFERENCES IN CARDIOMETABOLIC OUTCOMES IN OFFSPRING**

Damián Soria[1,2], Melanie Oviedo[1], Juan Gaetani[2], Analía Tomat[1,2], Cristina Arranz[1,2], Rosana Elesgaray[1,2], Tamara Zaobornyj[3,4], Carolina Caniffi[1,2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Fisiología, Buenos Aires, Argentina. [2]CONICET - Universidad de Buenos Aires, Instituto de Química y Metabolismo del Fármaco - CONICET (IQUIMEFA), Buenos Aires, Argentina. [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Fisicoquímica, Buenos Aires, Argentina. [4]CONICET - Universidad de Buenos Aires, Instituto de Bioquímica y Medicina Molecular (IBIMOL), Buenos Aires, Argentina

The intrauterine environment associated with maternal high-fat diet (HFD) increases the risks of non-communicable diseases later in life. The aim is to evaluate the effects of maternal HFD on metabolic homeostasis and oxygen consumption in left ventricle (LV) in female (f) and male (m) offspring. Experimental design: Wistar f rats fed either HFD (60% calories from fat) or standard diet (SD) from pregnancy to offspring weaning. At 21 days of life, f and m offspring were weighted (body weight, BW, g), and systolic blood pressure (SBP, mmHg) and fasting glycemia (mg/dL) were measured. After sacrifice, retroperitoneal adipose tissue (rAT, g), soleous muscle (SM, g), and LV were removed and weighted. Also, tibial length (TL, cm) was measured. In LV, oxygen consumption was measured, and the respiratory control (RC) was calculated. The protocol was approved by CICUAL-FFyB-UBA. Results are expressed as mean±SEM. Statistical analysis: 2-way ANOVA, n=5-6 rats/group; interaction diet x sex (i): \$p<0.05; *p<0.05; ***p<0.001 vs SDf; #p<0.05; ##p<0.01; ###p<0.001 vs SDm. Results: SBP was similar in all groups. BW was higher in HFDm (SDf=52.0±2.2; SDm=49.7±1.5; HFDf=51.6±0.9; HFDm=56.8±0.8###; i:\$). rAT/TL was higher in HFDf and HFDm compared to SDf and SDm, respectively (SDf=45.9±7.6; SDm=46.3±5.9; HFDf=84.6±3.5***; HFDm=101.0±2.3###) and SM/TL was lower in SDf (SDf=10.2±0.6; SDm=10.6±0.6; HFDf=7.6±0.3*; HFDm=9.4±0.9). Glycemia was lower in pups from HFD mothers (SDf=111.0±1.6; SDm=113.4±3.1; HFDf=92.3±4.9*; HFDm=92.8±6.5#). RC was higher in SDm compared to SDf, and HFDm showed lower RC than SDm (SDf=6.89±0.38; SDm=8.29±0.32*; HFDf=6.28±0.54; HFDm=5.43±0.43##; i:\$)

Conclusions: Maternal intake of HFD during pregnancy and lactation induces alterations in body composition with differences between f and m pups. These alterations are accompanied by a drop in RC in LV, showing that m offspring exposed early in life to maternal HFD have impaired cardiac mitochondrial functional integrity.

344. (393) **EFFECT OF A HYPERCALORIC DIET ON HYPOANDROGYNOUS RAT LUNG**

Biaggio Veronica[1, 2,3], Aballay Federico[1], Salinas Marysol[1], Zelarayan Sarmiento Daniela[1], Piguillem Silvana[1], Ciminari Eugenia[1], Razzeto Gabriela[1], Salinas Eloy[1], Álvarez Silvina [1, 2,3], Pérez Chaca Veronica[1, 2], Gomez Nidia N. [1, 2 ,3]

1- Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. E-mail: vbiaggio@gmail.com

2- Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL- CONICET)

3- Proyecto de Unidad Ejecutora (PUE). Resolución N°-2018-930-APN (IMIBIO-SL-CONICET)

Obesity is a systemic state of inflammation and oxidative stress that affects normal respiratory functioning. The presence of andro-

gen receptors in lung reveal that these hormones might play a key role in lung physiology. Obesity-mediated oxidative stress produced in adipose tissue is one of the main factors considered as oxidant source and inflammation mediator. The aim of this work was to study the effect of diet-induced obesity on the lung of androgen-deficient rats (castrated). Wistar male rats (200 ± 20 g) were separated in four groups: Control with normal diet (CoN), castrated with normal diet (KN), control with hypercaloric diet (CoOB) and castrated with hypercaloric diet (KOB) and sacrificed 30 days after castration. Biochemical parameters were analysed in serum and the expression of antioxidant enzymes and NOX-2, FOXO, HO-1 and RA in lung. ANOVA and Tukey test were used for statistical analyses. The results showed TBARS levels increased in KOB group compared to CoN ($p<0.001$) and KN ($p<0$, 01) groups, respectively. CAT activity was increased in KN ($p<0.05$) group. HDL levels increased in CoOB ($p<0.001$), KN ($p<0.001$) and KOB ($p<0.01$) groups. Both, urea and TG determinations were increased in KN ($p<0.001$); $p<0.01$ and KOB ($p<0.001$) groups. CL was increased in CoOB ($p<0.001$); KOB ($p<0.01$) compared to CoN group and increased in KN ($p<0.001$) and KOB ($p<0.001$) compared to CoOB group. CAT expression decreased in CoOB and KN groups, and RA expression increased in KN group compared to control group. Antioxidant enzymes NOX-2 and SOD-2 ($p<0.01$) and GPx-1($p<0,05$) increased in KOB group compared to control. FOXO-1 and HO-1increased in castrated group, with obesogenic diet ($p<0.01$). We previously demonstrated an important oxidative stress state in a castrated animal model. In conclusion, obesity added to androgen deficiency modifies different serum parameters. In fact, some inflammatory molecular pathways reveal a potential relationship between both situations (androgen deficiency-obesity).

345. (919) **ADIPOSE TISSUE AND INSULIN-RESISTANCE. BENEFICIAL EFFECTS OF GLP-1 AGONISTS**

María Florencia Quintanilla[1], Vanessa Touceda[2,3], Magali Barchuk[1], Paola Finocchietto[4], Celina Morales[5], Graciela Lopez[1], Silvia Friedman[3], Laura Schreier[1], Gabriela Berg[1], Veronica Miksztowicz[2,3].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica. Departamento de Bioquímica Clínica. Laboratorio de Lípidos y Aterosclerosis. [2]Instituto de Investigaciones Biomédicas (UCA-CONICET). Laboratorio de Patología Cardiovascular Experimental e Hipertensión Arterial. [3]Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal. [4]Universidad de Buenos Aires. Facultad de Medicina. Instituto de Inmunología, Genética y Metabolismo (INIGEM UBA-CONICET). Laboratorio de Metabolismo del Oxígeno. [5]Universidad de Buenos Aires. Facultad de Medicina. Instituto de Fisiopatología Cardiovascular (UBA-CONICET).

Insulin-resistance (IR) is characterized by adipose tissue (AT) expansion associated with extracellular matrix (ECM) remodeling. Metalloproteinases (MMPs) are endopeptidases involved in adipogenesis and angiogenesis, and they are proposed as pharmacological targets. Liraglutide (L), a glucagon-like peptide type 1 agonist, has emerged for the management of IR, and its effects on AT are still investigated. Aim: to evaluate the effect of L on MMPs activity in an animal model of IR. Methods: male Wistar rats (180-200 g) were divided in: Control (C, n=11) fed with standard diet, and sucrose rich diet group (SRD, n=14) fed with standard diet and sucrose 30% in drinking water during 15 weeks. Then, both groups were subdivided according to subcutaneous administration of L (0.6 mg/kg/day) for 5 weeks. The study was approved by the Ethic Committee-FFYB (UBA). Serum glucose, and lipid and lipoprotein profile were measured. Visceral AT (perirrenal, intestinal and epididymal) was removed and weighed. In epididymal AT (EAT) histological characteristics, MMPs activity by gelatinolytic zymography and antioxidants enzymes (SOD and Catalase) were evaluated. Results: as expected, SRD presented higher visceral AT mass ($p<0.05$), TG and glucose levels ($p<0.05$) than C. In SRD+L group, a significant decrease in body weight ($p<0.01$), EAT mass ($p<0.01$), TG ($p=0.045$) and glucose ($p=0.05$) levels compared to SRD was observed. As expected, SRD presented lower density of larger adipocytes than C ($p<0.05$). In turn, vascular density was lower in SRD ($p<0.05$ vs C). L decreased adipocyte size ($p<0.05$ vs SRD), as well as significantly increased vascular density in SRD+L ($p<0.001$ vs SRD). MMP-2 activity decreased in EAT from SRD ($p<0.05$ vs C) and increased in SDR+L ($p<0.05$ vs SRD). L decreased SOD activity in EAT in the SRD group ($p<0.01$) with no change in catalase activity. Conclusions: In IR, liraglutide would improve AT functionality by preventing disfavored features during MEC remodeling.

METABOLISM AND NUTRITION II

Friday, November 18, 9-10:30 hr
Chairs: Gustavo Hein - Gabriela Berg - Liliana Monasterolo

346. (147) **RENAL INFLAMMATION INDUCED BY HIGH FAT DIET IN RATS IS ASSOCIATED WITH RENAL DOPAMINERGIC SYSTEM ALTERATION AND SODIUM EXCRETION IMPAIRMENT**

Silvana M. Cantú[1,2], Hyun Jin Lee[1,2], Gabriel Kim, Ludmila Fenocchio[1], Christian Höcht[3], Adriana S. Donoso[1,2], Ana María Puyó[1,2], Marcelo R. Choi[1,4]

1 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Biológicas, Cátedra de Anatomía e Histología. Buenos Aires, Argentina. 2 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. 3 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Farmacología Cátedra de Farmacología. Buenos Aires, Argentina. 4 CONICET – Universidad de Buenos Aires. Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional (IATIMET). Buenos Aires, Argentina.

High fat diet (HFD) can cause metabolic alterations and a proinflammatory state that leads chronically to organ damage. In the kidneys, the renal dopaminergic system (RDS) plays an important role through its anti-inflammatory action by D2 like receptors (D2DR) activation. The aim of this study was to demonstrate the relationship between the RDS and natriuresis alterations with inflammation induced by a HFD in an animal model. For 8 weeks, Sprague Dawley rats (n=4-6/group) were randomized to HFD (50% w/w of bovine fat added to standard rodent diet) and control group (C) (standard rodent diet) and tap water to drink for both groups. The following determinations were performed; Insulinemia (commercial ELISA kit), L-dopa and dopamine urinary excretion (HPLC, L-dopa/dopamine index was calculated), expression of D2DR and pro-inflammatory molecules in renal cortex (Western blot), fibrosis (Picro Sirius red stain), and natriuresis through urinary sodium excretion (UNa.V). Results: L-dopa/dopamine index (HFD 3.7 ± 0.7 vs C 1.1 ± 0.1; $p<0.05$); insulinemia (HFD 3.2 ± 0.3 vs C 0.7 ± 0.1; $p<0.0005$); fibrosis % (HFD 28.0 ± 1.8 vs C 15.0 ± 0.8; $p<0.005$); NFkB (HFD 6.2 ± 0.8 vs C 2.2 ± 0.5; $p<0.05$); TGF-beta (HFD 8.3 ± 1.4 vs C 4.4 ± 0.6; $p<0.05$), were significantly improved in HFD vs C. D2DR (HFD 5.1 ± 0.8 vs C 12.3 ± 0.3; $p<0.005$) and UNa.V (mEq/24hs) (HFD 0.5 ± 0.1 vs C 1.8 ± 0.2; $p<0.0005$) were significantly decreased in HFD vs C. We observed positive correlations between L-dopa/dopamine index vs insulin ($r^2=0.90$, $p<0.001$), fibrosis % ($r^2=0.89$, $p<0.001$), NFkB ($r^2=0.83$, $p<0.001$); and fibrosis % vs insulin ($r^2=0.90$, $p<0.001$). Additionally, negative correlations between UNa.V vs L-dopa/dopamine index ($r^2=0.80$, $p<0.001$), D2DR ($r^2=0.93$, $p<0.001$), fibrosis % ($r^2=0.91$, $p<0.001$), and fibrosis % vs D2DR ($r^2=0.80$, $p<0.01$) were found. In conclusion, inflammation caused by a HFD leads to alterations of the RDS contributing to a reduction of renal sodium excretion.

347. (153) **OXDIDATIVE STRESS AND RESPIRATORY BURST STIMULATED BY URIDINE DIPHOSPHATE GLUCOSE (UDP–G) IN HUMAN NEUTROPHILS**

Claudio Carbia[1], Iris Chiesa[2], Fabiana Lairion[2,3], Alberto Lazarowski[1], Marisa G. Repetto[2,3]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bio-

química, Departamento de Bioquímica Clínica, Bioquímica Clínica II, Hematología, Instituto de Fisiopatología y Bioquímica clínica (INFIBIOC), Buenos Aires, Argentina, [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Química General e Inorgánica, Buenos Aires, Argentina, [3]Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL, UBA-CONICET), Buenos Aires, Argentina

The purinergic agonist uridine diphosphate glucose (UDP-G) activates chemotaxis of human neutrophils (PMN). The aim of this study is to show that PMN activation by UDP-G is associated to oxidative stress (OS) and respiratory burst (RB). Methodology: OS was evaluated by measuring spontaneous chemiluminescence (CL) of cells with a scintillation photon counter, and RB by measuring oxygen consumption with an oxygen electrode type Clark at 37 ºC, at basal condition (control, C) or after activation (15 min) with lipopolysaccharides (LPS, 2 μg/mL), phorbol myristate acetate (PMA, 20 ng/mL) or UDP-G (100 μM). To establish the activation effect of the three agonists, the stimulation index (SI) was calculated for each different treatments as the ratio (quotient) between the mean of the parameter evaluated in activated cells and non-activated cells. To establish the effect of UDP-G on PMN function, increasing concentrations of UDP-G were evaluated (0-200 μM). Results: The optimal cell concentration to evaluate OS in PMN was $1x10^5$ PMN/mL with an enhancement in CL of 35% ($p<0.05$) and 56% ($p<0.01$) and SI of 1.56 and 2.20 for both LPS and PMA respectively, and to evaluate the oxygen consumption in activated PMN with PMA was $1x10^5$ cells/mL, showing a significantly increase of 40% ($p<0.001$), and $5x10^5$ PMN/mL, with increment of 100% ($p<0.05$). When UDP-G was used as stimuli, an increase of 80 to 100% in the RB was observed ($p<0.001$) with 1 or $5x10^5$ cells/mL and SI of 1.2, 1.4 and 1.8 for LPS, PMA and UDP-G respectively for $1x10^5$ cells/mL. Oxygen consumption increased (38-50%, 100-200 μM) with UDP-G concentration (C: 87±1 nmol O_2/min/10^6 PMN, $p<0.0001$) and CL decreased with increments of UDP-G concentration (60%, 25 μM, $p<0.05$; 90%, 50-150 μM, $p<0.001$). Discussion: UDP-G is able to activate neutrophils associated to OS and RB; LPS and UDP-G have a synergistic effect on PMN activation suggesting a key role in infection and/or sepsis.

348. (233) **COPPER(II)-INDUCED IgG AGGREGATES BECOME IRREVERSIBLY AGGREGATED BY HYDROGEN PEROXIDE**

Christian Saporito-Magriñá[1,2], Lila Lopez-Montañana[1], María Laura Facio[3], Fabiana Lairion[1,2], Marisa Gabriela Repetto[1,2]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Química General e Inorgánica, Buenos Aires, Argentina, [2]Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL, UBA-CONICET), Buenos Aires, Argentina, [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Bioquímica Clínica I, Química Clínica Especial: Proteínas, Instituto de Fisiopatología y Bioquímica clínica (INFIBIOC), Laboratorio de Proteínas, Buenos Aires, Argentina

Proteins are prone to aggregation and free cupric ions (Cu(II)) promote this process. Such aggregates are found in plasma as detergent resistant aggregates (DRA). Cu(II) ions are found in the micromolar range in plasma but rise in pathologies such as Wilson Disease. We have shown that IgGs are selectively aggregated by Cu(II). Therefore, plasma Cu(II) may be a driving force for IgG aggregation. Additionally, DRA have been reported to increase in elders. We speculate that the oxidative modifications of the aggregates impair their clearance and drive their accumulation over the years. Methods: Optic density (OD), Lowry assay, Carbonyl content (CO), SDS-PAGE. Results: Followed by OD, Cu(II) and Zn(II) induce IgG aggregation at 5 μM and 20 μM, respectively (n=3). Zn(II) was employed as a non-redox active metal. Upon addition of H_2O_2, Cu(II)-induced aggregates (Cu(II)IA) become irreversibly insoluble while Zn(II)-induced aggregates (Zn(II)IA) are fully dissolved upon metal removal by EDTA. Cu(II)IA in the presence of H_2O_2 are also SDS-resistant whereas Zn(II)IA are completely soluble (n=3). Cu(II) IA become irreversibly insoluble in the presence of H_2O_2 (4 mM). However, when followed by SDS-PAGE, concentrations of 50 μM H_2O_2 yet yield Cu(II)IA with higher mass than monomeric IgG, indicating the formation of soluble DRA (n=3). In contrast, when Zn(II)IA with H_2O_2 mixtures are assessed by SDS-PAGE, no soluble higher mass aggregates are observed. Furthermore, the CO content significantly rises when IgGs are incubated with H_2O_2 in the presence of Cu(II) when compared to H_2O_2 alone ($p<0,01$). Discussion: Cu(II) reacts with H_2O_2 in an oxidative step which may drive the formation of DRA. While Zn(II) ions are also capable of inducing protein aggregation, this metal is not able to participate in this oxidative step. Thus, Zn(II)IA do not become DRA. Likewise, Cu(II), among other stimuli, could promote the oxidation of proteins undergoing aggregation in circulation, yielding DRA.

349. (236) **IRON(III) AND IRON(II) INDUCE SELECTIVE AGGREGATION OF IgG IN HUMAN PLASMA**

Christian Saporito-Magriñá[1,2], Lila Lopez-Montañana[1], Aldana Rodríguez[1], Guadalupe Belén[1] María Laura Facio[3], Marisa Gabriela Repetto[1,2]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Química General e Inorgánica, Buenos Aires, Argentina, [2]Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL, UBA-CONICET), Buenos Aires, Argentina, [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Bioquímica Clínica I, Química Clínica Especial: Proteínas, Instituto de Fisiopatología y Bioquímica clínica (INFIBIOC), Laboratorio de Proteínas, Buenos Aires, Argentina

Fe(II) is released from the enterocytes into the plasma, oxidized to Fe(III) by ferroxidases and transported by transferrin. Non-transferrin bound Fe(III) is present in plasma at a concentration of 1 μM. This labile Fe(III) drastically rises in pathological conditions such as thalassemia, hemochromatosis, etc. Here we show that both Fe(II) and Fe(III) selectively precipitate IgG, yielding a detergent resistant aggregate (DRA). DRA are found in elder patients and may be relevant in pathology. However, their nature is unclear. Methods: Optic density (OD), Lowry assay, SDS-PAGE, proteinogram, Mass Spectrometry (MS). Results: Followed by OD, Fe(II) and Fe(III) induce aggregation of plasma proteins at concentrations lower than 25 μM. This was confirmed by protein quantification in the aggregate fraction ($p<0.01$). The analysis of the aggregates by SDS-PAGE shows albumin depletion and an enrichment of a possibly IgG (n=3). The proteinogram of the aggregate shows enrichment of γ-globulin fraction when compared to control plasma (n=3). The analysis of the aggregate by MS indicated confirmed the identity of the IgG present in the aggregate and the depletion of albumin. Additionally, minoritarian proteins could be observed enriched such as fibrinogen, and serum amyloid A1, among others. The incubation of isolated IgG with Fe(II) or Fe(III) highlighted the sensitivity of this protein to aggregation, followed by OD. This was confirmed by quantification of the protein content on the aggregate ($p<0.01$). Fe(II) and Fe(III)-induced aggregates were shown to be irreversibly insoluble as they were not redissolved upon metal removal with EDTA. Additionally, the aggregates were only partially soluble in SDS as observed when followed by OD, indicating that they are DRA. Discussion: The formation of DRA occurs in plasma of healthy patients and may be increased over the years as reported in the literature. Fe(II) and Fe(III) may be contributing forces to the formation of such aggregates.

350. (313) **GLP-1 AGONISTS AND BONE QUALITY IN OBESITY. EFFECTS OF LIRAGLUTIDE ON BONE STRUCTURAL AND BIOMECHANICAL PROPERTIES**

Vanessa Touceda [1,2], Romina De Lucca [2], Florencia Fontana-Estevez [1], Romina Bustos [1], Clarisa Bozzini [3], Leonardo Cacciagiú [2,4], Silvia Friedman [2], Germán Gonzalez [1], Verónica Miksztowicz [1,2].
[1] Instituto de Investigaciones Biomédicas (UCA-CONICET). Laboratorio de Patología Cardiovascular Experimental e Hipertensión Arterial, [2] Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal, [3]

Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Fisiología, [4] Hospital General de Agudos Teodoro Álvarez. Laboratorio Central. Sección Bioquímica,

Obesity is associated with increase bone resorption and loss of tissue architecture. GLP-1 agonists have shown beneficial effects in reducing body weight and glycemic control, however their action on bone tissue is unknown. Aim: to evaluate the effect of liraglutide (L), GLP-1 agonist, on bone histological characteristics and biomechanical properties in an animal model of obesity. Methods: Male C57BL/6 mice (8 weeks old) were divided in Control (n=6) fed with standard diet, and high fat diet group (HFD, n=10) fed a diet with 40% of total calories from fat for 15 weeks. Then, both groups were subdivided according to subcutaneous administration of L (200ug/kg/day) or vehicle for 5 weeks. Body weight and caloric intake were registered weekly. The study was approved by the Ethic Committee of BIOMED. Glycemia, lipid profile and calcaemia were measured. Femur, tibia and visceral adipose tissue (VAT) were removed and weighed. Tibia length was measured to calculate femur/tibia index. Histological characteristics and biomechanical properties of femurs ((maximum fracture load (Wf max), limit elastic load (Wy), and diaphyseal stiffness (Wydy)) were evaluated using a three-point bending test. Results: In HFD VAT mass was higher compared to Control ($p< 0.01$), and decreased in HFD+L ($p=0.002$). Total and non-HDL cholesterol increased in HFD ($p<0.01$ vs Control) and decreased in HFD+L group ($p<0.01$ vs HFD). Femur weight and femur/tibia were lower in HFD ($p<0.05$ vs Control), while femur weight increased in HFD+L ($p<0.01$ vs HFD). Among biomechanical properties, Wy decreased in HFD ($p<0.05$ vs Control) and increased in HFD+L ($p=0.05$ vs HFD); Wydy presented a similar behavior. A lower bone tissue volume was observed in HFD which was accrual in HFD+L. Conclusions: in obesity, liraglutide could prevent deleterious effects observed in femurs improving structural and biomechanical properties. Further studies are necessary to elucidate the mechanisms of liraglutide on bone quality.

351. (314) **EFFECTS OF HIGH SALT DIET CONSUMPTION ON VISCERAL ADIPOSE TISSUE BEHAVIOR**

Vanessa Touceda [1,2], Leonardo Cacciagiú [2,3], Florencia Fontana-Estevez [1], Romina Bustos [1], Celina Morales [4], Germán Gonzalez [1], Verónica Miksztowicz [1,2].

[1]Instituto de Investigaciones Biomédicas (UCA-CONICET). Laboratorio de Patología Cardiovascular Experimental e Hipertensión Arterial, [2]Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal, [3]Hospital General de Agudos Teodoro Álvarez. Laboratorio Central. Sección Bioquímica, [4]Universidad de Buenos Aires. Facultad de Medicina. Instituto de Fisiopatología Cardiovascular (UBA-CONICET).

High salt intake (NaCl) is associated with high blood pressure and cardiovascular disease. In Argentina, the average consumption of salt per person is 11 g/day, twice the amount recommended by the World Health Organization, which suggests an intake <5 g/day. It has been proposed that chronic salt intake would alter the functionality of different tissues, independently of blood pressure. Our aim was to evaluate the effect of high salt diet (HSD) on adipose tissue (AT) remodeling in an animal model. Methods: Male C57BL/6 mice (8 weeks old) were divided into Control (n= 8) fed with standard diet and HSD group (n=4) fed a diet with NaCl 8% during 20 weeks. Body weight, food and water consumption were registered weekly and arterial pressure (AP) was evaluated by plethysmography at the beginning and end of experience. The study was approved by the Ethic Committee of BIOMED. Glycemia, lipid profile and sodium levels were measured. Epididymal AT (EAT), as representative of visceral AT, and liver were removed and weighed. Histological characteristics of EAT (adipocyte area and adipocyte and vascular density) were evaluated, and oxidative stress was determined through TBARS levels and antioxidant enzyme activity (SOD and Catalase) measurement. Results: HSD group presented a higher water ($p<0.0001$) and caloric ($p=0.01$) intake. Body weight, EAT and liver mass were significantly lower in HSD ($p<0.01$). AP was similar between groups. In HSD, triglycerides levels were decreased ($p<0.01$). In reference to histological characteristics, though adipocytes density and size were similar between groups, EAT of HSD presented lower vascular density ($p=0.03$). TBARS levels were higher accompanied by an increase in SOD ($p=0.05$) and Catalase ($p<0.01$) activity in HSD. Conclusion: high salt intake could modify AT functionality by altering vasculature and oxidative stress, independently of blood pressure. Future studies are necessary to elucidate the impact of this micronutrient in AT pathways.

352. (322) **EFFECT OF PROANTHOCYANIDINS-ENRICHED EXTRACT FROM LIGARIA CUNEIFOLIA (*Lc*) TREATMENT FOR 10 DAYS ON HEPATIC CHOLESTEROL METABOLIZATION AND EXCRETION IN WISTAR RATS FEEDING WITH HYPERLIPEMIC DIET**

Paula Belén Santiago Rahlf[1], Carolina Galiasso[1], José Gonzalvez[1], Leda Urli[1], Alicia Dominighini[1], María Teresa Ronco[2], Daniel Francés [2], Marcelo Wagner [3], Cristina Carnovale[2], Alejandra Luquita[1].

[1]Cátedra Biofísica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario; CIURN. [2]Cátedra de Fisiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario- IFISE-CONICET. [3]Cátedra de Fármaco-botánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

In folk medicine, *Ligaria cuneifolia (Lc*) is used to increase blood fluidity by lowering plasma cholesterol (Cho).Previous results showed that a proanthocyanidin-enriched fraction (P*Lc*) led to decreased levels of plasmatic Cho and Triglycerides (TG) in rats fed with high fat diet (HFD).We aimed to evaluate the effect of P*Lc* on hepatic metabolization and biliary excretion of Cho in Wistar rats fed with HFD (standard diet added with 40% of the first bovine juice) for 28 days and treated with P*Lc* for 10 days. Animals were divided into HFD Group (n=6) and HFD-Plc 10 days Group (P*Lc* 30mg/kg b.w, n=6). At day eleven, rats were anesthetized with ketamine/xylazine (100 mg/kg/3mg/kg,i.p.) bile flow was measure and bile collected. Then, blood was obtained by cardiac puncture and the liver was removed. In blood we determined total Cho, LDL-Cho, HDL-Cho and TG by using commercial detection kits. In microsomal-enriched liver fraction we determined the protein expression of the enzyme Cholesterol-7-alpha hydroxylase (Cyp7a1) by Western Blot. Plasmatic levels of Cho (mg/dL): HFD=191.7±4.7, HFD-P*Lc* 10d=105.8±4.2; LDL-Cho (mg/dL): HFD=22.6±1.07, HFD-P*Lc* 10d=20.00±0.71*; HDL-Cho (mg/dL): HFD=32.20±1.46, HFD-P*Lc* 10d=28.00±2.39(ns); TG (mg/dL): HFD=191.8±21.45, HFD-P*Lc* 10d=133.0±9.68*. Bile Flow (ml/min/100gr liver weight): HFD=2.6±0.4, HFD-P*Lc*10d=2.91±0.06* (mean±SEM; *p<0.05vs.HFD. Student's t-test for unpaired data). Also, we found a significantly augmented bile salt excretion rate (nmol/min/g liver weight) in HFD-P*Lc* 10d (54.50±6.5 vs 34.15±3.66 in HFD). In this regard, P*Lc* treatment increased Cyp7a1 protein expression by 5.3-fold. We propose that P*Lc* treatment showed a lipid-lowering effect. Lower levels of plasma Cholesterol would be explained, in part, by the induction of the expression of the Cyp7a1 enzyme after treatment with P*Lc*, which leads to an increase in the synthesis and biliary excretion of bile salts.

353. (337) **EFFECT OF NATURAL ANTIOXIDANTS ON LIPID ACCUMULATION IN 3T3-L1 ADIPOCYTES**

Laura Andrea Montaldo[1], Gabriela Fernanda Rocha[1,2], Mauricio Cesar De Marzi[1,2], Liliana Noemí Guerra[1,2,3]

[1]Universidad Nacional de Luján. Departamento de Ciencias Básicas. Luján, Buenos Aires, Argentina, [2]INEDES-CONICET (Instituto Nacional de Ecología y Desarrollo Sustentable), Universidad Nacional de Luján, Luján, Buenos Aires, Argentina, [3]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Buenos Aires, Argentina.

Components of natural sources beneficial to human health in preventing fat accumulation have been recently reported. We showed that antioxidant N-acetylcysteine (NAC) inhibited lipids in mature adipocytes 3T3-L1 (AD). Our aim is to study antioxidants with this possible effect. Here, we obtained extracts (EXT) with antioxidant

activity (AA) and evaluated their antiadipogenic effect on AD. EXT were prepared from boldo (*Peumus boldus*) leaves, at 37°C for 1h and from cassis (*Ribes nigrum*) fruit by enzymatic extraction. We determined AA by inhibition of DPPH radical technique, glucose content through GOD POD method and, polyphenol concentration (PPh) by Folin assay (which renders gallic acid content). We performed 24h treatments on AD with EXT and NAC, and evaluated intracellular accumulation of neutral lipids by Oil Red O (ORO) staining technique. Comparing to 5mM NAC AA (96.11 ± 0.63 %), cassis showed high AA (67.28 ± 5.38 %), while boldo reached 52.11 ± 1.21%. PPh cassis and boldo content were 9.03 ± 0.34 gallic acid/mL and 0.20 ± 0 mg gallic acid/mL, respectively. We evaluated ORO content in treated AD compared to non-treated AD, which is set to 100 in arbitrary units (AU) (100 ± 0.04 AU [AD] vs 80.44 ± 2.10 AU [AD with 5mM NAC], $p < 0.05$). Both extracts produced a significant decrease in lipid content, as well as NAC, (AD vs 90.45 ± 0.65 AU [AD with cassis], $p<0.05$; AD vs 75.37 ± 4.72 AU [AD with boldo], $p<0.05$). Glucose concentration was 17.17 ± 0.07 µmol/ml and 0.89 ± 0.04 µmol/ml in cassis and boldo, respectively. We assessed PPh as a possible antioxidant bioactive, but others component should be responsible for antiradical activity, since boldo showed similar AA than cassis with very lower PPh content. Moreover, boldo produced a higher decrease in lipid content than cassis. High glucose content in cassis could contribute to reverse its antiadipogenic effect. We suggested that natural antioxidants could be effective to decrease lipids in this in vitro model.

354. (341) **EFFECT OF INFUSIONS WITH ANTIOXIDANT CAPACITY IN A FATTY LIVER DISEASE MODEL MADE WITH HEP-G2 CELLS**

Francisco Luis Gualdieri[1], Laura Andrea Montaldo[1], Liliana Noemi Guerra[1,2], Gabriela Fernanda Rocha[1,2]
[1]Universidad Nacional de Luján, Buenos Aires, Argentina, [2]INEDES-CONICET-UNLu, Luján, Buenos Aires, Argentina

Non-Alcoholic Fatty Liver Disease (NAFLD) is associated with obesity; NAFLD treatment has low efficiency. Our laboratory has demonstrated that antioxidant N-acetilcysteine could inhibit lipid accumulation on *in vitro* cell models; here we analyzed effects of plant infusion with antioxidant capacity (AC) on NAFLD model using Hep-G2 cells treated with 0.05mM Oleic Acid (OA) for 48 hs. We applied a Central Composite Rotatable Design to establish the extraction conditions that give infusions with maximum AC from Orange (*Citrus sinensis,* O) Carqueja (*Baccharis articulate*, C) and Quebracho (*Schinopsis balansae*, Q). These conditions were: 30 mg dried leaves/mL, 70°C and 14 min. AC was determined by the DPPH radical method and Flavonoid content (FL) by the aluminum chloride method (standard solution: rutin). Hep-G2 cells were treated for 48 hrs with OA and nontoxic dilutions of the infusions, non-treated Hep-G2 cells were considered controls. Intracellular neutral lipid content was evaluated using the Oil Red O (ORO) technique, by quantification at 505nm and with an image-processing program (ImageJ). AC: 71.6 ± 2.2% (O), 51.5 ± 1.3% (C) and 60 ± 9.56% (Q); FL: 0.14 ± 0.04 mg/mL (O), 0.44 ± 0.08 mg/mL (C) and 0.72 ± 0.24 mg/mL (Q) were observed. NAFLD cells show twice the lipid content than control (1.07 ± 0.14 AU [Control] vs 2.19 ± 0.32 AU [NAFLD], $p<0.05$). C and Q decreased lipid content (1.11 ± 0.21 AU [C] vs 2.19 ± 0.32 AU [NAFLD], $p<0.05$) and (1.06 ± 0.24 AU [Q] vs 2.19 ± 0.32 AU [NAFLD], $p<0.05$) and presented the highest flavonoid concentration. Orange infusion showed no significant effect. Our results demonstrated that some infusions could inhibit lipid accumulation in NAFLD cells, although this result is not the consequence of AC. Despite the different components of the infusions, only those with high FL concentration showed significant lipid inhibitory capacity, suggesting these compounds could be involved in such activity.

355. (344) **SHORT-TERM INTERMITTENT COLD INDUCES BROWNING OF WHITE ADIPOSE TISSUE AND INCREASES GLUCOSE CLEARANCE**

Paloma Bivort Haiek*, Bárbara Valente*, Esteban Martin Repetto, Cora B. Cymeryng and Camila Martinez Calejman
Laboratorio de Endocrinología Molecular (LEM). Centro de Estudios Farmacológicos y Botánicos – CEFYBO/CONICET. Facultad de Medicina, Universidad de Buenos Aires.
**both authors contribute equally to this work.*

Introduction: Adipocytes are typically classified as being white or brown. White adipocytes (WA), which are the most abundant, store energy in a single large lipid droplet. Brown adipocytes (BA) have multiple lipid droplets, many more mitochondria, and specialize in burning energy to generate heat through non-shivering thermogenesis, which requires the expression of Uncoupling Protein 1 (UCP1). In response to cold environments, the sympathetic nervous system stimulates thermogenesis to BAT utilize large amounts of glucose, fatty acids, and other nutrients to fuel heat production. Notably, some WA can also adopt the metabolic characteristics of classic BA under certain conditions in a process called *browning*. These so-called brown-in-white (brite) adipocytes mainly appear in subcutaneous fat depots express Ucp1, and can also contribute to whole body energy expenditure. The energy expenditure properties of brown or beige adipocytes is garnering interest in developing strategies to increase BA number and/or activity to treat obesity and other metabolically related diseases. Aim: To study the effect of a short-term intermittent cold (IC) protocol on the activity of BAT and the browning capacity of WAT of mice. Materials & methods: We subjected 8 week-old male C57bl 6 mice to increasing short-term periods (5-15 min/5 days a week) at 4ºC for a month IC, and we measured its effect on BAT and WAT and in serum parameters. Results & Conclusions: Although there were no significant changes in BAT morphology as well as UCP1 expression, WAT histology showed a marked reduction in lipid droplet size and a higher expression of UCP1 in the IC group. Serum glucose levels were markedly reduced in the fed state (156±2 mg/dL vs 210±12 mg/dL $p<0.05$) in the IC group with no significant effects on TG or cholesterol levels. These results indicate that a short period of IC might induce the generation of brite adipose tissue and boost its metabolic capacity promoting serum glucose clearance.

356. (354) **CARBON STABLE ISOTOPES AS POTENTIAL BIOMARKERS OF SUGAR INTAKE**

Liliana Soledad Marchesi Olid[1,2,3], Paula Mantero[1], Laura Oliva[2], Gustavo Cernadas[4], Florencia Scochera[1], Marcela Zubillaga[1,3], Mariana Janjetic[1,3,5], Cinthia Goldman [1,3].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Física, Buenos Aires, Argentina; [2]Universidad de Buenos Aires, Facultad de Medicina, Escuela de Nutrición, Buenos Aires, Argentina; [3]CONICET, Argentina; [4]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Anatomía e Histología, Buenos Aires, Argentina; [5]Universidad de Buenos Aires, Facultad de Medicina, Escuela de Nutrición, Centro de Investigaciones sobre Problemáticas Alimentarias y Nutricionales (CISPAN), Buenos Aires, Argentina.

Introduction: A biomarker of added sugar intake might be useful to objectively measure its consumption, and to prevent its associated health implications. The Breath $^{13}C/^{12}C$ Carbon Isotope ratio (CIR) has been recently reported as a potential biomarker of added sugar intake. We aimed to evaluate the association of breath CIR with total sugar, added sugar and sugar sweetened beverages (SBB) intake of an adult population. Methods: Fasted dyspeptic adults (18-70y) referred to the Gastroenterology Unit of the Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo", Buenos Aires, Argentina, were included; weight, height and waist circumference were determined using validated tools. A sociodemographic survey was administered, and nutrient intake was assessed by 24 h dietary recalls. Duplicate breath samples were obtained for CIR measurement in a mass spectrometer coupled to a gas chromatographer (GC-MS). Statistical analysis was performed using the SPSS software. Results: 277 patients (41.7±13.4y) were included, of which 63.2% were female. Anthropometric analysis showed that 65.9% were overweight/obese, and 55.5% had an elevated cardiometabolic risk. Overall, 58.5% exceeded the WHO recommendations for added sugar intake: mean total sugar and added sugar intakes were 87.8 g/d and 56.7 g/d, which represented 20.5% and 12.7% of the total energy intake, respectively, whereas mean SSB intake

was 29.2 g/d. A positive correlation was found between breath CIR and the consumption of SSB (r=0.281; p=0.0042); however, no correlation was observed with added sugar intake (r=0.104; p=0.167). Conclusions: Total sugar, added sugar and SSB intakes were high in the studied individuals. Our preliminary results support the use of breath CIR as a potential biomarker of SSB intake, which should be further studied in a wider population.

357. (403) **EVALUATION OF REDOX STATE AND EXTRACELLULAR MATRIX OF SKELETAL MUSCLE OF RATS CHRONICALLY FED A SUCROSE-RICH DIET. EFFECTS OF SALVIA HISPANICA (CHIA SEED)**

Paola Guadalupe Illesca[1], María del Rosario Ferreira[1,2], Adriana Benmelej[3], Noelia Villafañe[3], María Eugenia D'Alessandro[1,2].

[1]Laboratorio de Estudio de Enfermedades Metabólicas Relacionadas con la Nutrición. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). [3]Cátedra de Morfología Normal. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral.

Skeletal muscle (SM) lipid accretion is associated with insulin resistance (IR). We showed in SM (gastrocnemius) of rats fed a sucrose-rich diet (SRD) for 6 months an altered oxidation of fatty acids and increased lipogenic pathway. Replacement of the fat source with chia seed reversed or ameliorated these disorders. In addition, it was reported that ectopic lipid deposition is associated with alterations in tissue redox state and remodeling. We aimed to explore possible SM changes in redox state and extracellular matrix in SRD-fed rats and the effects of a dietary substitution with chia seed. Male Wistar rats were fed a SRD for 3 months, after were divided into two subgroups. One subgroup continued with SRD up to 6 months and the other received SRD where chia seed was incorporated as source of dietary fat for the next 3 months (SRD-C). A reference group consumed a control diet all the time. In SM were analyzed: i) reactive oxygen species (ROS) and ii) thiobarbituric acid reactive substances (TBARS) levels, iii) reduced glutathione (GSH) levels, iv) ferric ion reducing antioxidant power (FRAP), v) catalase activity (CAT), vi) collagen deposition (Picrosirius red staining) and vii) hydroxyproline (HXP) levels. Statistical analysis was performed by one-way ANOVA and Scheffé's test, p<0.05 was considered significant. In SRD-fed rats reduced FRAP levels and lower CAT activity without significant changes in ROS, TBARS or GSH levels were observed. Chia seed restored the decreased levels of FRAP and increased TBARS levels. Collagen increased in SM of SRD-group and was restored in SRD-C group whereas HXP levels remained unchanged. The results of this work expand the current understanding of the mechanisms involved in metabolic disorders in the MS of SRD-fed rats and the effects of chia seed as a possible therapeutic nutritional intervention.

358. (412) **CHIA SEED IMPROVES GLUT-4 LEVELS, MODULATES LIPOGENIC ENZYMES AND EXTRACELLULAR MATRIX COMPONENTS IN DIFFERENT ADIPOSE TISSUES IN A SUCROSE-RICH DIET RAT MODEL**

Victoria Aiassa[1] María del Rosario Ferreira[1,2] Dante Selenscig[1] María Eugenia D'Alessandro[1,2]

[1]Laboratorio de Estudio de Enfermedades Metabólicas relacionadas con la Nutrición. Facultad de Bioquímica y Cs. Biológicas. UNL. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Body fat accretion is strongly linked to insulin resistance and others associated metabolic disorders. Chia seed appears a promising intervention in showing beneficial effects in adipose tissue dysfunction. An improvement of the altered insulin signaling pathway and lipolysis in different fat pad depots was recently reported by our group. Our research work focused at evaluating the effect of chia seed administration in a diet-induced adiposity rodent model upon GLUT-4 levels, lipogenic enzymes and some changes in extracellular matrix remodeling in different adipose tissues (epididymal- eAT and retroperitoneal- rAT). Male Wistar rats were fed a SRD for 3 months. Half of the animals continued with the SRD until month 6, the other half were fed with a SRD in which the source of fat, corn oil, was replaced by chia seed from month 3 to 6 (SRD+chia). Another group consumed a reference diet for 6 months (RD). It was analyzed in eAT and rAT: glucose transporter-4 (GLUT-4) protein levels in basal and insulin stimulated conditions, lipogenic enzyme activities: fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase (G6PDH), hydroxyproline levels and MMP-2 metalloproteinase activity. Statistical analysis was performed by one-way ANOVA post Newman Keul's test, p<0.05 was considered significant. Compared to the SRD-fed rats, the SRD+chia group showed in both eAT and rAT: a) an improve in the altered GLUT 4 levels, b) a significant decrease (p<0.05) in FAS and G6PDH enzyme activities, c) a decrease (p<0.05) in hydroxyproline content. Besides a significant decrease (p<0.05) in pro-MMP2 activity in rAT was also observed. This study provides new data regarding the beneficial effects of α-linolenic acid-rich chia seed upon several abnormalities developed in different fat pad depots of SRD-fed rats.

359. (414) **BONE-VASCULAR- ADIPOSE TISSUE INTERPLAY: MODULATION BY ESTROGENS**

Sabrina Belén Cepeda, Marisa Julia Sandoval, Pablo Cutini, María Ivon Valle, Adrian Campelo, Virginia Massheimer.

Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), CONICET-UNS, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina

Nitric oxide (NO) promotes bone cells proliferation, differentiation and survival. An adequate vascularization that provides cell progenitors and hormones is required for skeletal homeostasis. The menopausal hypoestrogenism is tightly associated with bone and cardiovascular diseases, and also with changes in adipose tissue (AT) distribution. In this work we studied the role of the estrogens estradiol (E_2) and estrone (E_1), and phytosestrogen genistein (Gen) on bone-vascular or bone-AT interactions. Two experimental designs were employed: 1) conditioned medium obtained from endothelial cells (EC) exposed to Gen (CM-EC), or conditioned medium obtained from osteoblasts (OB) exposed to Gen (CM-OB); 2) co-cultures OB-AT. A bidirectional regulation between OB and EC was revealed. CM-EC added to OB monolayers stimulated bone cells proliferation (150% a/C, p<0.05). In the presence of NO synthase (NOS) inhibitor, NAME compound, OB growth was blunted suggesting the participation of NOS system. On the other hand, CM-OB added to EC cultured enhanced EC proliferation and migration (44; 150% a/C respectively, p<0.01). Since these two events are involved in angiogenesis, tubes formation from aortic rings seeded on a collagen matrix was quantified. CM-OB induced a 0.5 fold increase (p<0.05) in tubes formation around the rings. To assess bone-AT interactions, cocultures OB-AT were used. After 3 days, E_2 E_1 and Gen stimulated NO production (64; 34; 37% s/c, respectively). At a longer co-culture time, a reduction in NOS activity accompanied by an increase in oxidative stress, measured as hydrogen peroxide production, was detected (1090 ± 37.1 vs 1220 ± 31.2 nmol H_2O_2/mg prot, C vs E2, p<0.01). When AT slices were removed, OB diminished their capability to enhance NO synthesis and, to mineralize extracellular matrix (Alizarin staining) in response to estrogens. In summary, estrogens favour bone vascular interactions, but in the presence of AT osteoblastogenic response is delayed.

360. (453) **MATERNAL ADVERSE DIET AND WHITE ADIPOSE TISSUE BROWNING CAPACITY OF ADULT OFFSPRING.**

Ana Alzamendi[1], Alejandro Bozzano[1], María Amanda Rey[1], Eduardo Julio Spinedi[2], Andrés Giovambattista[1].

[1]Unidad de Neuroendocrinología, IMBICE. CONICET-CIC PBA-UNLP, [2]CENEXA (CONICET-UNLP-FCM)

Beige adipocytes dissipate energy as heat through uncoupling protein-1 (UCP1) activity. Our aim was to assess whether maternal fructose rich diet (FRD) intake during lactation affects development of browning capacity of retroperitoneal white adipose tissue (WRPAT) from adult male offspring. Adult female rats were mated, at birth pups were counted and they were equal to 8 per mother.

Dams were provided with either tap water (control) or FRD (10 % w/v; in tap water) and fed *ad libitum* with chow up to day 21 of birth. Weaned pups received water and chow *ad libitum* up to 60 days of age (experimental day). C and F indicate pups born to control and FRD dams, respectively. At the age of 53 days, C and F pups were kept at 4ºC for one week. Body weight and food intake was registered every day. On experimental day, trunk blood was collected, WRPAT pad was dissected and weighted. mRNA expression levels of beige adipogenic markers were assessed in WRPAT pads, and additional tissue was used for H&E staining and IHC. In basal conditions, F animals gain more body weight as they consumed more calories, and moreover, ucp1 WRPAT expression was significantly higher in F than C animals. Cold exposure induced, in both groups, an increase in BAT mass, a decrease in body weight, in WRPAT mass, and in circulating triglycerides levels. Moreover, when submitted to cold F animals increased ucp-1 expression above C cold animals. And this increase was consistent with H&E stain showing beige areas in WRPAT, and UCP1 positive cells by IHC. This ucp1 gene expression pattern was accompanied by a higher pgc-1α expression in F cold animals, significantly higher than all other groups. Our results support the ability to mount a differential response to low temperatures by the animal in its adult stage, when it is subjected to metabolic programming by administration of a diet rich in fructose to the mother, in the infant stage, probably due to a compensatory mechanism (PICT2020-3064).

361. (461) **CEREBRAL CORTEX BIOMARKERS OF OXIDATIVE STATUS AND COGNITIVE PERFORMANCE ARE ALTERED IN HIGH-SUCROSE DIET FED RATS**

María del Rosario Ferreira[1,2], María de los Milagros Scalzo[1], Silvia Rodríguez[1,2], María Eugenia D´Alessandro[1,2].
[1]Laboratorio de Estudio de Enfermedades Metabólicas relacionadas con la Nutrición, Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

We previously shown that rats fed a high-sucrose diet for a short period of time (3 weeks) develop several features of the human Metabolic Syndrome (MS), such as dyslipidemia, insulin resistance, hyperinsulinemia, glucose intolerance and hypertension. In recent years, it was postulated that MS would be correlated with the development of neurodegenerative diseases, and an inverse association between MS and cognitive performance has also been proposed. On the other hand, it was demonstrated that oxidative stress plays a key role in the pathogenesis of both MS and neurodegenerative diseases. The **aim** of this work was to evaluate: a- cerebral cortex redox biomarkers and b- cognitive performance in the diet-induced MS rodent model previously described. Male Wistar rats initially weighing 180–200 g were randomly divided into 2 groups and placed for 3 weeks with a high-sucrose diet (HSD) or a control diet (CD). We analyzed in cerebral cortex: a- Reactive oxygen species (ROS) and TBARS - as a marker of lipid peroxidation- levels, b- reduced glutathione levels (GSH), c- the activities of GSH related antioxidant enzymes (glutathione peroxidase – GPx-, glutathione reductase- GR-, glutathione -S- transferase-GST-) and catalase (CAT). Non-spatial and spatial memory was analyzed by conducting the Novel Object recognition (NORT) and the T- maze tasks, respectively. Brain weight was also determined. The statistical analysis was performed by student-t test. Compared with CD-fed rats, HSD-fed group shown a significant increase ($P<0.05$) in both cerebral cortex ROS and TBARS levels and GPx and GR enzyme activities. These changes were accompanied by a significant cognitive decline evidenced by an altered performance in both NORT and T-Maze tasks. Brain weight, CAT and GST remained similar in both dietary groups. The results show that peripheral metabolic disorders developed in HSD-fed rats are accompanied by cerebral cortex oxidative status changes and impaired cognitive function.

362. (792) **EFFECTS OF CHLORIDE ANION ON THE DEVELOPMENT OF ARTERIAL HYPERTENSION AND OXIDATIVE KIDNEY DAMAGE**

María Julieta Rudi[1]; Nicolás M. Kouyoumdzian[1, 2]; Gabriel Kim[1]; Natalia L. Rukavina Mikusic[1,2]; Mónica Galleano[3, 4]; Belisario Fernández[4], Ana M. Puyó[1]; Marcelo R. Choi[1, 2, 4]
[1]Universidad de Buenos Aires (UBA).Facultad de Farmacia y Bioquímica (FFyB). Departamento de Ciencias Biológicas, Cátedra de Anatomía e Histología. Buenos Aires, Argentina. [2]CONICET-UBA, Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional (IATIMET). Buenos Aires, Argentina. [3]CONICET-UBA, Instituto de Bioquímica y Medicina Molecular (IBIMOL). Buenos Aires, Argentina. [4]UBA. FFyB. Departamento de Química Analítica y Fisicoquímica, Cátedra de Fisicoquímica. Buenos Aires, Argentina.

The excessive consumption of sodium chloride (NaCl) in the diet leads to the development of high blood pressure (HBP) and target organ damage. The contribution of the chloride anion (Cl^-) to these deleterious effects is unknown. The objective was to evaluate whether Cl^-, in addition to sodium (Na^+), would be involved in the renal inflammatory and oxidative response and in the development of hypertension. Male Wistar rats were divided into four groups (n=8/group) and fed with different diets for 3 weeks: control (group C); NaCl 8% (NaCl group); high Na^+ without Cl^-, $Na_3C_6H_5O_7$ 11.8% (Na group); high in Cl^- without Na^+, $CaCl_2$ 3.80%, KCl 3.06% and $MgCl_2$ 1.30% (Cl group). Systolic blood pressure (SBP), renal function and oxidative parameters in the renal cortex were determined: production of thiobarbituric acid reactive substances (TBARS) and activity and expression of enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The renal expression of the chloride channels CLCNKa and CLCN5 as key transporters involved in this process was also determined; the expression of p50-NFkB and the AT1 receptor. Protein expression was determined by Western Blot. Differences with a *p value*<0.05 were considered statistically significant. Results: Increased SBP, GPx activity, and renal p50-NFkB and AT1R expression were observed in the NaCl and Cl groups compared to the other groups (*p<0.05). The NaCl and Cl groups showed a higher expression of CLCNKa*, while CLCN5 decreased in the NaCl group compared to C*. In the experimental groups, the production of TBARS increased with respect to C, without changes in the activity or expression of SOD and CAT. Conclusion: Cl^- would be co-responsible, together with the Na^+, in triggering renal oxidative damage and increasing blood pressure; thus, the importance of reducing the intake of both ions as a non-pharmacological preventive measure for the prevention and control of hypertension is deduced.

363. (812) **NITRIC OXIDE BIOAVAILABILITY AND INFLAMMATION IN PERIVASCULAR ADIPOSE TISSUE: MODULATION BY DIET.**

Ezequiel Hid[1,2], Fiorella Lista[3,4], Mailén Massetelle Espósito[1,2], Ana Maria Balaszczuk[3,4], Cesar G. Fraga[1,2], Noelia Arreche[3,4], Monica Galleano[1,2].
1 Fisicoquímica, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. 2 CONICET- Universidad de Buenos Aires (IBIMOL), Buenos Aires, Argentina. 3 Fisiología, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. 4 CONICET- Universidad de Buenos Aires (IQUIMEFA), Buenos Aires, Argentina.

In rodents, thoracic aorta perivascular adipose tissue (taPVAT) constitutes an example of “beige” adipose tissue. Our group has demonstrated that dietary (-)-epicatechin (EC) attenuated taPVAT expansion, adipocyte hypertrophy and systolic blood pressure increasing in high-fructose (HF) fed rats (Hid et al., 2021, Abstract SAIC 433). The aim of this study was to investigate the mechanisms behind this effect focusing on the determinants of nitric oxide (NO) bioavailability and inflammation in taPVAT. Male Sprague-Dawley rats were divided into 4 groups: C: control diet and tap water; CE: EC (20 mg/kg BW/d) in the diet and tap water; F: control diet and 10% (w/v) fructose in the water, and FE: EC in the diet and fructose in the water. After 8 w, animals were euthanized and blood (plasma) and taPVAT were obtained. EC attenuated NADPH-depend superoxide anion production vs F (53%, $p<0.05$) and it correlated to a decrease in the expression of subunits of NADPH oxidase (NOX) iso-

forms, such as gp91, p47 and NOX4 (29, 11, and 26%, respectively, p<0.05), without changes in NO production determinants. Parameters related to oxidative stress were evaluated: EC decreased 3-nitrotyrosine (3-NT) and thiobarbituric acid reactive species (TBARS) detection respect to F (39, and 55%, respectively, p<0.05) and did not change 4-hydroxinonenal (4-HNE) levels. HF diet increased the levels of NF-κB signaling pathway proteins: p-p65, IL-6 and iNOS vs C (75, 13, and 51%, respectively, p<0.05) and EC mitigated these parameters to control values. In summary, in taPVAT of HF fed rats, EC was associated with i) increase in NO bioavailability mediated by inhibition of oxidants production in the same direction as observed in the aorta in previous studies, ii) attenuation of NF-κB pathway activation. These results could explain how taPVAT may contribute to the antihypertensive effect of EC in our experimental model, modulating NO bioavailability and inflammation. UBACyT 2018, PIP 2017-2019, PICT 2018.

METABOLISM AND NUTRITION III

Saturday, November 19, 14-15:30 hr
Chairs: Verónica D´Annunzio - Gabriela Marina Prendes

364. (481) **ACUTE INTERMITTENT PORPHYRIA AS A RISK FACTOR FOR HEPATOCELLULAR CARCINOMA FIRST CASE IN ARGENTINA**

Viviana Melito[1,2], Laura Varela[1], Florencia Antinucci[3], Julia Brutti[3], Rafael Maurette[4]
Lucía Tomassi[5], Ana Buzaleh[1,2], Victoria Parera[1], Margarita Anders[3]
(1) Centro de Investigaciones Sobre Porfirinas y Porfirias (CIPYP), Hospital De Clinicas, UBA-CONICET
(2) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
(3)Hepatology, Sanatorio Anchorena, Ciudad de Buenos Aires, Argentina.
4)Surgery, Sanatorio Anchorena, Ciudad de Buenos Aires, Argentina
5) Hospital General Agudos José Ramos Mejía, Ciudad de Buenos Aires, Argentina

The risk of hepatocellular carcinoma (HCC) is significantly increased in patients with Acute Intermittent Porphyria (AIP), a metabolic disease due to heme synthesis dysfunction, compared to the general population. Most HCCs are asymptomatic and up to one-third develop in non-cirrhotic patients. The aim is to report the first case of an AIP patient and a giant HCC. This is a 57-year-old female diagnosed with AIP in 1986: urinary porphobilinogen (PBG) (49.1mg/24h, NV:≤2) and 5-aminolevulinic acid (10.3mg/24h, NV:≤4), PBG deaminase (30.03U/mlGR NV: 81.51±11.96) and genetic study (c.612G>T mutation). The patient has been asymptomatic for more than 20 years. She was negative for HCV, HBV and HIV and no alcoholic. In June 2022, she consulted the emergency room for abdominal pain and, an ultrasound was performed showing a large liver mass. An abdominal triphasic abdominal MRI showed a focal lesion of 105x79mm that was enhanced with intravenous contrast compatible with a primary liver tumor (alpha-fetoprotein: 4400 ng/ml). Extension studies (chest tomography and bone scintigraphy) excluded secondary disease and there was no evidence of vascular invasion. The patient had a performance status of 0 and presented no weight loss. As a finding, she had hyponatremia with inadequate secretion of antidiuretic hormone. She was diagnosed with an early-stage HCC of the BCLC classification, and surgical resection was suggested. After performing liver volumetry, it was decided to do a two-stage surgery to hypertrophy the left lobe (ALPPS surgery). Postoperative complications were ascites with parameters of portal hypertension secondary to a small size syndrome and spontaneous bacteremia due to E. Coli. Currently, the patient is still hospitalized, and we are planning to repeat alpha-fetoprotein and MRI one month after surgery. The pathological anatomy confirmed the diagnosis of HCC.
In conclusion, this case highlights the importance of an HCC screening program in AIP patients.

365. (482) **BENEFICIAL EFFECT OF ZINC SUPPLEMENTATION ON CARDIOMETABOLIC ALTERATIONS INDUCED BY HIGH FAT AND FRUCTOSE DIET IN WISTAR RATS**

Diamela T Paez[1,2], Agustina Medina[1,2], Franco Polero[1,2], Juan Manuel Gaetani[1,2], Gabriela Noceti[1,2], Moriondo Marisa Mabel[5], Domínguez Juana[1,2], Carolina Olano[3,4], Valeria Zago[3,4], Rosana Elesgaray[1,2], Carolina Caniffi[1,2], Cristina Arranz[1,2], Analia Tomat[1,2]
[1]Cátedra de Fisiología, Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.
[2]Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), CONICET. Universidad de Buenos Aires, Buenos Aires, Argentina.
[3]Laboratorio de Lípidos y Aterosclerosis. Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
[4]Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
[5]Instituto "Alberto C. Taquini" de Investigaciones en Medicina Traslacional.

Introduction:We demonstrated that zinc deficiency during fetal and postnatal life induced cardiometabolic alterations in adult male rats. Zinc could play an import role attenuating these alterations due to its antioxidant properties and the multiple functions in glucose metabolism. Objective:To evaluate the effect of postweaning zinc supplementation on intermediate metabolism, retroperitoneal adipose tissue (RAPT), plasma lipid peroxidation (TBARS) and systolic blood pressure (SBP) in adult male Wistar rats fed a high fat and fructose diet during growth. Methods:Female rats were fed a control diet (C,30ppm) during the pregnancy and lactation periods. At day 21 male offspring were weaned and randomly fed a control diet (CC,30 ppm), high fat-control zinc diet and fructose 10% in drinking water (CHF, fat 60 % kcal, zinc: 30 ppm) or high fat-supplemented zinc diet and fructose 10% in drinking water (ZHF,190 ppm). At day 81 serum metabolic profiles, plasmatic TBARS, SBP and RPAT morphology were evaluated. Values are mean±SEM. Two-way ANOVA, Bonferroni post-test:***p<0.001,**p<0.01,*p<0.05 Vs CC;$$$p<0.001,$$p<0.01 Vs CGF (n=5). Results:High fat-control zinc diet during postweaning growth induced an increase of body weight (CC:456±15; CHF:515±10*; ZHF:476±10 g), uricemia levels, TBARS and SBP compared to CC. Adipose tissue from CHF showed higher weight (CC:1.4±0.1;CHF:3.1±0.4***;ZHF:2.7±0.1g) and adipocyte area (CC:4705±193; CHF:7450±838**;ZHF:7435±484 μm^2) than CC. Zinc supplementation reversed TBARS (CC:3.1±0.2;CHF:12.1±1.6***;ZHF:2.5±0,7$^{\$\$\$}$ nmol TBARS/ml), glycemia (CC:119±4,CHF:140±5**,ZHF:125±2$^{\$\$}$mg/dl),uricemia (CC:0.9±0.1;CHF:1.9±0.3*;ZHF:1.0±0.1$^{\$\$}$mg/dl) and showed lower SBP levels (CC:133±2;CHF:159±2*;ZHF:144±1$^{\$\$}$mmHg) than CHF. Conclusions:High fat and fructose diet during postweaning growth induced cardiometabolic alterations in adult life. Zinc supplementation attenuated cardiometabolic damage associated with metabolic syndrome.

366. (504) **DISCOVERING THE OUTCOMES OF SGSH ENZYME DEFICIENCY ON CELLULAR PHYSIOLOGY: THE LYSOSOMAL/AUTOPHAGIC PATHWAY AND MITOCHONDRIAL INTEGRITY IN NEURONAL MODELS OF MUCOPOLYSACCHARIDOSIS III TYPE A**

María Colonna[1], Soledad Porte Alcon[1], Marcos Gabriel Francia[2], Alejandra Sonia Guberman[2], Mónica Lidia Kotler[1], Roxana Mayra Gorojod[1].
[1]Laboratorio de disfunción celular en enfermedades neurodegenerativas y nanomedicina. [2]Laboratorio de regulación de la expresión génica en células madre. QB-FCEN-UBA. IQUIBICEN- CONICET.

Mucopolysaccharidosis type III (MPSIII), also known as Sanfilippo syndrome, is a rare metabolic, neurodegenerative disorder that first appears in early childhood. MPSIIIA results from mutations in the gene coding for N-sulfoglucosamine sulfohydrolase (SGSH) that is involved in heparan sulphate degradation in lysosomes. Currently, there are no nervous system-based cellular models of

MPSIIIA available. In our lab, we have developed HT22 neuronal cell lines deficient in SGSH activity —named 12 and 124. In this work, we aimed to characterize these models and determine the effect of enzyme deficiency on lysosomal/autophagic pathway and mitochondrial integrity. By Lysotracker Red DND-99 staining, we compared acidic vesicles (AVs) between MPSIIIA cells and a control line. We detected an increase in AVs number (124: 34.6±7.4% p<0.001), size (12: 12.8±1.6%; 124: 17.2±1.4% p<0.001), AVs area/cell area (12: 41.5±9.4% p<0.01; 124: 92.3±9.7% p<0.001), and a greater diffusion of the dye to the cytosol (12: 22.4±3.8% p<0.01; 124: 52.5±6.1% p<0.001) suggesting an expansion of the lysosomal compartment together with the loss of the lysosomal membrane potential in MPSIIIA cell lines. On the other hand, the levels of the lysosomal protein LAMP-1 remained unchanged when measured by western blot (WB, p>0.05). Despite AVs alterations, the autophagic flux remained functional as determined LC3-II expression (WB). Mitochondrial network displayed a fragmented morphology in MPSIIIA cells stained with Mitotracker Red CMXRos, likely related to cellular stress. However, ROS production was decreased in these cell lines when compared to controls (12: 30.8±7.4% p<0.05; 124: 43.6±7.6% p<0.001). These results contribute to characterize our novel neuronal MPSIIIA models and provide new evidence suggesting the occurrence of lysosomes and mitochondria alterations. We expect that these models, together with our findings, could be useful for the design of future therapies for this intractable disease.

367. (523) **EFFECT OF BIOACTIVE COMPOUNDS DERIVED FROM GRAPE POMACE ON OBESITY-RELATED KIDNEY INJURY**

Tatiana Silvina Figueras[2], Rodrigo Damián García[1], Diahann Perdicaro[2], Victoria Muscia Saez[2], Valeria Cacciamani[1,2], Patricia G. Vallés[1,2], Marcela Vazquez-Prieto[1,2], Valeria Victoria Costantino[1,2].
[1]Facultad de Ciencias Médicas, UNCuyo.
[2]IMBECU-CONICET.

Obesity is an important risk factor for the kidney disease development. Epidemiological studies have shown that the prevalence of chronic kidney disease is increasing concomitant with obesity. The physiopathology of obesity-related kidney disease is multifactorial. Several mechanisms by which lipids can cause kidney damage have been proposed, including inflammation, oxidative stress, and fibrosis. The intake of foods rich in bioactive compounds, such as polyphenols attenuates pathologies associated with obesity and reduces inflammation and oxidative stress. Grape pomace extract (GPE) is obtained from grape pomace, a residue of the winemaking process. GPE is mainly constituted of berry skins and seeds, which still contain high amounts of phenolic compounds. Given that in Mendoza, the wine industry each year generates great amounts of pomace, this can be used for implementing strategies in the prevention of obesity-associated pathologies. We propose to evaluate the GPE effect in renal injury related to obesity. For this, C57BL/6J mice (20–25 g) were divided into 4 groups (n = 8 each) and fed for 10 weeks as follows: 1) Control group (Ctrl): standard diet; 2) Ctrl group + diet supplemented with GPE 300 mg/Kg of weight/day; 3) HFD group (high fat), and 4) HFD + GPE 300 mg/Kg of weight/day. At the end, blood samples were taken, adipose tissue was removed and the mice were nephrectomized to obtain tissue samples. The concentrations of biochemical parameters were analyzed using commercial kits. We shown that GPE supplementation significantly reduced body weight, visceral and perirenal adipose tissue in the HDF+GPE group compared to the HFD group. In addition, the biochemical parameters concentrations such as cholesterol and serum urea were significantly decreased in the HDF+GPE group compared to the HFD group. These results highlight the potential use of grape-derived bioactive compounds to reduce obesity-related kidney injury.

368. (550) **EFFECTS OF OLIGONUCLEOTIDE IMT504 IN A METABOLIC SINDROME AND TYPE 2 DIABETES MODEL INDUCED BY HIGH-FAT DIET IN MICE**

María Marta Bonaventura[1], Laila Domé[1], Alejandro Montaner[2], Victoria Adela Lux-Lantos[1], María Silvia Bianchi[1]
[1]Instituto de Biología y Medicina Experimental (CONICET), Buenos Aires, Argentina.
[2]Instituto de Ciencia y Tecnología "Dr. Cesar Milstein" (CONICET – Fundación P. Cassará), Buenos Aires, Argentina.

We have shown that the immunomodulatory oligonucleotide IMT504 improves glucose homeostasis in animal models of type I diabetes by regulating expression of immune modulatory factors and by improving beta cell function. Here we evaluated the effects of IMT504 in a murine model of metabolic syndrome and type 2 diabetes induced by a high-fat diet. Male C57BL/6LP mice were fed either a standard diet (SD) or a high-fat diet (HFD: ResearchDiet, D12492) for 12 weeks. HFD animals showed higher non-fasting glycemia (Gly: p<0.01), and body weight (BW: p<0.01). Mice received one daily dose of IMT504 for 12 consecutive days (IMT: 20mg/kg/day, 6mg/kg/day or 2mg/kg/day) or saline. Glucose tolerance tests (GTT, day 10) and insulin tolerance tests (ITT, day 11) were performed. On day 12, food intake, BW, and non-fasted Gly were recorded; after 3 hours fasting, mice were sacrificed and blood samples collected. Gly did not vary with time in SD and HFD mice, while it significantly diminished with IMT treatment [Gly (mg/dl): repeated measures ANOVA: interaction, p<0.05, IMT6 and IMT20: Day1 vs Day12, p<0.05]. Glucose homeostasis and insulin resistance also improved with IMT treatment [GTT- Area under curve (AUC): ANOVA, p<0.01; HFD vs STD: p<0.01; HFD vs IMT6: p<0.01; HFD vs IMT20: p<0.01], [ITT-AUC: ANOVA, p<0.01; HFD vs STD: p<0.01; HFD vs IMT6: p<0.01; HFD vs IMT20: p<0.01]. Food (F) and calories (C) consumed decreased with IMT treatment [F(gr): ANOVA, p<0.01, IMT20 different from HFD, IMT2, IMT6: p<0.01] [C (Kcal): ANOVA, p<0.01, IMT20 different from HFD, IMT2, IMT6: p<0.03], which was reflected in a decrease in BW [repeated measures ANOVA: interaction, p<0.01, IMT6: Day1 vs Day12, p<0.01, IMT20: Day1 vs Day12, p<0.01]. These results show that IMT504 treatment promotes a significant, dose dependent, improvement in diabetic condition and food intake in HFD mice. Further investigation is underway to understand its mechanism of action. Funding: CONICET, ANPCYT, F. R Barón, F. Williams

369. (576) **ALTERATION IN LIPOPROTEIN-ASSOCIATED ENZYMES AND PROTEINS IN CHILDREN AND ADOLESCENTS WITH UNDERWEIGHT OR OVERWEIGHT /OBESITY IN ASSOCIATION WITH NOVEL ATHEROGENIC INDEXES**

Davico B*[1], Martin M*[1], Verona J[2], Tetzlaff W[1], Lozano Chiappe E[1], Verona MF[2], Gilligan L[2], Ballerini MG[1], Boero L[1], Gómez Rosso L[1], Brites F[1].
[1] Departamento de Bioquímica Clínica. Facultad de Farmacia y Bioquímica. UBA.
[2] Hospital Jose Fossati. Ciudad de Balcarce.
** Contributed equally*

Introduction: Overweight/obesity (OW/OB) is associated with modifications in lipoprotein (Lp)-associated enzymes and proteins, such as cholesteryl ester transfer protein (CETP), Lp-associated phospholipase A_2 ($LpPLA_2$) and paraoxonase (PON)1, while no evidence is available regarding underweight (UW). Moreover, the following indexes have been proposed to better assess atherogenic risk related to weight alterations: triglycerides-glucose index (TG-G), visceral adiposity index (VAI) and height-corrected lipid accumulation product (HLAP). Our objective was to analyze the presence of alterations in Lp-associated enzymes and proteins in children and adolescents with UW and OW/OB and their relation to novel atherogenic indexes. Methods: Thirty male children and adolescents with UW, 66 with normal weight (NW) and 30 with OW/OB were included. Anthropometric parameters were registered. Glucose, Lp profile and the activities of CETP, $LpPLA_2$ and PON1 were evaluated. Body mass index (BMI)-z, TG-G, VAI and HLAP were calculated. Results: UW presented lower TG than OW/OB. UW and NW showed lower CETP activity than OW/OB (Mean±SD) (218±38 *vs.* 224±26 *vs.* 237±26 %/mL.h; p<0.05), while both UW and OW/OB showed lower PON1 activity than NW (318±170 *vs.* 409±200 *vs.* 310±184 nmol/mL.min; p<0.05). TG-G was higher in OW/OB than UW (p< 0.01), whilst both HLAP (p<0.05) and VAI (p<0.01) followed a linear trend across

weight categories. Moreover, after adjusting for age and BMI-z, TG-G was an independent predictor of CETP (r^2= 0.25, β=-0.22, p<0.01) and $LpPLA_2$ (r^2= 0.21, β=-0.21, p<0.05), while VAI (r^2=0.21, β=-0.32, p<0.01) and HLAP (r^2= 0.20, β=-0.31, p<0.01) of CETP. Conclusions: Both UW and OW/OB showed impaired antioxidant PON1 activity, despite lack of alterations in HDL levels. Moreover, TG-G, VAI and HLAP were all capable of predicting alterations in crucial modulators of Lp metabolism and vascular inflammation in children and adolescentswith varying degrees of alterations in body weight.

370. (578) **EFFECT OF BARIARITRIC SURGERY ON LIPOPROTEIN LEVELS AND FUNCTIONALITY**

Lozano Chiappe, E[1]; Gómez Rosso, L[1]; Martín, M[1]; Davico, B[1]; Tetzlaff, W[1]; Jiménez, G[2]; Cerrone, G[3], Frechtel, G[3]; Sáez, MS[2]; Beskow, A[2]; Boero, L[1]; Gutt, S[2]; Woo, D[2]; Brites, F[1].

[1] Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires; [2] Hospital Italiano, Buenos Aires; [3] INIGEM – CONICET, Universidad de Buenos Aires.

Introduction: Morbid obesity is associated with different comorbidities, including dyslipidemia and cardiovascular disease (CVD), being bariatric surgery (BS) the most successful therapeutic option. The objective of this study was to evaluate the effect of BS on lipoprotein quantitative and functional aspects. Materials and methods: Twelve patients with morbid obesity were recruited from the Italian Hospital of Buenos Aires and were evaluated before and 12 months after BS. Weight and height were recorded, and body mass index (BMI) calculated. Plasma levels of glucose, high-sensitivity C-reactive protein (hsCRP), free fatty acids (FFA), and lipid profile were determined. The triglycerides (TG)/HDL-C ratio was calculated. Cholesteryl ester transfer protein (CETP) and PON1 activities were evaluated by developed methods. Data were analyzed employing parametric and non-parametric tests for paired samples, as appropriate. Results: Twelve months after BS, weight and BMI were significantly reduced. Decreases were detected in plasma levels of glucose, hsCRP and TG, as well as in TG/HDL-C and apo B/apo A-I ratios. HDL-C (43±7 *vs.* 57±9 mg/dL; p<0.001), apo A-I (141±16 *vs.* 155±42 mg/dL; p<0.05) and PON1 activity [145 (116-236) *vs.* 154 (113-173) *μ*mol/mL.min; p<0.05] significantly increased. Moreover, CETP activity decreased after the intervention (187±32 *vs.* 160±42 %/mL/h; p<0.05). Conclusions: BS produced improvements in anthropometric parameters, atherogenic potential of the lipoprotein profile, inflammatory status and the TG/HDL-C ratio, an indicator of insulin resistance and the proportion of small and dense LDL. HDL-C, its main apolipoprotein, apo A-I, and its antioxidant function, reflected by PON1, increased after BS, while CETP activity significantly decreased. These changes evidence different benefits of BS in morbidly obese individuals against the risk of developing atherosclerotic CVD.

371. (581) **IMPAIRED REVERSE CHOLESTEROL TRANSPORT IN CHILDREN AND ADOLESCENTS WITH ABDOMINAL OBESITY: ASSOCIATION WITH FATTY ACID PROFILE IN APO B-DEPLETED PLASMA.**

Martin M, Impa Condori A, Gómez Rosso L, Gaete L, Tetzlaff W, Lozano Chiappe E, Davico B, Saez MS, Lorenzon Gonzalez MV, Godoy MF, Osta V, Trifone L, Ballerini MG, Boero L, Tonietti M, Feliu S, Brites F.

[1] Departamento de Bioquímica Clínica. Facultad de Farmacia y Bioquímica. UBA.

[2] Departamento de Bromatologia y Nutrición. Facultad de farmacia y Bioquímica. UBA.

[3] Servicio de Nutrición y Diabetes. Hospital Ricardo Gutierrez. CABA.

[4] Hospital Italiano de Buenos Aires. CABA.

Introduction: Abdominal obesity is an important cardiovascular risk factor, closely related to lipid alterations. On the other hand, plasma fatty acids display a complex network of both pro and antiatherogenic effects. In this context, high density lipoproteins (HDL) carry out the antiatherogenic pathway called reverse cholesterol transport (RCT), which involves cellular cholesterol efflux (CCE), and lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities. Our aim was to characterize RCT and its relation to fatty acids present in plasma in children and adolescents with abdominal obesity. Methods: Seventeen children and adolescents with abdominal obesity and 17 healthy controls were studied. Anthropometric parameters were registered. Glucose, insulin and lipid levels were measured by automated methods, CCE employing THP-1 cells, LCAT and CETP activities by developed radiometric assays, and fatty acids in apo B depleted plasma by gas chromatography. Results: The obese group showed a more atherogenic lipid profile. Regarding RCT, the obese group displayed lower CCE (Media±SD) (6±2 *vs.* 7±2 %; p<0.05) and LCAT activity (11±3 *vs.* 15±5 umol/dL.h; p<0.05). With respect to fatty acids, the obese group showed higher myristic (1.1±0.3 *vs.* 0.7±0.3; p<0.01) and palmitic acids (21.5±2.8 *vs*, 19.6±1.9; p<0.05) in addition to lower linoleic acid (26.4±3.3 *vs.* 29.9±2.6; p<0.01). Eicosapentaenoic acid correlated with HDL-C (r=0.4; p<0.01), arachnoid acid with CCE (r=0.37; p<0.05), myristic acid with HDL-C (-0.38; p<0.05) and LCAT activity (-0.37; p<0.05), palmitic acid with HDL-C (r=-0.34; p<0.05), linoleic acid with HDL-C (r=0.38; p<0.05), apo A-I (r=0.40; p<0.05) and CCE (r=0.37; p<0.05), and lignoseric acid with LCAT (r=-0.5; p<0.01). Conclusion: Children and adolescents with abdominal obesity presented impaired RCT, which was associated with modifications in fatty acid profile, thus contributing to an increased cardiovascular risk.

372. (627) **AQUAPORIN 7 IN ADIPOSE TISSUE: MODULATION BY AGE AND HIGH-FRUCTOSE DIET**

Fiorella Lista[1,2], Ezequiel Hid[3,4], Daniel Keber[1,2], Larisa Maidana[1,2], Gustavo Cernadas[5], Andrea Fellet[1,2], Ana Maria Balaszczuk[1,2], Monica Galleano[3,4], Noelia Arreche[1,2]

1 Fisiología, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.2 CONICET-Universidad de Buenos Aires (IQUIMEFA), Buenos Aires, Argentina.3 Fisicoquímica, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.4 CONICET- Universidad de Buenos Aires (IBIMOL), Buenos Aires, Argentina. 5 Anatomía e Histología, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Adipose tissue (AT) is the main tissue involved in storage of fat in the body and is a key organ in metabolic homeostasis that can occur with aging. Among the aquaporins (integral membrane proteins functioning as water-selective channels), aquaporin-7 (AQP7) is an aquaglycerolporin, that is also permeable to glycerol. In AT, AQP7 deficiency is linked to increased triglycerides and glycerol accumulation, leading to adipocyte hypertrophy and development of obesity. The objective of this study was to investigate changes in AQP7 expression and glycerol levels in epididymal white adipose tissue (EWAT) in high-fructose (HF) fed rats with age progression. Male Sprague Dawley rats were grouped as follows: C1:1-month old rats, F1:1-m old rats + fructose, C12:12-m old rats, F12:12-m old rats + fructose. At 1-m or 12-m old, F groups started treatment receiving fructose in the drinking water (10% w/v) for 8 w, and C groups received tap water. At the end of treatments, animals were euthanized. Blood and EWAT were obtained. AQP7 expression and glycerol levels in EWAT, showed differential behaviors depending on age and fructose diet. Aging and HF diet in 1-m old rats produced significant increases in AQP7 expression (32% -C1 vs. C12-, and 98% -C1 vs F1-, p<0.05), without changes in EWAT glycerol. However, in 12-m old rats, AQP7 expression remained unaltered by HF diet, and was associated with a 92% of increase in EWAT glycerol. Triglycerides level in EWAT was associated with tissue expansion in 1-m old rats receiving HF, although this effect was not observed in 12-m group. Considering the role of AQP7 in the AT in the efflux of glycerol, the observed changes in its expression should represent adaptive responses to avoid cell hypertrophy and fat mass expansion. These results could explain the higher susceptibility to develop obesity with aging under overnutrition conditions. UBACyT 20020170100087BA (AB) and UBACyT 20020170100586BA (MG), PIP-CONICET 11220170100585CO, PICT 2018-03052.

373. (637) **CHARACTERIZATION AND FREQUENCY OF THE METABOLIC SYNDROME AND LIFESTYLE IN AN ADULT POPULATION OF SANTA FE- ARGENTINA**

Paola Guadalupe Illesca[1], Diego Manni[2], Florencia Cuneo[3], Carlos García[4,5], Damián Soria[5,6], María Jesús Pace[5], Sebastián Del Pazo[5,7], Marcela Inés Martinelli[8].

[1]Laboratorio de Estudio de Enfermedades Metabólicas Relacionadas con la Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Departamento de Matemática, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [3]Cátedra Seminario y Trabajo Final de Licenciatura en Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [4]Cardiología, Facultad de Ciencias Médicas, Universidad Nacional del Litoral. [5]Clínica Santa Fe. [6]Anatomía, Facultad de Ciencias Médicas, Universidad Nacional del Litoral. [7]Clínica Médica, Facultad de Ciencias Médicas, Universidad Nacional del Litoral. [8]Química Biológica, Metabolismo y Nutrición, Facultad de Ciencias Médicas. Universidad Nacional del Litoral.

The convergence of factors that increase the risk of noncommunicable diseases (NCDs) such as cardiovascular diseases, type 2 diabetes mellitus, as well as morbidity and mortality, is known as Metabolic Syndrome (MS). Several studies have reported that MS is associated with poor LS habits. In this context, the objective of this study was to evaluate the frequency of MS, its and its relationship with LS in patients from a private clinic of Santa Fe city. A descriptive and comparative cross-sectional study was carried out. Consecutive individuals who spontaneously attended the outpatient cardiology and medical clinic, between August 2021 and July 2022, were included. The inclusion criteria were age (40-75 years), without CVD diagnosis at the beginning of the study, agree to participate in the study by signing an informed consent. Anthropometric data and biochemical analyzes were recorded. Through surveys were recorded alcohol consumption, smoking, consumption of fruits and vegetables and physical activity. The presence of MS was determined by the unified clinical criteria proposed by Alberti (2009). LS was evaluated using the optimal LS score proposed by Van Wormer (2017). Qualitative variables were expressed as percentages and compared using the chi-square test and z-test. A value of $p<0.05$ was considered statistically significant. The frequency of MS was 70%. Regarding individual factors, the high frequency of hypertension (70%) stands out, followed by waist circumference (63%) and hypertriglyceridemia (42%). About the LS components, 21% of patients had a body mass index <25, only 11% reported a recommended consumption of fruits and vegetables, 27% reach recommended levels of physical activity, 46% do not consume alcohol and 85% indicated not to smoke. The identification of individuals with or at risk of developing MS as well as behavioral risk factors would help apply preventive actions to avoid its evolution to NCDs.

374. (718) **CHILDHOOD-ONSET TYPE 1 DIABETES AND CELIAC DISEASE: A POSSIBLE ROLE OF VITAMIN D IN HDL QUALITY ASSESSMENT**

Walter Tetzlaff1*, María Gabriela Ballerini1*, Maria Laura Arzamendia2, Nuria Grimberg2, Adriana Bottero3, Daniela Neder3, Maximiliano Martin1, Ezequiel Lozano Chiappe1, Belén Davico1, Leonardo Gómez Rosso1, María Luz Muzzio1, Mabel Ferraro2, Fernando Brites1, Laura Boero1.

**Contributed equally*

1 Departamento de Bioquímica Clínica. Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires. Hospital de Clínicas "José de San Martín", Argentina

2 Servicio de Nutrición y Diabetes, Hospital General de Niños Pedro de Elizalde, Ciudad Autónoma de Buenos Aires, Argentina.

3 Servicio de Gastroenterología Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Ciudad Autónoma de Buenos Aires, Argentina

Pediatric guidelines recommend screening for celiac disease (CD) at type 1 diabetes (T1D) onset, conditions that have been individually associated to HDL dysfunction. HDL promotes the inhibition of LDL oxidation (mainly dependent on paraxonase activity, PON) and indirectly transport cholesteryl esters to the liver by the cholesteryl ester transfer protein (CETP) activity. Epidemiological evidence has associated vitamin (V) D deficiency with immune and lipid dysregulation. Objective: To characterize serum HDL functionality in pediatric patients with both CD and T1D in comparison to subjects with either CD or T1D and to evaluate the possible role of VD in HDL quality in these entities. Subjects: Recently diagnosed children: 37 T1D, 34 CD and 13 T1D+CD, age: 3-18 years, with similar BMI-SDS. The protocol was approved by the ethic committee; written ascents/consents were obtained. Methods: Lipids by standardized methods, PON and CETP activities (in house methods), VD (ECLIA-Roche). Data expression: median (Pc 3rd-97th) range. Results: The T1D+CD group presented significantly higher TG: 73(33-356) mg/dL ($p< 0.01$) and lower PON activity ($p<0.05$): 87(30-251) nmol/ml.min as compared to T1D: 70(42-257) and 127 (39-270) and CD: 63(36-139) and 117(54-344) groups, respectively. Higher CETP activity ($p<0.01$) was observed in T1D+CD: 167(109-207) and T1D: 140(106-211) %/ml.h groups as compared to CD: 112 (89-147)%/ml.h. CETP activity was associated to higher TG levels ($r= 0.41$; $p<0.001$). No difference was obtained for VD among the groups. However, VD was inversely associated to TG ($r= -0.35$), TG/HDL-C index ($r= -0.31$), $p<0.001$, and CETP activity ($r= -0.27$), $p<0.05$. Conclusion: In children, T1D+CD displayed altered lipoprotein metabolism and HDL functionality thus increasing cardiovascular risk as compared to either T1D or CD. Our preliminary results suggest that VD status may be associated to a lower atheroprotective capacity of HDL.

375. (725) **DETERMINATION OF ZINC IN HAIR AS AN EARLY BIOMARKER OF DEFICIENCY**

Antonella de las Mercedes Biasi[1,2], Marina Mercedes Ramírez[3], Flavia Gimena Luna[2], María Verónica Pérez Chaca [5], Nidia Noemí Gómez[1,4,5]

[1]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Luis, Argentina. [2]Facultad de Ciencias de la Salud, Universidad Nacional de San Luis (UNSL). [3]Universidad Nacional de Villa Mercedes (UNVIME). [4]Facultad de Ciencias Médicas (UCCuyo-SL). [5]Laboratorio de Morfofisiología, FQByF - UNSL, Argentina.

The measurement of zinc in hair represents an advantage in terms of sampling, which is non-invasive and widely accepted, as well as being simple and effective. The present work intends to evaluate the possibilities of determining zinc in hair as an early biomarker of this deficiency, especially in cases of moderate deficiencies of this micronutrient (produced by deficiencies in absorption or by diet quality), since they do not present symptoms, however they can aggravate different clinical conditions, such as pulmonary pathologies, surgeries or infectious conditions. The technique for measuring zinc in hair was standardized. Acid digestion followed by microwave digestion was performed. The digestion process was tested in microwave equipment used in analytical techniques and in common microwaves. The results did not present significant differences between both teams, so it is concluded that the latter can be used, making it possible to manage times, costs and reagents. The results obtained from the analyzed samples (n=100) were between 200 mg/kg and 1000 mg/kg of zinc, in adults who consume a diet with an adequate amount of zinc and without any pathology that could modify the homeostasis of the mineral. It should be noted that there are no universally accepted reference values for zinc concentrations in hair, thus there is an urgent need to compile reference values. The values found are similar, even higher than the available values derived from studies published in countries with different characteristics: 53.7-327 mg/kg. This may be due to dietary differences between different cultures, mainly variations in dietary zinc and phytate content. It is expected to continue with this study to evaluate if there is a correlation between zinc consumption and/or other variables and its concentration in hair. By relating these parameters, the possibility of proposing the concentration of zinc in hair as an early biomarker of this deficiency will be analyzed.

376. (738) **PROTEIN KINASE B SIGNALING PATHWAY IN EPICARDIAL ADIPOSE TISSUE FROM CORONARY PATIENTS**

Barchuk Magali[1,2], Cebrón Julieta[3], Narvaez Pardo Andrés[3], Campos Jeanniel[1], Fariña Gregorio[1], Rubio Miguel[4], Schreier Laura[1], Dominici Fernando[3], Berg Gabriela[1,2].

1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires, Argentina.

2. Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Argentina

3. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) , Buenos Aires, Argentina.

4. Universidad de Buenos Aires, Hospital de Clínicas "José de San Martín", División de Cirugía Cardiovascular, Argentina.

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, despite the efforts of scientific community to find new causes and therapies for it. Directly related to CVD are obesity and insulin-resistance (IR), as main causes of the disease. In the last decades, epicardial adipose tissue (EAT), a visceral AT in direct contact with coronary arteries, has been proposed as an independent risk factor for CVD. We have previously reported a paradoxical behavior of EAT in IR, with an increase in Lipoprotein Lipase activity and its activators in coronary artery disease (CAD)-IR patients. To date, little is reported about insulin pathways in EAT. In this opportunity, our aim was to evaluate protein kinase B (AKT) activation as a step in insulin signaling pathway in EAT from CAD patients, and its association with IR markers. Materials and methods: we studied patients undergoing by-past graft surgery (CAD,n=10), and patients without CAD (No CAD,n=9). Serum lipoprotein and IR profiles were evaluated by colorimetric assays. In EAT and subcutaneous AT (SAT), we assessed Insulin Receptor (InR), AKT and Ser^{473}-phospho-AKT (pAKT) expression by Western Blot. Results: despite higher IR markers in CAD, pAKT/AKT index was higher in EAT ($p=0.02$) and SAT ($p=0.01$) in this group compared to No CAD. pAKT/AKT index tended to be lower in EAT than SAT from the same group. No differences were found neither between groups nor tissues in InR levels ($p>0.05$). Conclusion: insulin pathway would be more activated in EAT from CAD patients, despite IR, highlighting the paradoxical behavior of the tissue. Nevertheless, the role of the different cell types in the tissue metabolism cannot be yet differentiated. These results may help guide future drug discoveries that target EAT for CAD prevention.

377. (740) **HIGH FAT DIET AND CHRONIC STRESS EXPOSURE ALTER GLUCYDIC METABOLISM, GUT MICROBIOTA AND LIVER AND SMALL INTESTINE HISTOLOGY**

María Paula Marcone, Mara Roxana Rubinstein, Sofía Quiroga, Gabriel Valdivieso, Miriam Ruth Wald, Ana María Genaro.
Instituto de Investigaciones Biomédicas (BIOMED)/UCA CONICET

In recent decades, overweight and obesity have become a growing health problem. The main risk factors include increased fat intake and exposure to stressful situations. In the last years, gut microbiota (GM) has become increasingly important, contributing to the regulation of energy homeostasis. It has been described that stress and diet could alter the composition of GM, causing dysbiosis and increased intestinal permeability, inducing inflammation. In this context, the aim of this work was to investigate the relationship between high-fat diet (HFD) and chronic exposure to environmental stressors in metabolism, composition of the gut microbiota and histological alterations in liver and small intestine. The investigation was carried out in male C57Bl/6J mice consisting of four experimental groups (n=7): mice fed a normal diet (ND), DN+chronic stress (CS), high-fat diet (HFD) and HFD+CS. We observed a significant increase in body weight, fasting blood glucose and blood glucose overload test in mice fed HFD ($p<0.05$) and a decrease with stress exposure. Regarding the intestinal microbiota, Firmicutes, Bacteroidetes and Actinobacteria phyla were analyzed and total Bacteria was used as the reference gene. A significant decrease ($p<0.05$) in Firmicutes with CS or DAE was observed. There were no significant change in Bacteroidetes and Actinobacteria with both treatments. Histological analysis with hematoxylin and eosin staining indicated congestion and steatosis in the liver and inflammation in the small intestine with diet and stress treatments. We can conclude that HFD and/or stress caused alterations in body weight, glucose metabolism and modified the composition of the GM. These changes could be implicated in the pro inflammatory state of the liver and small intestine.

378. (763) **EVALUATION OF GUT ENVIRONMENT IN HEALTHY AND ALZHEIMER DISEASE PATIENTS IN THE CONTEXT OF A MEDITERRANEAN DIET**

Juana I. Mosele[1], Silvia Yuste[2], César G. Fraga[1], Mónica L. Galleano[1], Belinda Matute Tobías[3], Sara López-Álava[3], Cristina Lozano-Ochoa[4], Patricia Pérez-Matute[5], María José Motilva[2]

[1]Fisicoquímica, Facultad de Farmacia y Bioquímica-IBIMOL, Universidad de Buenos Aries-CONICET, Buenos Aires, Argentina, [2]Instituto de Ciencias de la Vid y el Vino-ICVV (CSIC, Gobierno de La Rioja, UR), Logroño, La Rioja, España, [3]Servicio de Neurología. Hospital Universitario San Pedro, Logroño, La Rioja, España, [4]Unidad Predepartamental de Enfermería. Universidad de La Rioja, Logroño, La Rioja, España, [5]Unidad de Enfermedades Infecciosas, Microbiota y Metabolismo. Dpto. Enfermedades Infecciosas. CIBIR-Hospital Universitario San Pedro, Logroño, La Rioja, España.

Introduction. Mediterranean diet (MD) has been proposed as a dietary pattern model to prevent or delay brain dysfunction by gut manipulation. Alterations of colonic environment including microbial population and derived metabolites have been associated with neurological alterations explained by gut-brain connection. Differences in metabolic profile between healthy and Alzheimer disease (AD) patients may offer the possibility to identify potential target to treat or delay the onset of the disease. The aim of this study was to identify possible metabolic alterations in the colon environment of AD patients compared with matched controls in the context of a MD. Material and methods. Healthy subjects (n=19) and AD patients (n=25) of both sexes, between 55-75 y from La Rioja, Spain, participated in this observational study. Data concerning dietary habits were obtained during personal interview and processed to calculate MD adherence score and nutrients intake. Plasmatic and fecal short chain fatty acids (SCFA) were determined by GC-MS/MS and fecal biomarkers of intestinal inflammation (IL-1β, TNF-α, calprotectin) were quantified by ELISA. Results. Dietary data and intestinal metabolites are reported in control and AD volunteers. Despite similar MD score, the consumption of animal proteins ($p=0.048$), fruits ($p=0.006$) and phenol flavones ($p=0.013$) were different between groups. Feces of controls were enriched in caproic acid ($p=0.008$) whereas propionic acid ($p=0.017$) was more abundant in fecal samples of AD patients. In plasma, isovaleric acid was higher in the control group ($p=0.041$). Regarding the biomarkers of intestinal inflammation, we observed that IL-1β was increased in feces of control volunteers ($p=0.008$). Correlations among SCFA, IL-1β and dietary parameters were also evaluated. Conclusions. These initial data suggest difference in microbial metabolism among healthy and AD patients which could be considered as possible participants in the gut-brain axis communication.

379. (775) **THE PHOSPHATIDYLCHOLINE TRANSFER PROTEIN STARD7 PARTICIPATES IN CELLULAR LIPID METABOLISM**

Jesica Flores-Martín [1], María Laura Rojas [1], Lucille Kourdova [1], Sofía Nardi [1], Ana Cristina Racca [1], Graciela Panzetta-Dutari [1], Susana Genti-Raimondi [1].

[1] Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBI-

CI-CONICET).

The increased prevalence of metabolic diseases has become a health problem that decreases life expectancy. Reports indicate that a diet deficient in methionine/choline or decreased phosphatidylcholine (PC) synthesis induces hepatic steatosis, at least in part due to reduced VLDL secretion. StarD7 is a PC transfer protein that belongs to the START superfamily, which are involved in metabolism, transport and intracellular signaling of lipids. In the present study we explored the participation of StarD7 in cellular lipid metabolism. Stable HepG2 cell lines silenced of StarD7 (shD7), and its control (shC) were generated. Data from qPCR demonstrated that StarD7 silencing lead to a significant increase of the mRNA levels of ATP-citrate Lyase (ACLY) and stearoyl-CoA desaturase (SCD) enzymes responsible for fatty acid synthesis. Also, lipid droplets stained by the fluorescent dye BODIPY (493/503) confirmed an increase of the neutral lipid storage in shD7 cells vs shC showed by microscopy. In addition to the CDP-choline pathway for PC synthesis, the liver also uses an additional pathway catalyzed by the enzyme phosphatidylethanolamine N-methyltransferase (PEMT) enzyme. Western bot assays carried out from HepG2 cells stably depleted of PEMT showed higher StarD7 expression compared to shC. Also, the addition of PC to the culture medium restores the levels of StarD7. Additionally, shPEMT cells exposed to a lipotoxic environment (200 μmol/L C16:0 palmitic acid or C18:1 oleic acid) for 24 hours expressed higher StarD7 levels than control, suggesting the involvement of StarD7 in the maintenance of lipid homeostasis. Collectively, these results highlight the role of StarD7 in the regulation of lipid biosynthesis. We consider it is essential to study the mechanisms that regulate lipid metabolism at cellular level to understand biological processes and develop effective therapies in patients with metabolic diseases.

380. (824) **NUTRITIONAL CONTRIBUTION OF SEA LEVEL QUINOA ECOTYPE TO DIET QUALITY**

María del Carmen Sanahuja[1], Viviana Rodriguez[2], Héctor Daniel Bertero[3], Anabel Nora Pallaro[1], Silvina Mariela Vidueiros[1]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Nutrición, [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Bromatología, [3]Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Producción Vegetal

Quinoa (*Chenopodium quinoa* Willd.) is considered a strategic crop to contribute to food security, being recognized as a nutritious grain due to the quantity and quality of its protein and lysine content. Lysine is an essential amino acid which is deficient in cereal proteins and determinant for protein quality. The aim of this work was to determine the protein quality through the evaluation of physiological parameters by biological method and assess the available lysine content of sea level quinoa ecotype grown in the humid pampas of Argentina. The protein quality of quinoa seeds cultivated in Hilario Ascasubi, Buenos Aires province (QHA), was evaluated using the Net Protein Utilization (NPU) method in rats. Body weight (BW, g), nitrogen intake (NI, g), body nitrogen (BN, g), NPU (%), digestibility (D, %) and biological value (BV, %) were determined. Protein content (AOAC Nº 984.13) and available lysine content (ALys, mg. g^{-1} protein), by the Albalá-Hurtado method, were determined in QHA and compared to maize flour (MA), casein (CAS) and whole milk powder (MI) values. BW, NI, BN, NPU and D of QHA (48.0 ± 3.1; 0.8 ± 0.1; 1.4 ± 0.1; 66.3 ± 6.7; 89.6 ± 0.1) were statistically lower than those in the control group that received casein (C) (66.7 ± 7.8; 1.1 ± 0.2; 1.8 ± 0.2; 84.0 ± 9.0; 98.6 ± 0.04)($p<0.01$). BV of QHA (74.0 ± 7.4) was statistically lower than C (85.2 ± 9.1) ($p=0.04$); moreover, this biological value points out a high protein quality in plant based sources. ALys content in QHA (38.4) was lower than CAS (85.8) and MI (79.8) but higher than cereals like MA (18.3). Sea level quinoa ecotype has nutritional characteristics, regarding its protein, that promote its inclusion in the diet to address problems related to nutrition through biodiversity in food and agriculture. Supported by 20020190200133BA.

381. (835) **INVOLVEMENT OF GRAPE-DERIVED BIOACTIVE COMPOUNDS ON THE PROTECTION OF INTESTINAL BARRIER ALTERATIONS INDUCED BY HIGH-FAT DIET**

Diahann Perdicaro[1], Victoria Muscia [1], Valeria Cacciamani[2], Andrea Antoniolli[3], Patricia Oteiza[4], Marcela Vazquez Prieto[1].
1. Laboratorio de Nutrición y Fisiopatología de la Obesidad, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo e Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)-CONICET, Mendoza, Argentina, 2. Laboratorio de Fisiopatología Renal Facultad de Ciencias Médicas, Universidad Nacional de Cuyo e Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)-CONICET, Mendoza, Argentina, 3. Laboratorio de Bioquímica Vegetal, IBAM, FCA, CONICET-UNCuyo, Mendoza, Argentina, 4. Departments of Nutrition and Environmental Toxicology, University of California, Davis, USA.

We previously observed that grape pomace extract (GPE), concentrated in polyphenolic compounds, attenuates metabolic and cardiovascular alterations such as dyslipidemia, hypertension and insulin resistance and prevents adipose tissue (AT) hypertrophy and inflammation in experimental models of metabolic syndrome induced by high-fat and/or high-fructose diets. Since the gastrointestinal tract, and in particular the microbiota, plays a very important role in pathologies associated with excess caloric intake, overweight and obesity, the aim of this study is to evaluate how dietary intervention with GPE can modulate the integrity of the intestinal barrier and metabolic parameters associated with high-fat diets. Male C57BL/6 mice (20-25 g) were divided into 4 groups (n = 7 each) and fed for 14 weeks as follows: i) Control group (Ctrl): standard diet; ii) Ctrl group + diet supplemented with GPE: 300 mg/kg body weight (bw)/day iii); iv) HF (high fat) group; control diet containing 60% of total calories from fat and v) HF + GPE 300 mg/kg bw/day. Consumption of a HF diet significantly increased body weight gain, visceral and subcutaneous AT increase and adipocyte hypertrophy. These parameters were associated with higher levels of fast glucose in the HF diet group. The supplementation with GPE significantly prevented body weight and AT gain and attenuated fast glucose levels. Also, the HF diet treatment decreased colon crypts length and altered goblet cells which are critical players in the preservation of barrier function. GPE supplementation to the HF diet restored these changes. Overall, consumption of GPE can protect against HF diet induce adipocyte hypertrophy and metabolic alterations and could protect the gastrointestinal tract from the damage caused by the intake of diets rich in fats. These findings support the potential relevance of consuming GPE-rich foods to attenuate high-fat diet-induced alterations.

382. (839) **SUPPLEMENTATION WITH GRAPE POMACE EXTRACT, RICH IN PHENOLIC COMPOUNDS, MITIGATES HIGH FAT DIET-INDUCED LIVER STEATOSIS IN MICE**

Victoria Muscia Saez[1], Diahann Perdicaro[1], Joana Asensio[1], Rodrigo García[1,2], Valeria Costantino[2], Marcela Vazquez Prieto[1]
1. Laboratorio de Nutrición y Fisiopatología de la Obesidad, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo e Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)-CONICET, Mendoza, Argentina, 2. Laboratorio de Fisiopatología Renal Facultad de Ciencias Médicas, Universidad Nacional de Cuyo e Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)-CONICET, Mendoza, Argentina.

Obesity is one of the major public health concerns worldwide. Obesity can increase the risk to develop insulin resistance, cardiovascular disease, dyslipidemia and non-alcoholic fatty liver disease among others. Grape pomace (GP) is a waste product, containing predominantly a left-over of skins and seeds, generated in the winemaking process which contains relatively high amounts of bioactive components like polyphenols. The aim of this study is to evaluate the effect of grape pomace extract (GPE), containing high amount of phenolic compounds, on the consequences and the metabolic complications associated with high fat diet (HDF)-induced obesity in mice. 8- week-old C57BL/6 male mice (20-25g) were divided into 4 different groups (n=7 each one) and fed for 14 weeks as follow: i)

control group: standard diet, ii) control with GPE group: control diet supplemented with GPE 300mg/kg body weight/day, iii) high-fat diet group (HFD): control diet containing 60% of the total calories from fat (lard), iiii) HFD supplemented group: high fat diet supplemented with GPE 300mg/kg body weight/day. Consumption of a HFD significantly increased body, adipose tissue and liver weight. In addition, the HFD increased plasma cholesterol, decreased glucose tolerance as evidence by glucose tolerance test and increased liver fat infiltration. GPE supplementation decreased these variables and consequently prevented fat infiltration into hepatocytes in mice fed with HFD. Overall, these findings highlight the utilization of GPE rich in bioactive compounds in the attenuation of liver steatosis and obesity-related alterations. PICT 2018-03056.

MUCOSAL IMMUNITY

Wednesday, November 16, 13:30-15 hr
Chairs: Rodrigo Papa Gobbi - Virginia Gentilini - Martin Rumbo - Renata Curciarello - Paola Smaldini - Agustina Errea - Carolina Ruera - Lorena Laporte

383. (96) **AZITHROMYCIN DISTINCTIVELY AFFECTS MESENTERIC LYMPH NODE CELL PHENOTYPE AND FOXP3 INDUCTION AFTER *IN VITRO* STIMULATION WITH BACTERIAL EXTRACTS**

Virginia, Piqueras, Luciana, Moine, Cristian, Jaime, Silvia, Correa.
Departamento de Bioquímica Clínica. Centro de Investigaciones en Bioquímica Clínica e Inmunología, CIBICI (CONICET). Facultad de Ciencias Químicas-UNC, Córdoba, ARGENTINA.

The gut microbiota plays a crucial role in influencing the development of host immunity. Most studies of the microbiome to date have focused on analyzing the organization of the microbial population. However, it is equally important to study how variability in microbial abundance and composition affects host functions. We previously described significant changes in microbial abundance in mice treated for 5 days in drinking water with 50 mg/kg/day of azithromycin (AZM), an antibiotic of the macrolide family. In this work, using C57BL/6 GFP-Foxp3 mice, we evaluated whether differences in microbial composition could modify cellular subsets of inductive sites in the intestinal mucosa. For this we prepared a) bacterial extracts (BE) and b) filtered bacterial extracts (f) from fecal samples from the control group (BEC and BECf) and from the AZM group after 5 days of treatment (BEA and BEAf). Mononuclear cells from mesenteric lymph nodes (MLN) draining small intestine (SI) and colon (C) from control and AZM-treated groups were cultured for 24 h with PBS (basal condition), BEC, BECf, BEA and BEAF. After stimulation, we determined the frequency (%) and mean fluorescence intensity (MFI) of CD3+, CD4+, CD4+Foxp3+, CD4+CD49b+, CD8+, and CD19+ lymphoid subsets by flow cytometry. The % of SI CD4+Foxp3+ cells increased in the AZM group both in basal cultures ($p=0.0385$) and after BEC ($p=0.0022$). The increase in the % of CD4+Foxp3+ cells was dependent on the presence of bacteria in the AZM group ($p<0.0001$). The frequency of C-draining MLN cells remained unchanged in the AZM group, while increases in MFI were observed for CD3 ($p = 0.0331$), CD4 ($p = 0.0347$), and CD19 ($p = 0.0064$) markers. Together, these results show that in AZM-pretreated animals, SI- and C-draining MLN cells exhibit differences in phenotype and ability to respond to stimuli. These findings suggest the association of the dysbiosis effects produced by AZM with changes in immune activity.

384. (120) **AGE, DIET AND SECTION: NEW INSIGHTS INTO THE IMMUNE BARRIER BUILDING IN AN AVIAN MODEL, THE QUAIL**

Cristian Jaime[1], Virginia Piqueras[1], Luciana Moine[1], Nicolás Nazar[2], Silvia Correa[1]
[1]Departamento de Bioquímica Clínica, Centro de Investigaciones en Bioquímica Clínica e Inmunología, CONICET, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina, [2]Instituto de Investigaciones Biológicas y Tecnológicas, CONICET, Instituto de Ciencia y Tecnología de los Alimentos, Facultad de Ciencias Exactas, Físicas y Naturales, UNC, Córdoba, Argentina

Colonization is the first step in the establishment of a diverse bacterial community in the gut. In mammals, the first contact with the microbiota occurs in the birth canal, and this inoculum is modulated by lactation. In birds, colonization begins during hatching by contact with environmental microorganisms. Therefore, studying immune parameters along a different colonization process in an avian model is highly relevant. We evaluated Japanese quail (Q, *C. coturnix*) at 7, 9, 14, 16, 21, and 28 days of age (DA); on DA 28, the rearing boxes were divided in two: maintained with starter food (SF) or changed to adult food (AF). Both groups were also studied on DA 35 and 42. Bi- or tri-factorial analyses were performed using ANOVA. Effects of Age, Section (duodenum vs colon) and Diet (starter vs adult) on bacterial density (flow cytometry), mucin production (colorimetric assay) and Bursa cellularity were studied. The bacterial density/mg of feces did not show differences with Age or Diet. Mucin analysis showed a significant interaction effect between Age and Section ($p=0.0164$) in SF-Q. Between 35 and 42 DA, significant effects were observed for the double interactions between Diet and Section ($p=0.0059$) and Age and Section ($p=0.0264$), and a trend for the interaction between Age and Diet ($p=0.0582$). Mucin values in the duodenum remained stable in the studied period, perhaps by early maturation in goblet cell density for this region. Regardless of the Diet, the colon's mucin values were significantly higher by 42 DA. In terms of bursal cellularity, between 35 and 42 DA there was a trend for the double interaction between Age and Diet ($p=0.0598$). The diet was able to modulate mucin production in the colon at different DA as well as the cellularity of the Bursa. Together, our data provide insights into the relevance of Age, Gut Section, and Diet on immune barrier development in Q along the first 42 DA, thus helping to understand the initial host-microbe interaction in birds.

385. (184) **CHITOSAN AND CHITINASE ACTIVITY IN THE INTESTINAL MUCOSA COULD HAVE IMPLICATIONS FOR THE IMMUNE RESPONSE**

Luciana Moine[1], Silvina Salinas[2], Carina Porporatto[3], Silvia Correa[1].
[1] Departamento de Bioquímica Clínica. CIBICI (CONICET). Facultad de Ciencias Químicas-UNC, Córdoba, Argentina. [2] Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR), CONICET, Argentina. [3] Instituto Multidisciplinario de Investigación y Transferencia Agroalimentaria y Biotecnológica (IMITAB-CONICET)- UNVM, Córdoba, Argentina.

Chitosan (Q) is a polymer derived from the partial deacetylation of chitin with a wide spectrum of biomedical applicability. Recently, it has been suggested that conserved chitin recognition and degradation systems in mammals could produce persistent immune activation affecting mucosal feedback mechanisms. Considering the role of epithelial cells (IECs) in intestinal immunity, we evaluated the activity of Q using *in vitro* and *in vivo* models. We evaluated the viability (MTT assay) and the migratory capacity (wound healing assay) with the IEC-18 cell line (rat small intestine cell line) after 24, 48 and 72 h of stimulation with different doses of Q. On the other hand, C57BL/6 mice were gavaged with PBS (control), 0.1 mM acetic acid (diluent), or a single dose of 5 mg/ml Q. After 16 h, IECs were isolated and E-cadherin expression was assessed (% and MFI) by flow cytometry *ex vivo* or after restimulation with Q. Finally, we measured chitinase activity in homogenized intestinal mucosa from untreated control mice aged 21-45 days (colorimetric assay). We found that Q did not alter the viability of IEC-18 cells at the doses (10 to 1000 μg/ml) and time studied. In addition, Q stimulated cell migration with a significantly higher percentage of wound closure at 24 h (200 to 1000 μg/ml) ($p<0.005$), although the effect was already observed at 12 h ($p<0.005$) with 1000 μg/ml. The frequency of IEC E-cadherin+ was higher in mice that received Q and the expression increased even more after restimulation *in vitro* for 48 h. Finally, we found a progressive increase in chitinase activity with age. In conclusion, Q interacts with IEC without affecting viability and stimulating

wound healing and the expression of molecules involved in intercellular junctions. As chitin is the second most abundant biopolymer in nature and the main structural component in a variety of living organisms, the increase in chitinase activity could be explained by sustained oral stimulation.

386. (203) **USE OF A POLYMERIC NANOPARTICLE AS AN ADJUVANT TO ACTIVATE MUCOSAL AND SYSTEMIC INNATE AND ADAPTIVE IMMUNITY**

Gastón Pascual Rizzo[1], Maia Lina Elizagaray[1], Camila Chavero[1], Daiana Bianchi[1], Eugenia Apuzzo[2], Santiago Esteban Herrera[2], Maximiliano Luis Agazzi[2], Griselda Moreno[1], Omar Azzaroni[2], Guillermo Horacio Docena[1], Paola Lorena Smaldini[1].

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP-UNLP-CONICET); [2]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA-UNLP- CONICET).

Nanotechnology plays an important role in vaccine development. It offers the opportunity to design different functional nanoparticles (Np) based on different composition, size, shape and surface properties for biomedical applications. This work aimed to characterize Np as a safe vehicle and adjuvant to be further used in vaccines. Nanoparticles were characterized using human and murine antigen-presenting cells (APCs) and epithelial cells. Cell interaction was evaluated by fluorescence microscopy (internalization and localization), flow cytometry (activation-MHCII and CD86 expression) and ELISA (IL-1β secretion). Furthermore, Balb/c mice were intraperitoneally and intranasally administered with Np-OVA and the pharmacokinetic was monitored using Np-FITC. Finally, humoral and cellular immune responses (cell subsets and cytokines), and lung-resident memory T cells (Trm) were evaluated by ELISA and flow cytometry. We found that Np were internalized only by APC and cells became activated, showing a significant increased expression of CD86 and activation of the inflammasome with secretion of IL-1β. The IL-1β production was abrogated with different inflammasome inhibitors. In vivo experiments showed that Np protected OVA through the mucosa passage, and Np-OVA reached the critical organs to promote immune activation. We observed a significant induction of serum OVA-specific IgG, increased secretion of IFN-γ by splenocytes with a high frequency of $CD8^+$- and $CD4^+$IFN-γ^+ secreting cells. Remarkably, lung $CD62L^-CD69^+$ Trm cells ($p<0.05$) and mucosal IgA were induced. In conclusion, we found that APC internalized Np and activated the inflammasome pathway promoting IL-1β secretion and B and T cells induction. Remarkably, this nanoparticle exhibited adjuvant properties for mucosal targeting to induce Th1- dependent immunity and Trm cells that could be exploited in preventive or therapeutic vaccine development for infectious and non-infectious diseases, respectively.

387. (297) **DIFFERENTIAL EXPRESSION OF THE TAM COMPONENTS AND ITS CORRELATION WITH IFN PATHWAY IN HUMAN SMALL INTESTINE OF CELIAC PATIENTS**

María Luz Iribarren[1#], Federico Perez[1#], Carolina N. Ruera[1], Luciana Guzman[2], Lorena Menendez[2], Laura Garbi[3], Cinthia M. Olexen[4,5], Juan Manuel Ortiz Wilczyñski[4], Andrea E. Errasti[5], Eugenio A. Carrera Silva[4], Fernando G. Chirdo[1]

[#] These authors equally contributed to this work.

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos-IIFP (UNLP-CONICET). Servicios de Gastroenterología de [2]Hospital de Niños and [3]Hospital San Martin. La Plata. [4]Instituto de Medicina Experimental – IMEX (CONICET). [5]Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Argentina

Objective: Celiac Disease (CD) is a chronic inflammatory disease caused by an abnormal T cell-mediated immune response to the gluten in the diet in genetic susceptible individuals. The TYRO3, AXL and MERTK (TAM) receptors and their ligands (GAS6 and PROS1) play significant roles in the immune response but also in inflammation and autoimmunity. Previously, we showed decreased PROS1 transcript levels along with increased GAS6 and AXL expression in duodenum of CD patients by RT-qPCR and immunofluorescence. Here, our aim was to determine the main TAM-expressing cell compartment in CD and its relationship with the IFNs pathways, which are involved in both CD pathogenesis and the regulation of TAM expression. Material-Methods: Duodenal biopsies were collected from untreated CD patients (CD) and non-celiac (NC) individuals and analyzed by western blot (WB) and fluorescent microscopy using specific immune linage markers. Principal component analysis (PCA) was also performed to correlate TAM axis component with IFNs pathways. Results: WB analysis showed that the expression of Pros1 and Gas6 has an inverse relationship in duodenum from CD patients. Furthermore, Gas6 was found increased in intraepithelial T cells (p-value <0.001) and *lamina propria* macrophages (p-value <0.05), whereas $Tyro3^+$ epithelial cells (p-value <0.05) and $Mertk^+$ $CD11c^+$ cells (p-value <0.05) were found decreased in CD samples. Surprisingly, no statistically differences on $Pros1^+$ and Axl^+ cells were observed on the evaluated populations comparing CD and NC. PCA analysis of target genes (TAM axis, IFNB1, IFNG, IRF1, SOCS1, USP18) separated CD from NC samples. Interestingly, two clusters of CD cohort were obtained. Conclusion: Components of the TAM axis have differential expression in distinct cell populations in duodenum. Their expression correlates with markers of the IFNs pathways with a critical role in the disease. These findings suggest a link between TAM components and CD pathogenesis.

388. (310) **PROBIOTIC YEAST TREATMENT ATTENUATES IRINOTECAN INDUCED INTESTINAL MUCOSITIS IN MICE AND IN VITRO MODEL**

Girardi Juan Ignacio, Ferreyra Compagnucci Malena María, Errea Agustina Juliana, Rumbo Martin.

Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP) - CONICET - Universidad Nacional de La Plata, La Plata, Argentina.

Irinotecan is a chemotherapeutic drug commonly used in colorectal metastatic cancer therapy. It can generate intestinal cytotoxic damage, triggering a drug induced mucositis. Given its severity, it can force the interruption of the chemotherapeutic scheme with consequent impact on disease progression. For that, we aimed to evaluate an intervention using the probiotic yeast *Kluyveromyces marxianus* CIDCA 8154 (*Km*8154), whose anti-inflammatory and anti-oxidative capabilities and protective properties in intestinal colitis models have previously been reported. *In-vitro*: reporter CACO-2 cell line with luciferase gene under CCL20 promoter were treated with Irinotecan and supplemented with *Km*8154. After incubation, cells luciferase activity was measured to assess inflammatory response. In addition, cell oxidative stress was measured using H_2-DCFDA probe by flow cytometry. *In-vivo*: Irinotecan 75mg/kg was administered to BALB/c mice to induce the drug associated mucositis and the *Km*8154 intervention group had the yeast administered daily by gavage at 10^9UFC/ml until endpoint. Seven days after the first irinotecan dose, mice were killed, and intestinal samples were obtained. Clinical, histopathological and biochemical parameters were evaluated.
In-vitro studies have shown that irinotecan treatment induce activation of the reporter Caco-2 cells and *Km8154* co-treatment is able to reduce the production of luciferase in irinotecan treated cells (p=0.0018). Furthermore, irinotecan induced oxidative stress in Caco-2 cell line that is attenuated by *Km8154* co-treatment (p=0.0255). *In-vivo* studies have shown that yeast treatment improved histopathological and diarrheal scores of mice treated with irinotecan while protecting against intestinal damage measured with a villus/crypt ratio (p=0.0006) and intestinal length shortening (p= 0.0049). The probiotic yeast *Km*8154 is able to confer protection against intestinal mucositis induced by irinotecan treatment both in vitro and in vivo models.

389. (332) **ANALYSIS OF INTESTINAL STEM CELLS IN INFLAMMATORY PATHOLOGIES**

María Belén Polo1, Emanuel Barbiera Romero1, Lorena Menendez2, Paula Borobia2, Cecilia Zubiri2, Anabella Zosi2, Luciana Guzman2, Viviana Bernedo2, Eugenia M. Altamirano3, Gustavo J. Correa4, Martín Yantorno4, Andrés Rocca5, Cecilia I. Muglia1, Guillermo H. Docena1

1Instituto de Estudios Inmunológicos y Fisiopatológicos

(IIFP), CONICET y Universidad Nacional de La Plata, La Plata (BA) Argentina, 2Servicio de Gastroenterología, Hospital de Niños Sor María Ludovica de La Plata, La Plata (BA), Argentina, 3Servicio de Anatomía Patológica, Hospital de Niños Sor María Ludovica de La Plata, La Plata (BA), Argentina, 4Servicio de Gastroenterología, Hospital General San Martin de La Plata, La Plata (BA), Argentina, 5Servicio de Gastroenterología, Hospital Dr. Carlos B. Udaondo, Buenos Aires, Argentina.

Intestinal epithelial cells (IECs) are key elements of the intestinal epithelial compartment. It is implicated in cross-talk with the microbiota and the underlying immune cells and in barrier integrity. A continuous replacement of differentiated cells maintains the homeostasis of the intestinal epithelium through the replication of stem cells (ISCs) located within the crypts. However, in different inflammatory settings, the renewal of epithelial cells may be altered, contributing to different chronic pathologies. This work aimed to identify and quantify human intestinal stem cell populations in different pathological conditions. We quantified stem cells in intestinal biopsies (23) and surgical pieces (8) of inflamed and surrounding tissue of adult patients with colorectal cancer (CCR), inflammatory bowel diseases (IBD) (19), and healthy controls (HC) (8), and from colorectal polyps of pediatric patients sensitized to cow´s milk protein (11) and biopsies surrounding the polyps (3). Stem cells were identified and quantified as LGR5+ cells by flow cytometry and confocal microscopy.
Our results showed a higher frequency of LGR5+ cells in inflamed IBD tissue compared to non-inflamed IBD tissue and gut from HC (p=0,005). In contrast, we found a smaller fraction of LGR5+ stem cells in polyps' samples compared to its control surrounding biopsies (p=0,03). Immunofluorescence microscopy revealed the presence of LGR5+ cells within the epithelial compartment, and mainly located in the crypts. In conclusion, we found an expansion of colonic stem cells in different pathologies and inflammatory conditions, being more expanded in inflamed tissues. Further analysis, including functional and next-generation sequencing assays, is in progress to get more insights into the role of these cells in the different inflammatory pathologies.

390. (401) **COLORECTAL POLYPS FROM PEDIATRIC PATIENTS SENSITIZED TO COW´S MILK SHOWED ELEVATED PRO-INFLAMMATORY MEDIATOR, IgE-EXPRESSING CELLS AND IgE MEMORY B LYMPHOCYTES**

Manuela Ilid[1], Julián Vaccaro[1], Emanuel Feregotti[1], Belén Polo[1], Elisa Moncada, Lorena Menendez[2], Paula Borobia[2], Cecilia Zubiri[2], Anabella Zosi[2], Luciana Guzman[2], Viviana Bernedo[2], Marcela García[3], Eugenia M. Altamirano[4], Renata Curciarello[1], Cecilia I. Muglia[1], Guillermo H. Docena[1] .

1 Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), CONICET y Universidad Nacional de La Plata, La Plata (BA) Argentina. 2 Servicio de Gastroenterología, Hospital de Niños Sor María Ludovica de La Plata, La Plata (BA), Argentina. 3 Servicio de Alergia, Hospital de Niños Sor María Ludovica de La Plata, La Plata (BA), Argentina. 4 Servicio de Anatomía Patológica, Hospital de Niños Sor María Ludovica de La Plata, La Plata (BA), Argentina.

Cow's milk allergy (CMA) is caused by an immune response to food dairy proteins and is mainly observed in the pediatric population. We previously described that colorectal polyps in children with CMA present an allergic cell infiltrate rich in eosinophils, Th2 inflammatory environment and germinal centers with IgE-producing cells. In this work, we aimed to get further insights on the inflammatory mechanisms that may contribute to polyp development and search for IgE memory B cells (IgE LB_{mem}). Juvenile polyps (n=23) were removed by colonoscopy followed by epithelial and stromal cell isolation. Surrounding control tissue (SCT) biopsies and peripheral blood were also obtained in every patient. Immunofluorescence was performed to evaluate IL-33 and TSLP production (n=8); IL-1β, IL-33, TLSP and CCL26 expression was quantified by RT-qPCR (n=10); the frequency of eosinophils (CD45+, CD14-, CD16-, Siglec8+), IgE-expressing cells (IgE+), and IgE+ LB_{mem} (CD19+, CD27+, CD138-, IgD-, IgE+) was determined by flow cytometry (n=10). We found IL-33+ and TSLP+ epithelial cells by confocal microscopy and a significant increase of IL-1β transcripts in polyp tissue compared to SCT (p=0,031), especially in the epithelial compartment (p=0,034). Similarly, IL-33 was higher in polyps than SCT (p=0,057), while TSLP and CCL26 expression was variable in different polyps and SCT. Concordantly, we found an eosinophil-dominant cell infiltration in polyps (1,55+/-2,79% of live cells) with IgE+ cells (35,41+/-20,36% of the total cells). Remarkably, IgE+ LB_{mem} cells were observed within polyps and higher than in peripheral blood (20+/-2 vs 12+/-2% of LB_{mem} cells, respectively, p=0,051). In conclusion, our results suggest that juvenile colorectal polyp tissue from cow's milk sensitized children produce proinflammatory signals that trigger a Th2 environment, favoring eosinophil recruitment, IgE production and LB differentiation to IgE+LB_{mem}+ cells. This is the first report of the presence of human colonic IgE_{mem}+ B cells in food allergy.

391. (450) **THE GALECTIN-4-GLYCAN AXIS IN INTESTINAL INFLAMMATION: FRIEND OR FOE?**

Mora Massaro[1], Alejandro J. Cagnoni[1,2], Montana N. Manselle Cocco[2], Anabela M. Cutine[1,2], Joaquín P. Merlo[1], Florencia Veigas[2], Sabrina G. Gatto[2], Rosa M. Morales[2], Bladimiro A. Lenis[3], Marta A. Toscano[3], Juan M. Pérez-Sáez[2], Gabriel A. Rabinovich[2], Karina V. Mariño[1].

[1] Laboratorio de Glicómica Funcional y Molecular, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

[2] Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

[3] Unidad de Conocimiento Traslacional, Hospital Doctor Arturo Oñativia, Gobierno de la Provincia de Salta, Argentina.

Inflammatory Bowel Diseases (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) constitute a group of chronic disorders that affect the gastrointestinal tract. Limitations of the currently available treatments generate a need for new therapeutic targets. Galectin-4 (Gal4) is a tandem-repeat type lectin with two carbohydrate recognition (CRD) domains that is predominantly expressed in intestinal epithelial cells, but its role in IBD is still under debate. In this work, we analyzed single cell RNAseq transcriptomic data from UC patients and found that Gal4 is downregulated when compared to healthy controls (p-val=$4.9e^{-68}$). We then evaluated the influence of Gal4 using transgenic mice with a specific depletion of Gal4 in the intestinal epithelium ($CreVill^{+/-}$/$FloxGal4^{+/+}$). Although development of dextran sulfate sodium (DSS) acute colitis in these mice did not show statistically significant differences in weight loss and colon weight/length ratio when compared to WT littermates, we found significant differences in the T cell compartment, as mesenteric lymph nodes showed increased proportion $CD4^+$ $CD62L^+$ $CD44^+$ memory T cells (p-val=0.009). No differences in the myeloid or B cell compartments were found. Next, and in order to explore the functional role of this protein, we have optimized the recombinant expression and purification of human Gal4 in *E. coli* BL21 (DE3) cells. As the His-Gal4 construct did not show optimal results, vector pET-28a-SUMO-Gal4 was finally selected for transformation. For purification, the SUMO tag was cleaved, and two sequential affinity columns (Ni-NTA and lactose) were used, obtaining 2 mg/L of active hGal4. Activity was biochemically tested by solid phase assays and *in vitro*, as hGal4 was able to induce interleukin-6 secretion in activated (anti-CD3 and anti-CD28) splenocytes. In summary, with these preliminary results we set up the basis for further characterization of the role of the Gal4-glycan axis in intestinal inflammation.

392. (463) **SEMEN IMPROVES THE PROTECTIVE IMMUNE RESPONSE GENERATED BY A SYSTEMIC VACCINATION AGAINST HSV-2**

F. Di Diego García; B, Mazzitelli; A. Varese; A. Paletta; G. Cabrerizo; J. Sabatté; F. Remes Lenicov; J. Geffner; A. Ceballos.

University of Buenos Aires, INBIRS (Institute for Biomedical Research on Retroviruses and AIDS), Buenos Aires, Argen-

tina

Semen is able to induce immunological tolerance which is determinant in fecundation. But it is also the principal vector of sexually transmitted infections (STI). Are the immunosuppressive properties of semen able to facilitate STI? In previous work, we demonstrated that the seminal vesicle fluid (VS) does not facilitate the infection, but improves the protective effect induced by an intravaginal vaccination with inactivated HSV-2 in a murine model. This effect could be due to a local inflammatory response and a robust memory immune response mediated by CD8+ T cells. Now, we are exploring whether semen is able to promote the homing of activated T cells at the vaginal tract after systemic immunization. Vagina is restrictive for memory T cell entry; this can be overcome in inflammatory processes, leading to the establishment of resident memory T cells. Our hypothesis sustains that semen by inducing a local inflammatory response will promote the recruitment of memory T cells generated in response to systemic immunization. We design a strategy based on two steps: 1) subcutaneous vaccination with UV-inactivated HSV-2 (10^6 PFU) and 2) vaginal application of VS in ten-week-old female BALB/c mice (6 per group). Twenty five days later, mice were challenged with a lethal dose of HSV-2 ($2x10^5$PFU). Clinical score, weight and survival were measured Mice stimulated with VS after vaccination showed higher survival probability after the challenge compared with the PBS group (67% vs 17%; p=0.0105; n=2 independent experiments). The VS group have lost less weight and showed less signs of effective infection. In addition, none of the animals in this group showed hind limb paralysis, an indicator of irreversible infection. Our preliminary results showed that the VS improved the protective response conferred by the vaccine. This subject acquires special relevance in the design of vaccines that confer protection against sexually transmitted infections at vaginal mucosa.

393. (477) **TRANSIENT RECEPTOR POTENTIAL VANILLOID 1-DEFICIENCY PROTECTS FROM IMMUNE-MEDIATED CORNEAL NERVE DAMAGE IN THE CONTEXT OF DRY EYE**

Manuela Pizzano, Alexia Vereertbrugghen, Florencia Sabbione, Irene Angelica Keitelman, Carolina Maiumi Shiromizu, Douglas Vera Aguilar, Federico Fuentes, Mirta Giordano, Analía Trevani, Jeremías Galletti

Innate Immunity Laboratory, Institute of Experimental Medicine (CONICET/National Academy of Medicine), Buenos Aires, Argentina

Introduction: Transient receptor potential vanilloid 1 (TRPV1) channels are involved in neurogenic inflammation and neurodegeneration in non-ocular tissues. TRPV1 channels are activated by inflammatory stimuli and we have reported increased TRPV1 signaling in dry eye. Here we hypothesized that TRPV1 channels could play a role in dry eye-associated corneal nerve damage. Methods: Dry eye was induced for 10 days in 6-8-week-old C57BL/6 (wildtype, wt) and TRPV1KO mice of both sexes by bilateral excision of the extraocular lacrimal glands. Conventional dry eye parameters were assessed along with corneal nerve function (mechanical sensitivity) and morphology (βIII tubulin staining). Results: Wt and TRPV1KO mice with dry eye had comparable tear deficiency (17±7 vs 23±18%, p=0.4), corneal epithelial damage (MFI 11.8±0.8 vs 11.1±0.6, p=0.6) and conjunctival CD4+ T cell infiltration (7.8±1.4% vs 3.1±0.1%, p>0.05). Also, there was no difference in interferon gamma- (2.85±3.62 vs 1.73±2.2, p=0.4) and interleukin 17-producing (2.4±4.6 vs 1.9±5.1, p=0.9) CD4+ T cells in the eye-draining lymph nodes, which are pathogenic in dry eye. Regarding the corneal nerves, TRPV1KO mice had significantly higher corneal mechanical sensitivity than wt mice at baseline (4.6±0.09 vs 3.75±0.1 cm, p<0.05). Intriguingly, corneal sensitivity in TRPV1KO mice was not affected by dry eye (-4%, p=0.3), which contrasted the observed drop in wt mice (-21%, p<0.01). In line with this finding, there was a trend for less dry eye-induced change in corneal nerve morphology in TRPV1KO than in wt mice (-4±9% vs -11±9%, p>0.05). Conclusion: These findings suggest that dry eye-induced corneal neuropathy depends on TRPV1 signaling and that it is a separate entity from corneal epitheliopathy because TRPV1KO mice did not differ from the wt mice in any of the the non-neural aspects of the disease.

394. (506) **FOOD ALLERGY: LESSONS FROM JUVENILE POLYPS**

Cecilia Muglia[1]

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), CONICET-Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, asociado CIC PBA, La Plata, Argentina.

Food allergies, including cow´s milk protein allergy (CMA), are adverse immune response to food proteins, which are growing worldwide, affecting 5-10% of children and 1-3% of adults. These disorders can be classified as IgE-, non-IgE- and mixed allergic reactions. IgE mediated allergies are of concern because of the risk of severe reactions such as anaphylaxis. Evidence regarding the mechanisms of IgE production in the human gut are scarce, and little is known about IgE memory cells. We have been working for several years on colorectal juvenile polyps (JP) from children sensitized to cow´s milk proteins (CMP). These polyps, which promote rectal bleeding, are routinely removed by colonoscopy, and constitute an excellent human tissue to study the mucosal underlying mechanisms that provoke food allergies. We reported a prominent cellular infiltrate in JP rich in mononuclear cells, eosinophils, and mast cells. We demonstrated the presence of active germinal centers, with a high frequency of CD20+ cells (B cells), ki67+ cells (proliferating cells) and AID+ cells (cells undergoing class switch recombination and hypermutation). The stroma also showed to contain IgE+ plasma cells (IgE+CD138+ cells). All these features refer as active and Ig-producing germinal centers in the stroma of polyps, and we demonstrated for the first time that the human colon is a mucosal site for production of IgE. Furthermore, we described in the polyp stroma a Th2 inflammatory environment, with high levels of IL-4 and a high IL-13/IFN-γ ratio compared to the surrounding tissue, and the presence of milk-specific T cells expressing gut homing integrins. Finally, JP are rich in pro-inflammatory and type-2 chemokines secreted by epithelial cells, which may play a central role in the promotion of the local allergic response and development of polyps. In conclusion, our findings reveal the role of epithelial cells in the inflammatory environment and IgE synthesis found in JP and indicate a potential link between JP and food allergy.

395. (520) **MICRO-RNA 21 BUT NOT MIR-155 IS DIFFERENTIALLY EXPRESSED IN THE GUT MUCOSA OF PATIENTS WITH INTESTINAL INFLAMMATORY CONDITIONS**

Emanuel Barbiera Romero[1], Anahí Rendón Soria[1], Malena Ferreyra Compagnucci[1], María Belén Polo[1], Gustavo J. Correa[2], Martín Yantorno[2], María Victoria Mercader[3], Paula Chavero[3], Julio De Maria[3], Andrés Rocca[4], Alicia Sambuelli[6], María Virginia Gentilini[7], Gabriel Gondolesi[7,8], Guillermo H. Docena[1], Martín Rumbo[1], Cecilia I. Muglia[1] and Renata Curciarello[1].

1-Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), CONICET- Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Asociado CIC PBA, La Plata, Argentina. 2-Área de Enfermedad Inflamatoria Intestinal, Sala de Endoscopía, Servicio de Gastroenterología, Hospital Interzonal General de Agudos General San Martín, La Plata, Argentina. 3-Servicio de Gastroenterología, Hospital Italiano de La Plata, La Plata, Argentina. 4-Unidad Endoscopía, Hospital de Gastroenterología Dr. Carlos Bonorino Udaondo, Ciudad Autónoma de Buenos Aires, Argentina. 5-Unidad de Proctología, Departamento de Cirugía, Hospital de Gastroenterología Dr.Carlos Bonorino Udaondo, Ciudad Autónoma de Buenos Aires, Argentina. 6-Sección de Enfermedades Inflamatorias Del Intestino, Hospital de Gastroenterología Dr. Carlos Bonorino Udaondo, Ciudad Autónoma de Buenos Aires, Argentina. 7-Laboratorio de Investigación Traslacional e Inmunología Asociada al Trasplante. Instituto de Medicina Traslacional, Inmunología, Trasplante y Bioingeniería (IMeTTyB-CONICET), Universidad Favaloro, Ciudad Autónoma de Buenos Aires, Argentina. 8-Unidad de Soporte Nutricional, Rehabilitación y Trasplante

Intestinal, Hospital Universitario Fundación Favaloro, Ciudad Autónoma de Buenos Aires, Argentina.

Mucosal myofibroblasts are key stromal cells involved in IBD pathogenesis and in the CRC tumor micro-environment. Here, we aimed to study the expression of miR-21 and miR-155 in the mucosa and intestinal fibroblasts from IBD and CRC patients, as potential biomarkers to predict CRC outcome in patients with chronic intestinal inflammatory disorders. Target genes and proteins regulated by these miRNAs were also analyzed. Total RNA was obtained from mucosal explants from IBD(n=25), polyp(n=8), CRC(n=7), and healthy control (HC) patients (n=16). Intestinal fibroblast primary cultures were established from colon surgical pieces (n=10). Microvesicles were obtained from fibroblast culture supernatant by ultracentrifugation. MiR-21, miR-155, PDCD4, CBX7, KRAS transcript expression was quantified by qPCR in mucosal and cell samples. Also, TNF-α was quantified by ELISA while FAP (Fibroblast Activation Protein) expression was evaluated by immunofluorescence in biopsies. We found that miR-21 was highly expressed in inflamed biopsies compared to uninflamed mucosa ($p<0.01$) and to HC ($p<0.01$), while PDCD4 levels were decreased in IBD biopsies compared to HC ($p<0.01$). No differences were found for miR-155 expression, and KRAS was not detectable. MiR-21 was overexpressed in the stromal compartment of the mucosa ($p<0.1$) compared to the epithelial layer. At the protein level, TNF-α was increased in IBD biopsies compared to HC (21.09 vs. 6.19 pg/μg of protein, $p<0.1$) and FAP was highly detected in inflamed intestinal biopsies from active IBD patients, polyp and CRC samples, compared to uninflamed mucosa. Fibroblast primary cultures from IBD patients and extracellular microvesicles overexpressed miR-21 ($p<0.1$) and miR-155 ($p<0.1$), while PDCD4 was downregulated ($p<0.001$) compared to HC fibroblasts. Our results highlight the relevance of miRNAs and fibroblasts in gut inflammation and CRC development. MiRNAs expression pattern studies would contribute to identification of CRC biomarkers.

396. (574) ***Corynebacterium pseudodiphtheriticum* STIMULATE ALVEOLAR MACROPHAGES AND IMPROVE THE RESPIRATORY HUMORAL IMMUNE RESPONSE**

Ramiro Ortiz Moyano[1], Fernanda Raya Tonetti[1], Mariano Elean[1], Stefanía Dentice Maidana[2], Leonardo Albarracín[1], Susana Alvarez[1], Julio Villena[1].

[1]Laboratory of Immunobiotechnology, Reference Centre for Lactobacilli (CERELA-CONICET), Tucuman, Argentina. [2]Laboratory of Antimicrobials, Institute of Microbiology, Faculty of Biochemistry, Chemistry and Pharmacy, National University of Tucuman, Tucuman, Argentina.

Previously, we demonstrated that nasally administered *Corynebacterium pseudodiphtheriticum* 090104 (Cp) or its bacterium-like particles (BLPCp) were able to increase the resistance of mice against bacterial and viral respiratory pathogens. In this work, we evaluated: a) the interaction of Cp and BLPCp with alveolar macrophages (AMs) and also b) their ability to enhance the humoral immune response induced by a commercial vaccine against *Streptococcus pneumoniae* (Sp). First, Cp or BLPCp were incubated with primary cultures of murine AMs, the phagocytic activity and the production of cytokines were evaluated. Optical and electron microscopy analysis revealed that Cp and BLPCp were efficiently phagocyted by AMs. Plus, they were able to trigger the production of TNF-α, IFN-γ, IL-6 and IL-1β ($p<0.05$ vs non-stimulated controls) by AMs. Next, 3 weeks-old-Swiss mice were intranasally immunized at days 0, 14 and 28 with 1:100 PBS dilution of the pneumococcal vaccine Prevenar®13 (PCV), Cp+PCV or BLPCp+PCV (10^8 cells or particles/ml). On day 33, samples of bronco-alveolar lavages (BAL) and serum were collected for the study of specific antibodies and parallel, challenged with Sp serotypes 6B or 19F (10^8 UFC/ml PBS), then sacrificed on day 35 to evaluate the resistance to the infection. Cp+PCV and BLPCp+PCV groups had higher specific serum IgG and BAL IgA antibodies than control mice ($p<0.05$). These groups also had lower lung pneumococcal cell counts ($p<0.05$) as well as lower levels of BAL albumin and LDH indicating a reduced alteration of the alveolar-capillary barrier and lower cellular damage than control mice. The results demonstrated that Cp and BLPCp are capable of stimulate the respiratory innate immune system serving as adjuvants to potentiate the adaptive humoral immune response. Our study is a step forward in the positioning of this respiratory commensal bacterium as a promising mucosal adjuvant for vaccine formulations aimed to combat respiratory infectious diseases.

397. (577) **UP TO NO GOOD: ABERRANT GLYCOSYLATION AND SECRETORY IGA FUNCTION DURING INTESTINAL INFLAMMATION**

Anabela M. Cutine[1,2], Alejandro J. Cagnoni[1,2], Joaquín Merlo[1,2], Luciano G. Morosi[1,2], Montana N. Manselle Cocco[2], Florencia Veigas[2], Verónica Martínez Allo[2], Rosa Morales[2], Sabrina G. Gatto[2], María May[3], Gabriel A. Rabinovich[2], Marta A. Toscano[4], Karina V. Mariño[1]

[1] Laboratorio de Glicómica Funcional y Molecular, Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina.

[2] Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina.

[3] Instituto de Investigaciones Farmacológicas, CONICET, Buenos Aires, Argentina.

[4] Hospital Dr. Arturo Oñativia, Salta, Argentina.

Ulcerative colitis (UC) is characterized by chronic and relapsing inflammation of the colon and has currently no cure. Secretory IgA (SIgA), a heavily glycosylated complex produced by plasma cells (PCs), plays a key role in maintaining gut immune homeostasis. In numerous inflammatory pathologies including UC, aberrant glycosylation affects the function of immune cells and glycoproteins; however, the specific role PCs and SIgA glycosylation in gut inflammation has not been characterized yet. Thus, we started by analyzing glycosylation-related genes in single cell RNA-sequencing data from UC patients' biopsies. Notably, IgA^+ PCs showed downregulation of genes involved in UDP-GlcNAc and CMP-sialic acid biosynthesis (*UAP1*, *NANS*) compared with controls ($p<0.05$), which could impair SIgA sialylation. We experimentally addressed this question in a chronic dextran sodium sulphate-induced colitis model, where we observed decreased **α(**2,6) sialylation both in *lamina propria* IgA^+ PCs ($p<0.05$) and SIgA ($p<0.05$). Moreover, B cells deficient in *St6gal1* (an enzyme that adds **α(**2,6) sialic acid to N-glycans) showed an impaired ability to ameliorate T cell-driven colitis in *Rag2*$^{-/-}$ mice, with increased histologic score compared with their WT counterparts ($p<0.05$). After ruling out potential mechanisms related to sialylation in B cell function, we focused on the role of desialylated SIgA, and found that sialic acid removal induced higher binding to fecal bacteria ($p<0.05$) and human monocytes ($p<0.05$). Furthermore, binding of desialylated SIgA potentiated the proinflammatory profile of human monocytes by upregulating IL-1β ($p<0.05$). Based on these findings, we propose a novel immune circuit potentially relevant to UC, where gut inflammation may promote decreased sialylation in PCs and SIgA and, in turn, desialylated SIgA may exacerbate colitis by heightened proinflammatory activity. These results postulate inflammation-related aberrant sialylation as a novel functional modulator of SIgA.

398. (634) **POTENTIAL ROLE OF NECROPTOSIS IN THE PATHOGENESIS OF CELIAC DISEASE.**

Ruera Carolina[1], Perez Federico[1], Iribarren María Luz[1], Guzman Luciana[2], Menendez Lorena[2], Garbi Laura[3], Chirdo Fernando Gabriel[1]

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos-IIFP (UNLP-CONICET). Servicios de Gastroenterología de [2]Hospital de Niños and [3]Hospital San Martin. La Plata. Argentina

Objective. Apoptosis is the cell death pathway commonly used for the massive elimination of cells, such as enterocytes in celiac disease (CD), an enteropathy developed in genetically predisposed individuals after gluten ingestion. Unlike the immunologically silent apoptosis, necroptosis results in the release of intracellular components with proinflammatory activity (danger signals), which may expand the inflammation and tissue damage. The aim of this work was to evaluate the expression of components of the necroptosis pathway in healthy human small intestine and samples from CD patients.

Material-Methods. Duodenal biopsies were collected from pediatric and adult patients during the routine procedure for CD diagnosis and used for confocal microscopy studies, Western blot (WB), and RT-PCR analysis. Ethics committees from Health Institutions approved this protocol. Results. TUNEL reaction showed an increased number of dead cells in the duodenum of CD patients compared with healthy controls (p< 0,002). TUNEL$^+$ cells were CD95$^+$ and in close contact with CD3$^+$ cells. In addition, p-MLKL expression was found in some CD3$^+$ T cells in *lamina propria* of CD patients and Paneth cells (βdefensin$^+$) in the crypts. WB and inmunofluorescence analysis showed an increment in RIPK3 and p-MLKL expression in CD patients (p <0.05). RT-qPCR analysis revealed that RNAm levels of ZBP1 were also increased in CD patients (p <0.05). Interestingly, Type I IFN and IRF1 (p< 0,05), inducers of ZBP1, were also found to increase in duodenum of CD patients compared with controls. Conclusion. Markers for necroptosis were detected in the duodenum of CD patients. Therefore, in addition to the immunologically silent apoptosis, proinflammatory cell death is also active in enteropathy. Since necroptosis releases danger signals, this pathway may contribute to amplify the inflammatory process and damage mechanisms in the intestinal mucosa in CD.

399. (652) **HUMAN OROPHARYNGEAL MUCOSAL IMMUNITY OVER TIME, A TALE OF TWO LYMPHOID POPULATIONS**

Juliana Puyssegur[1], Rocío A. Pastor[1], Lindybeth Sarmiento Varón[1], Gonzalo Husain[1], M. Elena Arabolaza[2], Bibiana Paoli[2], and Eloísa I. Arana[1,3]

[1]Institute of Immunology, Genetics and Metabolism (INIGEM), Clinical Hospital 'Jose de San Martín', University of Buenos Aires (UBA), National Council for Scientific and Technological Research (CONICET), Buenos Aires, Argentina, [2]Pediatric Otorhinolaryngology Service, Otorhinolaryngology Division, Clinical Hospital 'Jose de San Martín', UBA, Buenos Aires, Argentina, [3]Department of Immunology, School of Medicine, UBA, Buenos Aires, Argentina

Human paired palatine tonsils are lymphoid epithelial tissues of the oral mucosa around the oropharynx. We have used them as a model to explore the evolution of oropharyngeal mucosal immune responses from childhood to youth. Age-related functional changes were explored in our cohort of patients by scoring the percentage of germinal center B cells (B_{GC}) within isolated tonsillar mononuclear cells, as a read out of the effector immunological activity of these organs. We found that the proportion of B_{GC} steadily declined with increasing age (n=74, 4 groups of age, means statistically different, p<0.05, Anova test). On the other hand, memory B cell pool increased with increasing age (n=52, 4 groups of age, p<0.05, Anova test). Considering that Tfh cells are the drivers of T cell–dependent GC responses, we assessed tonsillar T CD4+ cell populations. Tfh cells locating within the GC (Tfh_{GC}) express high levels of CXCR5 and PD-1 ($CXCR5^{high}$ $PD\text{-}1^{high}$). They declined with increasing age (n=44, 4 groups of age, p<0.05, Anova test). There are also Tfh in secondary lymphoid organs which express intermediate levels ($CXCR5^{int}$ $PD\text{-}1^{int}$) of those markers and localize in the mantle zone of the follicle (mTfh). The latter increased with increasing age (n=44, 4 groups of age, p<0.05, Anova test). Finally, we showed that the impairment of GC reaction with ageing, correlated with an increment in the proportion of regulatory B cells (n=40, 2 groups, 37.7% ± SD 10% vs 25.8% ± SD 8.8% respectively, p<0.01). To conclude, our results show that different life stages present different immune landscapes. In early childhood, the immune system needs to be educated for its basic functions: to recognize pathogens and to set mechanisms to establish tolerance to harmless microbes. Post puberty, memory pools increase due to recurrent Ag exposure and regulatory mechanisms become relevant to avoid immune-pathology due to the overexposure with time.

400. (654) **A TH1-SKEWED IMMUNE RESPONSE IN THE OCULAR SURFACE FAVORS CORNEAL NERVE DAMAGE**

Alexia Vereertbrugghen, Manuela Pizzano, Florencia Sabbione, Irene Angelica Keitelman, Carolina Maiumi Shiromizu, Douglas Vera Aguilar, Federico Fuentes, Mirta Giordano, Analía Trevani, Jeremías Galletti

Laboratorio de Inmunidad Innata, Instituto de Medicina Experimental (CONICET/Academica Nacional de Medicina), Buenos Aires, Argentina

Introduction: Corneal nerve damage occurs by an unknown mechanism in dry eye and other immune-based ocular surface disorders with a Th1-based pathogenesis but not in ocular allergy. Therefore we hypothesized that a particular type of immune response could promote corneal neuropathy. Methods: OT-II mice [transgenic for an ovalbumin (OVA)-specific CD4-restricted TCR] were immunized with OVA+Freund's adjuvant or alum to induce a Th1 or Th2-skewed immune response and 2 weeks later challenged with OVA eye drops for 10 days. Also, CD4+ T cells from Th1 or Th2-skewed OT-II mice were adoptively transferred to RAG1KO mice that were given OVA eye drops for 5 weeks. Results: Th1-skewing in CFA-immunized mice was confirmed by higher OVA-induced delayed-type hypersensitivity (0.20±0.09 vs 0.05±0.08 mm, p<0.05) and a lower IL-4+/IFN-γ+ CD4+ T cell ratio (2.16±1.05 vs 7.97±8.67, p<0.05) than alum-immunized mice. After OVA challenge, Th1-skewed mice had lower corneal mechanical sensitivity (-25±12% vs +1±10%, p<0.05) and fewer intraepithelial nerve endings (42.4±11.44 vs 85.8±14.65, p<0.05) than Th2-skewed mice. Neither showed corneal epithelial damage (MFI 4.33±1.49 vs 4.06±0.63, p>0.05). By contrast, Th2-skewed mice showed a drop in corneal MS by 5 days after alum immunization (-12.18±10.84% vs +0.18±6.83%, p<0.05) that did not worsen by ocular OVA challenge. We found a trend towards more conjunctival CD4+ T cells in Th2-skewed than in Th1-skewed mice after OVA challenge (0.72±0.77% vs. 0.17±0.11%, p>0.05). Finally, only the adoptive transfer of Th1-skewed CD4+ T cells led to reduced corneal MS (-21.14±6.96% vs -3.06±7.06%, p<0.05) and less corneal nerve density (3.16±0.55 vs 4.83±0.85, % area, p<0.05) in RAG1KO recipients. Conclusion: These findings show that corneal nerve damage is immune-driven, favored by a Th1 immune response, and more importantly, not a direct consequence of corneal epitheliopathy. These models could serve to study the pathophysiology of corneal neuropathy.

401. (694) **EFFECT OF A PROBIOTIC STRAIN ON THYMUS T-LYMPHOCYTE POPULATION IN OBESE AND SENESCENT MICE MODELS**

María Florencia Balcells[1 2], María José Martínez Monteros[1], Silvia Inés Cazorla[1 3], Gabriela Perdigón[1] and María Carolina Maldonado-Galdeano[1 3].

[1]Centro de referencia para Lactobacilos (CERELA), San Miguel de Tucumán, Tucumán. Argentina, [2]Cátedra de Microbiología y Parasitología, Nutrición. Universidad del Norte Santo Tomas de Aquino (UNSTA), San Miguel de Tucumán, Tucumán. Argentina, [3]Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán. Argentina.

The thymus undergoes involution physiological processes that affect its functionality caused by senescence. The obesity can speed up this process with T lymphocytes population deterioration. Aim: to study the effect of a probiotic strain on the T population from the thymus of obese and senescent mice. Methods: Male Balb/c mice were divided in tow groups according to the experimental model: A) Obesity: was subdivided according to diet/treatment: Normal Control (NC), NC + Probiotic (NC+P), Obese Control (OC) and OC + probiotic (OC+P); B) Senescence: was subdivided according to treatment and age: NC and NC+P: 21, 28, 45, 90 and 180 days. A high fat diet was administered to obese mice group until they reached 25% more body weight compared to the NC group. Samples: Thymus (T) from obese and senescent mice were taken: T Lymphocyte (LT) were analyzed by flow cytometry and IL-7 was determined in culture supernatant of T stimulated with the probiotic bacteria (B) or its cell wall (W) by ELISA. Statistical analyses were performed by GraphPad Prism software using the ANOVA, with Tukey's correction for multiple comparisons, p< 0.05 was considered statistically significant. Results: In both obesity and senescence mice, decreased LT-CD4+, LT-CD4+/CD8+ (DP) population and increased LT-CD8+ population were observed. Probiotic normalized LT-CD4+ and increased DP and LT-CD4-/CD8- (DN) compared to NC. IL-7 levels decreased significantly in both models (P<0.0001). B and W significantly in-

creased IL-7 levels in both mouse models relative to NC (P<0.0001). Conclusion: the probiotic strain has a positive influence on the thymus, on the maturation of the T population and this improvement goes hand in hand with the significant increase observed in IL-7 level in all the experimental groups that received the probiotic strain both as oral suspension and also in T culture stimulated with B or W.

402. (732) **GALACTO-OLIGOSACCHARIDES, ANTAGONIZE TOXIC EFFECTS OF ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* ON CULTURED CELLS AND BRINE SHRIMP MODEL**

Hugo, A.A[1]; Bugallo A[1]; Pérez, P.F[1]; Ramos, V[2]; Palermo, M[2].
1-Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, CCT-La Plata). Calle 47 y 116. La Plata, Argentina. 2-Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, IMEX (CONICET)-Academia Nacional de Medicina, Academia Nacional de Medicina, Buenos Aires, Argentina.

Enterohaemorrhagic *Escherichia coli* (EHEC) is a food-borne zoonotic pathogen, responsible for bloody diarrhea, enterocolitis and hemolytic uremic syndrome. The main virulence factors responsible for disease development are Shiga toxins. Galacto oligosaccharides (GOS) are prebiotic compounds, with capacity to increase the number of beneficial microbes in the intestinal tract. They are composed of a variable number of galactose units linked to a glucose unit, forming di, tri tetra or pentasacharides. The aim of this work is to evaluate the inhibitory capacity of different GOS formulations against toxic effect of Shiga toxin using *in vitro* models of Vero cells and *Artemia salina.*. Biological activity of Shiga toxin (recombinant Stx2) was tested, in the presence or not of GOS, using Vero cells (LDH assay and crystal violet staining) and brine shrimp (lethality test). In Vero cells, GOS formulations exhibited a protection between 30% and 50% when GOS were added at a concentration ranging from 4% to 2%. Inhibitory effect was assayed with different GOS concentrations and fitted to a dose response curve. Formulations with higher percentage of GOS showed the highest protection. In the brine shrimp assay, nauplii decreased their viability in the presence of Shiga toxin, and the incubation with GOS at 4% and 2% GOS significantly reduced their mortality. Results revealed the capacity of GOS to antagonize the biological effect of Shiga toxin on Vero cells and the brine shrimp model. This protective effect could be ascribed to the interaction of the galactose units of GOS with the Shiga toxin. In fact, the globotriaosylceramide-3 (Gb3) receptor of the Shiga toxin is a ceramide trihexose formed by the alpha linkage of galactose to lactosyl-ceramide. The galactose units of GOS may bind to the toxin thus acting as a competitive inhibitor avoiding binding to receptors on the surface of eukaryotic cells. Our findings suggest the potentiality of GOS for antagonizing biological activity of bacterial toxins by competition with Gb3 receptors.

403. (742) **THE IMMUNOMODULATORY CAPACITY OF A POSTBIOTIC DEPENDS ON MUCOSAL ADMINISTRATION ROUTE**

Gutiérrez F[1], Vasile B[1], Ivir M[1], Castellano P[1], Alvarez S[1,2*], Salva S[1*].
[1]Laboratory of Immunobiotechnology, CERELA-CONICET. Tucuman, Argentina.
[2]Institute of Applied Biochemistry, Tucuman University. Tucuman, Argentina.

The nasal administration of *Lacticaseibacillus rhamnosus* CRL1505 peptidoglycan (PG), not their cell wall (CW), increases the infection resistance against *Streptococcus pneumoniae* (Sp), and improves innate and adaptive immune response in immunocompromised mice. In addition, the immunomodulatory effects of PG are a strain specific property. However, the oral administration of CW is more effective than the PG to improve bone marrow (BM) myelopiesis in immunocompromised mice. In this work, we compared the immunomodulatory capacity of PG and CW, before and after simulation of gastrointestinal digestion by *in vitro* and *in vivo* studies. First, *in vitro* colony-forming unit assays were performed to assess whether the clonogenic capacity of BM cells responded to direct interaction with *L. rhamnosus* (Lr) and its postbiotics. For this, mouse BM cells were plated in the presence or absence of Lr, PG or CW in culture medium for the granulocyte/macrophage forming unit (CFU-GM) (MethoCult™). Besides, the effect of splenocytes, HTL929 and RAW cells supernatants conditioned with Lr or its postbiotics was investigated. The counts and the phenotypic characterization of the colonies obtained were determined. We found that only Lr was able to increase clonogenic activity by directly stimulating BM cells. Surprisingly, in indirect assays, PG-conditioned HTL929 supernatants were the only ones to show increased clonogenic activity. Then, CW was incubated in a solution of simulated gastric and intestinal juice. Finally, 6-week-old Swiss albino mice received 2d nasal instillation of 8 μg/mice of PG (positive control), untreated CW (negative control), or treated CW. On d3, mice were challenged with Sp (10^7 CFU/ml). At 2d post-infection, we found that the treated CW was able to prevent the passage of Sp into the blood and reduce the lung counts as well as PG. Thus, the gastrointestinal passage of CW is necessary for the bioactive component of *L. rhamnosus* CRL1505 to exert its beneficial effect

404. (755) **BENEFICIAL EFFECTS OF PROBIOTIC ADMINISTRATION IN AN EXPERIMENTAL MODEL OF SMALL INTESTINE INFLAMMATION**

Emanuel G. Miculán, Carolina N. Ruera, Gerónimo M. Ducca, Federico Pérez, M. Luz Iribarren, Paula Carasi, Fernando G. Chirdo

Objective. Several animal models have shown the beneficial effect of probiotics in the control of inflammation in the colon. However, information about the role of probiotics in small intestine is limited. The aim of our work is to evaluate the effect of precolonization with two probiotic strains (*Lactobacillus kefiri* and *Enterococcus durans)*, in a previously characterised murine model of enteropathy induced by a gliadin-derived peptide (p31-43). Material-Methods. C57BL/6 wild type mice were treated by intragastric administration (IG) with (10^8 CFU) *L. kefiri* or *E. durans,* 3 times a week, for 3 weeks, or vehicle. Then, mice were treated by IG with p31-43 (20μg/mouse) or vehicle. After 16 hs, small intestines were collected. Sections from proximal small intestine were H&E stained for Villus height /Crypt depth ratio (V/C) analysis and counting of intraepithelial lymphocytes (IELs). TUNEL reaction was used to evaluate cell death. Protein extracts were used for western blot (WB) analysis. Results. P31-43-treated mice showed a decrease in V/C ratio and increased number of IELs, compared to control groups. Treatment with both probiotics inhibited the tissue damage upon p31-43 challenge. Similarly, TUNEL reaction showed a reduction on the number of dead cells in mice receiving *L. kefiri* or *E. durans* and p31-43 challenge, compared with mice treated with p31-43 alone (p< 0,001). WB analysis showed a decrease in active caspase-1/procaspase-1 ratio in small intestine of C57BL/6 mice treated with any of both probiotics and p31-43 peptide challenge compared with p31-43-treated mice (p<0,05). Similar results were obtained for the analysis by WB of active caspase-3/procaspase-3 ratio. Conclusion. Treatment with L. kefiri or E. durans reduced tissue damage and inflammation induced by intragastric p31-43 in mice. These results show that this experimental model is useful to examine probiotic-driven regulatory mechanisms in the proximal small intestine.

405. (808) **NASAL *KLEBSIELLA PNEUMONIAE* IMMUNIZATION GENERATES HETEROGENEOUS POPULATIONS OF RESIDENT MEMORY B CELLS AND PLASMA CELLS IN THE LUNG**

MC Amezcua Vesely[1]*, Ariel Berenstein[2]*.Valentina Brunotto[1], Laura Almada[1], Julio Gareca[1], Yamila Gazzoni[1], Richard Flavell[3], Eva Acosta Rodriguez[1], Carolina Montes[1], Adriana Gruppi[1].
**These authors equally contribute to this work.*
1) CIBICI-CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Argentina
2) IMIPP- CONICET
3) Yale University. New Haven. CT. USA

The development of a proper adaptive immune response against

different microbes protects against future challenges through the generation of memory B and T cells. Memory immune responses can be induced locally or systemically after immunization or infection. Systemic immunizations generate circulatory memory B and T cells but local immunizations induce both circulatory and tissue resident memory T and B cells (T_{RM} and B_{RM}). The development of memory cells in the lung is crucial to quickly deal with respiratory tract infections. Our previous results indicate that intranasal immunization with heat-killed *Klebsiella pneumoniae* (Kp) protects mice from infection through the generation and re-activation of CD4 T_{RM} cells. Based on these results, our goal was to characterize memory B_{RM} after intranasal immunization. C57Bl/6 mice were intranasally immunized on day 0 and 7 with heat killed Kp. On day 30 post immunization, mice were intravenously (iv) injected with anti-CD45-A700 antibody as a tool to exclude circulating immune cells in future analysis; 5 minutes after that mice were sacrificed, and lung collected. Then, we sorted lung CD45iv ^{negative} and CD4^{negative} cells from naïve and immunized mice to perform single cell RNA-seq and SingleR to identify clusters of hematopoietic and non-hematopoietic cells. Among hematopoietic cells, we selected the B cell cluster using a single-cell-Gate tool to evaluate B_{RM} heterogeneity. As expected, the number of lung infiltrating B cells was higher in immunized versus naïve mice. Immunized mice had heterogeneous populations of B_{RM} based on cluster numbers (6 clusters immunized mice vs 3 clusters naïve mouse) and also an increase in IgA^{+} resident plasma cells compared with naïve mice. Preliminary evaluation using flow cytometry shows agreement with single cell RNA-seq data. In conclusion, we determined that intranasal Kp immunization generates different types of B_{RM} whose specificity and function will be further evaluated.

NEFROLOGY *Friday, November 18, 14-15:30 hr*
Chairs: Néstor Lago - Elsa Zotta

406. (256) **EFFECTS OF A SUBLETHAL DOSE OF SHIGA TOXIN 2 ON RENAL REGENERATION IN PREGNANT AND NON-PREGNANT FEMALE RATS**

Lilian K. Fischer Sigel[1], Daiana S. Sánchez[1], Agostina Presta[1], Elsa Zotta[1,2], Claudia Silberstein[1].
1Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-Houssay) UBA-CONICET. Dpto. Cs Fisiológicas, Facultad de Medicina, UBA. 2Catedra de Fisiopatología. Dpto. de Cs Biológicas. Facultad de Farmacia y Bioquímica. UBA

Shiga toxin-producing *E. coli* causes acute renal failure and Hemolytic Uremic Syndrome. It is known that renal changes during pregnancy may protect maternal kidney from a renal damage. We have previously shown that a sublethal dose of Shiga toxin 2 (Stx2) caused lower renal injury and faster recover in pregnant rats than in non-pregnant female rats. Therefore, the aim of our work was to study the effects of a sublethal dose of Stx2 on renal tubular epithelial regeneration in pregnant rats and compare with non-pregnant female rats. Pregnant Sprague-Dawley rats, at the eighth day of gestation, and non-pregnant rats, were injected intraperitoneally with 0.5 ng Stx2/g body weight (PS and NPS) or with diluent for control pregnant and non-pregnant rats. Rats were euthanized at 4, 8 and 30 days post-injection (dpi). Kidneys were removed to study Ki67 (proliferation marker) and Vimentin (Vim, mesenchymal marker) expression by immunofluorescence. Besides, co-localization of Ki67 or Vim and Aquaporin 4 (AQP4, collecting ducts marker) were assayed. Apoptosis was evaluated by TUNEL assay. Tubular apoptosis was observed in renal tubular cells of NPS and PS rats, which corroborate our previous functional and histological results. At 4 and 8 dpi, NPS and PS rats significantly increased Ki67 and Vim expression in tubular cells of renal medulla with respect to controls, being significantly higher in NPS than in PS rats ($p<0.01$). At 8 dpi, 80% of both Ki67 and Vim positive tubular cells co-localized with AQP4 in NPS, while 80% of Ki67 and 65% of Vim co-localized with AQP4 in PS rats. At 30 dpi, Ki67 and Vim expression significantly decreased in tubular cells of NPS and PS rats, which coincided with a complete repair of kidney damage. In conclusion, our results demonstrate that after a moderate renal damage caused by Stx2, tubular epithelial cells have the ability to proliferate and dedifferentiate, proportional to the level of injury, resulting in complete tubular repair.

407. (441) **IMMUNE RESPONSE AFTER LOWERING-BLOOD PRESSURE IN SALT-SENSITIVE HYPERTENSION RAT MODEL**

Sandra G. Vlachovsky1, Pablo J. Azurmendi1, Elisabet M. Oddo1, Luis Di Ciano1, Romina S. Rodríguez1, Nora P. Goette2, Leonardo A. Paz3, Claudia Silberstein4, Fernando R. Ibarra1,4
1 Universidad de Buenos Aires, Laboratorio de Nefrología Experimental y Bioquímica Molecular, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
2 Universidad de Buenos Aires, Laboratorio Hematología Investigación, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
3 Universidad de Buenos Aires, Servicio de Anatomía Patológica, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
4 Universidad de Buenos Aires, Departamento de Ciencias Fisiológicas, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay)–CONICET, Facultad de Medicina, Buenos Aires, Argentina

In previous work we showed that ovariectomized (oVx) adult Wistar rats have a deranged renal sodium handling leading to salt-sensitive hypertension (HSS) development upon a high sodium intake (HS). We also showed an infiltration of immune cells in renal tissue. To differentiate if the infiltration occurs by the HS intake or the high blood pressure, oVx HS rats were treated with the vasodilator hydralazine (HDZ) in drinking water (20 mg/kg/day) to reduce blood pressure.
At 60 days of life, half of rats were oVx, and at 145 days intact female (IF) and oVx rats were divided into NS (0.24% NaCl) or HS (1% NaCl in drinking water) intake. oVx HS rats were divided into two subgroups: A) received HS and HDZ simultaneously (oVx HS+HDZ) for 14 days and B) received HS for 14 days but started with HDZ 7 days later (oVx HS-HDZ). During the treatment, systolic blood pressure (SBP, tail-cuff method) was recorded and animals were sacrificed at the end of study. Previously, blood samples were taken for peripheral blood lymphocytes (PBL) separation to analyze Na+K+ATPase (NKA) expression by Western Blot. Kidney histology was examined in hematoxylin-eosin stained, and common leukocyte antigen (CD45) was revealed by immunohistochemistry. oVx HS+HDZ were normotensive (SBP: 112±8.6 mmHg). Whereas oVx HS-HDZ under HS treatment became hypertensive, but after hydralazine, SBP decreased (SBP: 140±10 vs. 113±8.8, $p<0.01$). CD45 leukocytes were found in renal cortex and medulla in both A) and B) subgroups. In PBL of B) subgroups animals, NKA overexpression was not changed pre- and post-HDZ treatment. HDZ treatment was effective to avoid (oVx HS+HDZ) and ameliorate (oVx HS-HDZ) hypertension, but it was not effective to avoid infiltration in renal tissues. Besides, NKA overexpression remains unchanged in PBL. Overall, PBL are capable to respond under a HS intake independently of blood pressure level, suggesting a role in the immune response in renal tissue and therefore, in disease exacerbation.

408. (451) **EFFECTS OF ESTROGEN AND PROGESTERONE REPLACEMENT IN SALT-SENSITIVE HYPERTENSIVE OVARIECTOMIZATED RATS**

Sandra G. Vlachovsky1, Romina S. Rodríguez1, Elisabet M. Oddo1, Pablo J. Azurmendi1, Luis Di Ciano1, Nora P. Goette2, Leonardo A. Paz3, Claudia Silberstein4, Fernando R. Ibarra1,4
1 Universidad de Buenos Aires, Laboratorio de Nefrología Experimental y Bioquímica Molecular, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
2 Universidad de Buenos Aires, Laboratorio Hematología Investigación, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
3 Universidad de Buenos Aires, Servicio de Anatomía Patológica, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina
4 Universidad de Buenos Aires, Departamento de Ciencias Fisiológicas, Instituto de Fisiología y Biofísica Bernardo

Houssay (IFIBIO Houssay)–CONICET, Facultad de Medicina, Buenos Aires, Argentina

Our previous reports have shown a salt-sensitive hypertension model in which ovariectomized (oVx) rats develop hypertension with a high sodium intake (HS). In this model we observed an infiltration of immune cells in renal tissue. Now the aim of this study was to analyze if this infiltration occurs in other tissues, and if it could be avoided by estrogen or progesterone replacement in oVx HS rats. Female Wistar rat were used. At 60 days of life, half of the rats were oVx, and at 145 days intact female rats (IF) and oVx rats were divided into NS (0.24% NaCl) or HS (1% NaCl in drinking water) subgroups. Simultaneously, oVx rats were supplemented with 17 β estradiol 3-benzoate (oVx NS E2 and oVx HS E2, 60 µg/kg), progesterone (oVx NS P4 and oVx Hs P4, 10 mg/kg) or vehicle (vegetal oil, oVx HS-v) in subcutaneous injections twice a week for three weeks. During the treatment, systolic blood pressure (SBP, tail-cuff method) was recorded. At the end of experiment, the animals were sacrificed and samples of kidney, skeletal muscle and skin were taken to study hematoxylin-eosin stained sections and common leukocyte antigen (CD45) by immunohistochemistry. Results: SBP (mmHg), IF NS: 119 ± 4.5, IF HS: 120 ± 3.8, oVx NS-v: 122 ± 0.24, oVx HS-v: 134 ± 1.7*, oVx NS E2: 121 ±1.79, oVx Hs E2: 124±1.7, oVx Ns P4: 121 ±1.9, oVx HS P4: 135±2* (*p<0.05 oVx HS-v. and oVx HS P4 *vs* rest of the groups). CD45 leukocytes were found in hypodermis, renal cortex and renal medulla from oVx HS-v and oVx HS P4. Skeletal muscle samples did not have infiltration with CD45 cells. Results suggest that E2 or its absence has an important role in salt-sensitive hypertension pathophysiology, because only in oVx HS rats with E2 replacement, hypertension, renal tissue and hypodermis infiltration were prevented while P4 treatment did not avoid hypertension and CD45 cells infiltration.

409. 540. **IMPACT OF ISCHEMIC-KIDNEY INJURY ON INTESTINAL ABC TRANSPORTERS**

Romina P Bulacio[1,2], María Fernanda Fussi[1,2], Tomás Rivabella Maknis[3], María Herminia Hazelhoff[1], Romina V Campagno[1,2], Liliana A Monasterolo[1,2] Sara M Molinas[1,2], María Cecilia Larocca[3], Anabel Brandoni[1,2].

[1]Faculty of Biochemical and Pharmaceutical Sciences (UNR), Rosario, Argentina

[2]National Scientific and Technical Research Council (CONICET), Argentina

[3]Institute of Experimental Physiology of Rosario (IFISE-CONICET-UNR), Rosario, Argentina

rpbulacio@gmail.com

Acute kidney injury caused by ischemia-reperfusion (IR) is a complex pathophysiological process associated with numerous metabolic and structural changes, also affecting remote organs. P-glycoprotein (P-gp, ABCB1) and Multidrug resistance-associated protein 2 (Mrp2, ABCC2) belong to ATP-binding cassette (ABC) transporter superfamily. Both are expressed in kidney and intestine at the apical membrane of proximal tubular cells and enterocytes, respectively. They are involved in the efflux of endogenous compounds and xenobiotics. The aim of this study was to evaluate how renal IR injury affects the intestinal expression of P-gp and Mrp2. Male Wistar rats were subjected to 40 min of unilateral renal ischemia followed by 24 h of reperfusion (IR, n=3). Sham-operated controls (C, n=3) were also processed. P-gp and Mrp2 expression in total plasma membranes from post-ischaemic kidneys ($P\text{-}gp_{Km}$ and $Mrp2_{Km}$) and in apical membranes from jejunum and ileum regions of small intestine ($P\text{-}gp_{Jm}$; $P\text{-}gp_{Im}$; $Mrp2_{Jm}$ and $Mrp2_{Im}$) were evaluated by immunoblotting. t-Student *: $p < 0,05$, **: $p < 0,01$. Results: $P\text{-}gp_{Km}$ (%): C=100±9, IR=163±20*; $P\text{-}gp_{Jm}$ (%): C=100±6, IR=58±4**; $P\text{-}gp_{Im}$ (%): C=100±7, IR=91±7; $Mrp2_{Km}$ (%): C=100±9, IR=106±3; $Mrp2_{Jm}$ (%): C=100±11, IR=102±14; $Mrp2_{Im}$ (%): C=100±12, IR=171±18*. After renal IR injury, P-gp expression was increased in renal membranes and decreased in apical membranes from jejunum, whereas Mrp2 expression did not change in renal membranes but significantly increased in apical membranes from ileum. In conclusion, after inducing renal IR injury, expression of P-gp and Mrp2 is differentially regulated, not only in the kidney, where the insult occurs, but also in remote organs, such as the intestine, with region-specific alterations. Those modifications may impact on handling of P-gp and Mrp2 substrates during kidney injury. The mechanisms underlying those alterations will be the subject of further studies.

410. (721) **MOLECULAR MECHANISMS UNDERLYING DISEASE PROGRAMMING DURING POSTNATAL DEVELOPMENT IN A MODEL INDUCED BY THE INHIBITION OF ENDOTHELIN SYSTEM: OXIDATIVE STRESS**

Lucas H. Oronel[1], Analía Czerniczyniec[2,3], María del Carmen Ortiz[1], Silvia Lores-Arnaiz[2,3], María Àngeles Magnanelli[1], Florencia Albertoni Borghese[1,4] and Mónica Majowicz[1]

[1]Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Departamento de Química Analítica y Fisicoquímica, Cátedra de Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [3]Instituto de Bioquímica y Medicina Molecular (IBIMOL), CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina, [4]Cátedra de Biología Celular y Molecular, CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

We showed that Endothelin (ET) inhibition during the early postnatal period (PNP) with Bosentan (20 mg/kg/day), a dual ET receptor antagonist (ERA), increases TBARS levels in kidney homogenates of male rats, indicating an increase in lipid peroxidation. Given the importance of oxidative stress as one of the mechanisms underlying disease programming during development, in the present work we evaluated $O_2^{\cdot-}$ (superoxide anion) production and some pro- and anti-oxidant enzymes in crude mitochondrial fractions in 7 day old Sprague-Dawley rats. To this end, four experimental groups were studied: control males (CM), ERA-treated males (ERA-M), control females (CF) and ERA-treated females (ERA-F). We determined: $O_2^{\cdot-}$ production, Catalase (Cat) and Glutathione Peroxidase (GPx) activities by spectrophotometric methods, NADPH-Oxidase (NOX) activity by a fluorometric method and NOX-4 expression by Western Blot. Statistics: two-way ANOVA followed by Bonferroni post-test. We found a tendency to increase in $O_2^{\cdot-}$ production in ERA-M vs CM (13.27 ± 3.35 vs 8.68 ± 2.66 nmol/min.mg). We found a decrease in Cat activity in ERA-M vs CM ($p<0.01$) and an increase in ERA-F vs CF ($p<0.05$), while no significant changes in GPx activity were observed among experimental groups. Contrary to what we expected, there was a tendency to decrease in NOX activity (232,294 ± 85,163 vs 359,738 ± 50,543 AU mg protein^{-1}) and NOX-4 expression (1.0 ± 0.4 vs 1.3 ± 0.5 AU, change from control) in ERA-M vs CM. The last result suggests that the tendency to increase in $O_2^{\cdot-}$ is not due to an increased NOX-4 expression/activity. However, it could be due to the contribution of mitochondrial Complex I and III, to a decreased SOD activity and/or to peroxisomal reactive oxygen species production (also present in the mitochondrial fraction). The decrease in Cat activity in ERA-M and the increase in ERA-F indicates a sex difference, being females more protected than males against ERA inhibition in PNP.

411. (771) **GLOMERULAR DAMAGE CHARACTERIZATION UNDER THE EFFECT OF LETHAL AND SUBLETHAL DOSES OF SHIGA TOXIN 2 ALONG THE TIME**

Francisco Martella [1], Agustina Tanca [1], Mercedes Paciaroni *[1], Agostina Presta[2], Rocío Duete[1], Bruno Carabajal [1,] Alexa Barreto [1], Elsa Zotta[123], Federico Ochoa [123].*

[1]UA2. Depto. de Cs Fisiológicas. Facultad de Medicina. UBA

[2]Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-Houssay) UBA-CONICET. Dpto. Cs Fisiológicas, Facultad de Medicina, UBA.

[3]Catedra de Fisiopatología. Depto de Cs. Biológicas. Facultad de Farmacia y Bioquímica. UBA

In the Argentina, hemolytic uremic syndrome (HUS) is the most common cause of acute renal failure in children. Previously we informed survived rats to HUS showed epithelial to mesenchymal transition EMT in inner medulla with focal segmental glomeruloscle-

rosis and corticomedullary fibrosis. Moreover, we reported that the detection of podocyturia could be used as a biomarker of early renal damage in HUS. The objective of our study was to analyze the podocyte response to the effect of Shiga toxin type 2 (Stx2) during the evolution to chronicity, in two experimental models of HUS in rats. Adult Sprague-Dawley rats (150-200 g) were divided in four groups (n=6 in each group) to be injected by intraperitoneal route. Experimetal groups were injected with 3 and, 0,25 mL/ 200 g bw (lethal and sublethal doses) of culture supernatant of recombinant *E. coli* expressing Stx2. Controls were injected with 3 and 0.25 mL of culture supernatant of recombinant E. coli not expressing Stx2, respectively. Animals were sacrificed at 48 hs,1 week and 3 months post inoculation. Functional, histological and immunofluorescense studies were performed in all groups. Proteinuria and albuminuria were detected at 3 months of evolution (p<0,01). We studied by immunofluorescence the presence of the Shiga toxin in all groups to determine if the toxin colocalized with different markers of podocytes glomerular cells (Vimentin, uPAR). Shiga toxin was marked only at 48 hours and 1 week whereas vimentin was strongly colocalized in detachment intratubular podocytes at 48 hours and 1 week with a marked decrease expression in glomeruli (p<0,05). uPAR was higher expressed at 3 months than 48 hours and 1 week. In conclusion, at earlier HUS nephropathy detachment podocytes was increased in contrast that occur at 3 months, when podocytes were marked in glomeruli. The uPar increased expression at 3 months could be related with the development of proteinuria.

412. (825) **SEX DIFFERENCES IN THE RESPONSE TO ISCHEMIC ACUTE KIDNEY INJURY AND IN URINARY NGAL BIOMARKER**

María Fernanda Fussi[1,2], Tomás Rivabella-Maknis[3], Romina P. Bulacio[1,2], M Herminia Hazzelhoff[1], Susana B. Marquez[4], Romina Campagno[1,2], Daniel De Vuono[5], Claudia Massoni[5], Juan M. Riera[5], Susana Lioi[5], Jorge L. Molinas[6], Anabel Brandoni[1,2], Liliana A Monasterolo[1,2], M Cecilia Larocca[3], Sara M. Molinas[1,2]

[1]Área Farmacología. Facultad de Cs. Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario.
[2]CONICET.
[3]Instituto de Fisiología Experimental (IFISE-CONICET).
[4]Anatomía y Fisiología Patológica. Facultad de Cs. Médicas. Universidad Nacional de Rosario.
[5]Laboratorio Central del Hospital Centenario de Rosario
[6]Fisiología Humana. Facultad de Cs. Médicas. Universidad Nacional de Rosario.

One of the main causes of acute kidney injury (AKI) is ischemia-reperfusion (IR). Animal studies have shown that female sex protects against the development of ischemic renal injury. Neutrophil gelatinase-associated lipocalin (NGAL) is increasingly secreted by tubular cell during AKI, constituting a promising diagnostic and prognostic biomarker. However, there are limited studies in female animals and sex differences in the urinary excretion of this biomarker during AKI are not clear. Our aim was to characterize the renal response to IR damage in males and females rats, and evaluate the correlation with uNGAL levels. Male (MIR) and female (HIR) Wistar rats (n=6 per group) underwent 40 min of unilateral renal ischemia followed by 1 day of reperfusion. Controls underwent sham operation (MC and HC). Functional and histological studies were performed. α-SMA, an epithelial-to-mesenchymal transition marker, was evaluated in renal tissue by Western Blot. uNGAL was analyzed by Western Blot. IR damage produced a decrease in glomerular filtration rate, estimated by creatinine clearance, that was greater in MIR (-38% p<0.05 vs MC) than in HIR (no difference vs HC), and an increase in uremia that was greater in MIR (+65% p<0.05 vs MC) than in HIR (+36 p<0.05 vs HC). Histological analysis showed extensive acute tubular necrosis in MIR, while in HIR the tissue damage was moderate. IR kidneys showed an increase in α-SMA in male (+150% p<0.05 vs MC) but no change in female kidneys. By contrast, uNGAL excretion (arbitrary units/min) was higher in HIR than in MIR (+ 180% p<0.05). In conclusion, as described in other studies, we found that IR damage in female rats was less severe than in males. However, that diminished damage does not correlate with the increased uNGAL levels, which would suggests a sexual dimorphism in NGAL expression in response to IR kidney damage and reveals the need of further studies for its better clinical application as a biomarker.

413. (866) **PREVENTION OF SALT-SENSITIVE HYPERTENSION DEVELOPMENT IN CONDITIONS OF RENAL FAILURE AND SALT DIETARY OVERLOAD BY SUPPLEMENTATION WITH COENZYME Q10**

Daniela Josefina Porta1, Rivoira María Angélica1, Jorge Humberto Mudski2, María Eugenia Pasqualini1, Néstor Horacio García1

1 Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET) FCM-UNC, Córdoba, Argentina, 2 Centro de Microscopía Electrónica- FCM-UNC, Córdoba, Argentina.

Introduction: Hypertension (HTA) affects more than 30% of world's adult population, being the main risk factor for cardiovascular mortality. In those subjects with "non-modulating" phenotype, failures are manifested in different mechanisms of adaptation to salt overload and consequent renal alteration that culminates in the establishment of salt-sensitive HTA (SS-HTA). Thus, in this work we hypothesize that dietary supplementation with Coenzyme Q10 (CoQ10) slows the development of SS-HTS in moderate renal insufficiency. Objective: To determine the effect of CoQ10 with in prevention of SS-HTA development induced by salt overload in rats with UNX and its antioxidant effects in mitochondrial redox system as a preventive mechanism on tissue damage. Materials and methods: Four groups of uninephrectomized (UNX) rats were used as model of moderate chronic renal failure, G1=NNaD (NaCl 0.2%), G2= HNaD (NaCl 4%), G3= CoQ10 (200mg/Kg/day) + HNaD, and G4=CoQ10. Blood pressure (BP) and body weight were determined at baseline, and days 45 and 100. Urinary proteinuria ($U_{prot}V$) and sodium ($U_{Na}V$) excretion, creatinine clearance, systemic ROS, total glutathione mitochondrial content (GSHMt), superoxide dismutase activity (SODMt) (in renal isolated mitochondria), TGFβ1 expression, histology changes and the left ventricular hypertrophy index (LVHI) were analyzed. To evaluate pressure-induced natriuresis; G1 and G4 were subjected to a sodium challenge during 7 days and the change on BP was measured. Results: As we expected, BP increased in G2 group, but not in group G3 (Day 100, G2=142.2±5.7mmHg vs G3=126.0±7.5mmHg, p<0, 01). Additionally, G3 has less LVHI, $U_{prot}V$, systemic ROS and GSHMt and TGFβ1 renal expression. $U_{Na}V$ increased during CoQ10 supplementation, preventing BP increase. **Conclusion:** We conclude that supplemental CoQ10 prevents increased BP, target organ damage in UNX rats under high-sodium diet through an antioxidant effect.

414. (878) **THE ADAPTED-ULTRACENTRIFUGATION METHOD OF ISOLATION OF URINARY EXTRACELLULAR VESICLES (EVS) IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)**

Rosenberg ML [1], Villafañe M.[2] Yaneff A[2], Branca BE[1], Riera N[1], Vlachovsky S[1],Davio C[2], Goette NP[3], Peroni RN[2], Oddo EM[1], Azurmendi PJ[1]

[1] Lab. Nefrología Experimental y Bioquímica Molecular, IIM Alfredo Lanari, UBA
[2] Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica , UBA –CONICET
[3] Lab.Hematología investigación, IIM Alfredo Lanari, UBA-CONICET

ADPKD is characterized by an intracellular calcium depletion that belongs to deficient activity of the polycystin 1 or 2 complex which, in turn, stimulates cAMP production. Urine EVs are extracellular-microvesicles, which play an essential role in cell-to-cell communication, possibly by interactions between the primary cilium. The gold standard for EVs isolation is a 200,000x*g* ultracentrifugation, a method that is not currently accessible for clinical laboratories. Previous results (Rosenberg, et al., abstract #0689, SAIC 2019) obtained with EVs extracted by a commercial kit based on precipitation showed low protein and RNA recovery and aggregated vesicles that jeopardizes subsequent studies.Therefore, our objective was to iso-

late of EVs from morning urine by centrifugation method, equalizing time and speed (50,000xg for 4h at 4ºC) with respect to ultracentrifugation, and the enrichment step using membrane filters (0.22 um) and 200 mg/ml of dithiothreitol from urine of 4 patients and 2 control subjects. EVs were characterized as spherical, 30-150 nm particles using electron microscopy and dynamic light scattering, respectively. Exosomal marker CD63, evaluated by flow cytometry, showed 60 ± 16 % of positive particles. Protein content, measured by Bradford, ranged from 0.9-1.3 ug/ul, 9±1 times than the precipitation method. Preliminary cAMP quantification in EVs was 2.0 ± 0.2 nmol/ml, showing a 22 ±2 fold more recovery than precipitation method. The adapted-ultracentrifugation method presented here recovers particles that fulfill conditions of EVs, and enriches it improving future studies for biomarkers of disease progression.

NEUROSCIENCES I

Wednesday, November 16, 14-15:30 hr
Chairs: Pablo Barcelona - Sandra Zarate - Carlos Pomilio - Fernando Correa

415. (20) **THE ROLE OF ACETALDEHYDE IN THE DELETERIOUS EFFECTS TRIGGERED BY BINGE ETHANOL EXPOSURE ON SYNAPTIC MITOCHONDRIAL BIOENERGETICS**

Analia Karadayian, Analia Czerniczyniec, Lautaro Carrere, Juan Ignacio Guerra, Silvia Lores-Arnaiz
Instituto de Bioquímica y Medicina Molecular Profesor Alberto Boveris (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Junín 956, 2do piso, (C1113AAD) Buenos Aires, Argentina.

Alcohol hangover (AH) is defined as a combination of mental and physical symptoms experienced the day after a single episode of heavy drinking, starting when blood alcohol concentration (BAC) approaches zero. We demonstrated that AH induced strong oxidative stress, mitochondrial dysfunction and alterations in nitric oxide (NO) metabolism in brain cortex synapses, together with the induction of apoptosis signaling pathways. The aim of the present work was to assess the role of acetaldehyde in AH deleterious effects on mitochondrial function at brain cortex synapses using 4-methylpyrazole (4MP), an inhibitor of alcohol dehydrogenase (ADH). Swiss male mice were divided in four different experimental conditions which received i.p. injections of saline (control group), 4MP (10 mg/kg), ethanol (3.8 g/kg, AH group), and 4MP-ethanol. Animals were sacrificed 6h afterwards (BAC=0). Determinations were conducted in brain cortex synaptosomes. The reduction of acetaldehyde levels significantly restored respiration driving ATP synthesis and coupling efficiency by a 5-fold enhancement as compared with AH group ($p<0.05$). 4MP treatment was able to fully prevent the reduction in enzymatic activity of mitochondrial complex I-III due to AH, while the activity of complex IV was only partially recovered, remaining 50% lower than control group ($p<0.05$). The use of 4MP fully restored ATP production and mitochondrial membrane potential, which were 41% and 48% decreased in AH group ($p<0.05$). Respect to NO metabolism, the decrement in acetaldehyde levels would not seem to have any significant effects. Summing up, results show that acetaldehyde seems to be responsible for most of the deleterious effects on mitochondrial bioenergetics triggered by binge ethanol exposure at the synapses. The impairment of NO metabolism, which was not reverted by 4MP, could be probably due to the presence of the remaining ethanol at the systemic level after ADH inhibition.

416. (21) **IMPACT OF HYPERBARIC ATMOSPHERE ON BRAIN MITOCHONDRIAL FUNCTION**

Pablo La Padula*, Analía Karadayian#, Silvia Lores Arnaiz#, Guillermo Di Girolamo*, Lidia E. Costa*, Analía Czerniczyniec#.
**Instituto Alberto Taquini de Investigaciones en Medicina Traslacional (IATIMET), Facultad de Medicina, UBA-CONICET.*
 # Instituto de Bioquímica y Medicina Molecular Profesor Alberto Boveris (IBIMOL), UBA - CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Chronic hyperbaric treatment is often used to hasten tissue recovery and improve the physiological aspects of the damaged tissue. Taking into account that the brain is an organ highly sensitive to oxygen levels and possesses the highest energy demand, we evaluated the mitochondrial functionality and reactive oxygen species (ROS) production in cerebral cortex and hippocampus using an animal model of hyperbaria. Male Sprague Dawley rats were subjected to 30 sessions of 60 min in a hyperbaric chamber at 1.44 atm. and 100% O_2. Mitochondrial oxygen consumption, respiratory efficiency (ADP/O) and nitric oxide (NO) levels were preserved in the cerebral cortex after chronic hyperbaric treatment. In addition, a 31% increase in superoxide anion production and a decrease in blood supply were observed. Regarding hippocampus, hyperbaric conditions decreased mitochondrial oxygen consumption (16% and 20 % for state 3 and 4, respectively) with the consequent reduction in superoxide anion levels (38%) and an inhibition of NO production (32%), while preserving respiratory efficiency (ADP/O). Conclusions: after a stimulus in the variation of the oxygen pressure in the inspired air, the hippocampus displays physiological adaptations at the mitochondrial level which lead to a decrease in the production of ROS. While the cerebral cortex, a more robust tissue, maintains its respiratory function assuming the impact of the increase in ROS. These results are in agreement with our previous studies of the effect of low oxygen pressure in the inspired air, where the mitochondrial functionality in cerebral cortex was preserved and the hippocampal mitochondria displayed different strategies to adapt to different oxygen pressures.

417. (26) **PHYTOCANNABINOIDS ATTENUATE ASTRO AND MICROGLIOSIS REACTION FOLLOWING SPINAL CORD INJURY**

Julián Del Core[1], Ignacio Jure[1], Alejandro F De Nicola[1,2] and Florencia Labombarda[1,2]
1 Laboratorio de Bioquímica Neuroendócrina, Instituto de Biología y Medicina Experimental, CONICET. 2 Departamento de Bioquímica Humana, Facultad de Medicina, UBA.

Traumatic spinal cord injury (SCI) is a physically disabling and psychologically devastating condition. Reactive gliosis and microglial activation are involved in both secondary damage and the persistence of chronic neuroinflammation after SCI. Therefore, their regulation represents a therapeutic target. In this regard, Tetrahydrocannabinol (THC) and Cannabidiol (CBD), the main phytocannabinoids of *Cannabis Sativa*, emerge as anti-inflammatory molecules in some experimental models. In the present study we used a model of SCI in rats to evaluate the effects of oil extracted from a resin composed of THC: CBD 1:1. Spinal cord injured rats received an oromucosal dose (20mg/kg/day) during 15 days post-injury (dpi) and they were sacrificed at 60 dpi. Immunohistochemistry studies showed that the number of microglial cells (Iba1+ cells) increased with respect to sham rats ($p<0.001$ ANOVA two ways) in the epicenter and in both the white and grey matter of the rostral and caudal segment from the lesion 60 dpi. However, THC: CBD treatment decreased microglial density compared with injured rats ($p<0.05$ ANOVA two ways) in the white and grey matter of all the studied regions. Regarding astrocytes (GFAP+ cells), their number was upregulated after chronic SCI with respect to sham rats ($p<0.001$ ANOVA two ways) in the epicentre and in both the white and grey matter of the rostral and caudal region from the lesion. Unlike microglial cells, after THC:CBD administration, astrocyte density decreased only in the grey matter of the rostral and caudal region with regard to injured rats ($p<0.05$ ANOVA two ways). These results suggest that THC and CBD offer a promising perspective in reducing chronic neuroinflammation and gliosis, which eventually could lead to functional recovery.

418. (80) **MITOCHONDRIAL DYNAMICS IN THE PRIMARY VISUAL CORTEX IN GLAUCOMA**

Ailen G. Hvozda Arana[1,3], Laura Caltana[4], Juan Santiago Adán Arean[2,3], Claudia G. Reides[1,3], S. Fabián Lerner[1], Silvia Álvarez[2,3], Romina M. Lasagni Vitar[1,3], Sandra M. Ferreira[1,3]
[1] Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Químicas. Cátedra de

Química General e Inorgánica. Buenos Aires, Argentina.
[2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Químicas. Cátedra de Fisicoquímica. Buenos Aires, Argentina.
[3] CONICET- Universidad de Buenos Aires. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Buenos Aires, Argentina.
[4] CONICET- Universidad de Buenos Aires. Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis", Buenos Aires, Argentina.

Glaucoma is the first irreversible cause of blindness worldwide and affects eye structures and brain areas related to the visual system. Oxidative stress plays a key role in the development and progression of the disease. The aim was to evaluate the mitochondrial dynamics in the primary visual cortex in a glaucoma model. Three-month Wistar rats were operated by cauterizing two of the episcleral veins in the left eye: glaucoma group (G n=8); the control group (n=8) received a sham procedure. Seven days after surgery rats were euthanized, and the primary visual cortex was dissected. We separated both hemispheres in G, the ipsilateral (GI) and contralateral (GC) (CICUAL FFyB n° 3314). We evaluated OPA-1 and DRP-1 expression in both mitochondrial and cytosolic fractions, and PGC-1α expression in primary visual cortex homogenates. Mitochondrial ultrastructure was studied by transmission electron microscopy (TEM). When compared to control, GC and GI showed an increase of 47% and 58%, respectively, in OPA-1 expression in the mitochondrial fraction ($p<0.01$). However, only GI showed an increase of 41% in OPA-1 expression in cytosolic fraction ($p<0.05$). Regarding DRP-1 expression, only GI cytosolic fraction showed an increase of 22% ($p<0.01$), with no changes in GC and mitochondrial fraction. There were no changes in PGC-1α expression in GC and GI compared to control group. Finally, TEM images showed a slight clarification, swelling and disruption of mitochondrial internal structure in GC and GI compared with control. These results suggest that glaucoma alters mitochondrial dynamics, showing an increase in the fusion process, with no changes in fission process or biogenesis. In addition, mitochondrial ultrastructure is altered in the primary visual cortex. Understanding the key drivers of mitochondrial impairment in glaucoma are crucial to identify new therapeutic targets that would halt disease progression.

419. (197) **CORTICOSPINAL AXONS RECONNECTION PROMOTES THE RECOVERY OF VOLUNTARY LOCOMOTION AFTER ACUTE SPINAL CORD INJURY**

Julieta Schmidt[1], Ramiro Quintá[2,3] [1] Facultad de Medicina - UBA.
[2]Consejo Nacional de Investigaciones Científicas y Técnicas –CONICET; y [3]Laboratorio de Medicina Experimental, Hospital Alemán.

In traumatic spinal cord injury (SCI), the flux of information along the shaft of long motor and sensory spinal axons is interrupted. The goal is to overcome the deleterious effect produced by SCI promoting re-growth of the damage axons as well as a functional re- connection of its with the lower neuronal targets to achieve a recovery of locomotor functions. According to this, the aim of this research is focused in the design of a therapeutic approach in SCI. Netrin-1, a chemoattractant protein, is involved in axonal growth during the embryonic development. Netrin-1 drives the corticospinal axons growth and its navigation across the pyramidal decussation to the white matter spinal cord by a haptotaxis phenomenon. As I previously described, Netrin-1 promotes a significant recovery of locomotor activity in rats with a complete SCI at Th10 level, assessed by BBB score. Furthermore, this result correlates with a significant improvement in the control of voluntary locomotion, assessed by ladder rung test. In line with this, a stereotaxic surgery was carried out to trace the corticospinal axons. Using the clearing technique it was observed a significant regeneration of corticospinal axons at the lesion site only in Netrin-1 treated rats, as well as a significant preservation in the number of synaptic contacts downstream of the lesion. Besides, an in-vivo trans-synaptic interaction was revealed only in treated rats.
Finally, using a 3-Tesla MRI, a preservation of myelinated spinal tissue was shown in Netrin-1 treated rats after SCI. In conclusion, the administration of Netrin-1 in acute SCI promotes regeneration of corticospinal axons, prevents axonal dying back, stimulates neo-formation and re-arrangement of synaptic contacts and preserves the myelinated spinal tissue. All of these cellular processes could partially explain the pathway by which Netrin-1 addition produces a significant recovery of locomotor function after injury.

420. (206) **2-ARACHIDONOYLGLYCEROL METABOLISM IS MODULATED BY CANNABINOID RECEPTOR LIGANDS AND BY PHOTOTRANSDUCTION RELATED PROTEINS IN RETINAL ROD OUTER SEGMENTS**

Estefania Chamorro Aguirre, Virginia Lucía Gaveglio, Ana Clara Pascual, Susana Juana Pasquaré
Laboratorio de Cannabinología, INIBIBB-UNS-CONICET. Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Buenos Aires, Argentina.

The endocannabinoid 2-arachidonoylglycerol (2-AG) level in the central nervous system is regulated mainly by diacylglycerol- and monoacylglycerol-lipase (DAGL and MAGL) activities. Lysophosphatidate phosphohydrolase (LPAP) could also generate 2-AG from 2-arachinodonoyl lysophosphatidate. Previous studies demonstrated that MAGL activity is modified in rod outer segment (ROS) membranes treated with buffers of low (5 mM Tris-HCl) or moderate (100 mM Tris-HCl) ionic strength, which mostly extract rhodopsin kinase (RK) and arrestin (Arr), both being proteins related to the phototransduction process. One aim of this work was to analyze if cannabinoid receptor ligands WIN55212-2 (WIN), JWH-133 (JWH), SR141716 (SR1) and SR144528 (SR2) modulate these enzymatic activities in ROS isolated from dark-adapted bovine retinas and exposed to light (3000 lux, ROS B) or kept under darkness (ROS O) for 30 min at 37 °C. Thus, ROS O/B were incubated with cannabinoid ligands for 10 min before adding the radiolabeled substrate. It was observed that WIN and JWH agonists (5 μM) diminished MAG production from LPA by 32% and 60% in ROS O and ROS B, respectively. SR1 and SR2 antagonists (1 μM) also generated a MAG diminution of 71% and 51% in ROS O, respectively, while both antagonists diminished it by 48% in ROS B (n=5, $p<0.05$). On the other hand, DAGL activity was evaluated in ROS membranes treated at 5 mM or 100 mM in dark or light (3000 lux) for 30 min using radiolabeled diacylglycerol as substrate. DAGL activity diminished by 25% and 55% at low and moderate ionic strength in ROS O, respectively. A diminution of 60% in DAGL activity under both treatments was observed in ROS B (n=4, $p<0.05$). This suggests that RK or Arr modulate DAGL activity. Taken together, these results indicate that 2-AG metabolism is regulated by cannabinoid receptor ligands as well as by the phototransduction process, suggesting an important role of the endocannabinoid system in the visual cycle.

421. (325) **THE HYPOTHERMIA MIMETIC SYNTHETIC MOLECULE ZR17-2 PREVENTS RETINAL DAMAGE CAUSED BY PERINATAL ASPHYXIA IN THE RAT**

Ronan Nakamura [1], Manuel Rey-Funes [1], Juan Carlos Fernández[1] Rafael Peláez [2,] Manuel Soliño [1], Daniela Contartese [1], Nicolas Sebastián Ciranna [1], Verónica Berta Dorfman [3], José María Zapico [4], Ana Ramos [4], Beatriz de Pascual-Teresa [4], Juan José López-Costa [1], Ignacio Larrayoz [2], Alfredo Martínez [2,¶] and Cesar Fabián Loidl [1,¶]
[1]Instituto de Biología Celular y Neurociencia "Prof. E. de Robertis", Facultad de Medicina, Universidad de Buenos Aires, Argentina; [2]Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain; [3]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina; [4]Department of Chemistry and Biochemistry, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.
[¶]These authors contributed equally to this study and should be considered co-last authors.

Introduction. Perinatal asphyxia (PA) is responsible for a large proportion of neonatal deaths and numerous neurological sequelae, including visual dysfunction and blindness. During PA, the retina

is exposed to ischemia/reoxygenation, which results in neuronal cell death and aberrant angiogenesis and gliosis. Since we have previously demonstrated that hypothermia prevents retinal damage caused by PA. We hypothesized that small molecule mimetics of hypothermia may also prevent PA-induced retinal degeneration. Materials and methods. Male rat pups were subjected to an experimental model of PA. Four groups were studied: i) normally delivered (CTL); ii) normally delivered treated with 330 nmols/L zr17-2 (CTL-ZR); iii) exposed to PA for 20 min at 37ºC (PA); and iv) exposed to PA and, then, treated with zr17-2 (PA-ZR). Five days after birth, some rats were sacrificed and the eyes were studied by TUNEL assay. Forty five days after birth, other animals were subjected to electroretinography (ERG), sacrificed, and the eyes studied by histology. Results. Electroretinography showed that PA animals had significant defects in the a- and b-waves and oscillatory potentials. The same animals presented a significant increase in the thickness of the inner retina and a large number of TUNEL-positive cells. All these physiological and morphological parameters were significantly prevented by the treatment with zr17-2. Conclusions. zr17-2 protects from cell death and restores electrophysiological function in the retina. This molecule could be used as a treatment to prevent the deleterious visual consequences of PA.

422. (367) **PERSISTENT ASTROGLIAL DNA METHYLATION AND DOWNREGULATION OF HOMEOSTATIC GENES IN A LITHIUM- PILOCARPINE MODEL OF TEMPORAL LOBE EPILEPSY (TLE)**

Gomez DD1, Rossi A1, Villarreal A1, D´Alessio L, Ramos AJ1
1. IBCN UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires

Retrospective studies in TLE patients have shown the common feature of an initial precipitating injury (IPE) in early childhood, usually complex febrile with Status Epilepticus (SE). The IPE is usually followed by a silent period of years until chronic epilepsy emerges. We have previously shown extensive neuroinflammation and reactive astrogliosis in this silent period in the Li-pilocarpine rodent model of TLE (Rossi et al, 2013; 2017). Here, we studied if extensive astrogliosis induce epigenetic alterations in DNA methylation and homeostatic genes expression. Adult male Wistar rats (220-240 g) were treated with 127 mg/kg LiCl and 18 h later they received 30 mg/kg pilocarpine ip. Status Epilepticus (SE, IPE) were allowed to persist for 30 min and then seizures were stopped by 10 mg/kg diazepam. At different times during the latency period (7, 21 or 35 DPSE, days post SE), the abundance of 5 methyl Cytosine (5mC) in the astroglial DNA and the expression of several astroglial homeostatic genes (Kir4.1, aquaporin 4 (AQP4), glutamine synthetase (GS), In addition, we analyzed by RT-qPCR the changes in the expression of DNA methyl transferases DNMT1 and DNMT3a. Level of astroglial 5mC was also studied in hippocampal sections of surgical resections of TLE patients. We observed a global hypermethylation in astrocytes, accompanied by increased expression of DNMT1/DNMT3a and a reduction in the expression of GS, Kir4.1 and AQP4 at 7, 21 and persisted until 35 DPSE. Reactive astrogliosis and increased 5mC in astrocytes were also observed in the hippocampal sections of TLE patients. We conclude that astrocytes show persistent downregulation of homeostatic genes during the latency period that follows an experimental IPE. This correlates with increased 5mC in the DNA that may be responsible for the homeostatic gene repression. Supported by grants: PICT 2017-2203; UBACYT; PIP CONICET 479

423. (405) **ENRICHED ENVIRONMENT PROTECTS THE CENTRAL OUTER RETINA AGAINST EXPERIMENTAL NON-EXUDATIVE AGE-RELATED MACULAR DEGENERATION IN MICE**

Damian Dorfman[1], Nathaly Bernal Aguirre[1], Juan S. Calanni[1], Agustina Alaimo[2], Horacio E. Romeo[3], Ruth E. Rosenstein[1], Hernán H. Dieguez[1].
1. Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine/CEFyBO, University of Buenos Aires/CONICET, Buenos Aires, Argentina. 2. Interdisciplinary Laboratory of Cellular Dynamics and Nanotools, Department of Biological Chemistry, School of Exact and Natural Sciences/IQUIBICEN, University of Buenos Aires/CONICET, Buenos Aires. 3. School of Engineering and Agrarian Sciences, Pontifical Catholic University of Argentina, BIOMED/UCA/CONICET, Buenos Aires, Argentina.

Non-exudative age-related macular degeneration (NE-AMD), the main cause of blindness in the elderly, is characterized by retinal pigment epithelium (RPE) and photoreceptors (PR) atrophy circumscribed to the macular area. There are no effective therapies to prevent or delay the vision loss that affects patients with NE-AMD. We have developed a NE-AMD model induced by superior cervical ganglionectomy (SCGx) in C57BL/6J mice, which reproduces the disease hallmarks exclusively circumscribed to the temporal region of the RPE/PR. Environmental enrichment (EE) is a complex combination of physical and inanimate stimulation, which has proven neuroprotective effects against retinal neurodegeneration. The aim of this work was analysing the protective effect of the exposure to EE on the alterations induced by experimental NE-AMD. Adult male C57BL/6j mice were submitted to unilateral SCGx, whereas the contralateral side was submitted to a sham procedure. Animals were submitted to SCGx and exposed to a standard environment (SE) or EE for 10 weeks. SCGx induced a significant increase in ubiquitous choroid thickness ($p<0.01$), a significant decrease in the electroretinogram a-wave amplitude ($p<0.01$) and performance in visual behaviour tests ($p<0.01$), a significant decrease of RPE melanin content and RPE65-immunoreactivity ($p<0.01$), a significant decrease in mitochondria mass and increase in mitochondria superoxide and lipid peroxidation products ($p<0.01$), and RPE and PR ultrastructural alterations at the temporal region. Although, EE did not prevent choroid alterations, it completely preserved visual function, and RPE and PR structure ($p<0.01$). EE preserved mitochondria mass and structure ($p<0.01$) and prevented RPE oxidative stress ($p<0.01$). These results suggest that EE prevented the functional and structural damage induced by experimental NE-AMD, through protecting RPE mitochondria and preventing RPE oxidative damage, thus becoming as a promising therapeutic strategy for NE-AMD.

424. (419) **PAIN PATHWAY ASIC1 CHANNEL EXPRESSION IN A RODENT MODEL OF ACUTE PAIN: HIND-PAW FORMALIN INJECTION TEST**

María Natalia Gobetto[1], Libia Catalina Salinas Castellanos[1], Natalia Estefania Contreras[1], Alejandro Sodero[2], Damián Alejandro Cambiagno[3], Georgina Oriana Mingolo Malnati[4], Mayra Micaela Montes[4], Osvaldo Daniel Uchitel[1], Carina Weissmann[1].
[1.] Insituto de Fisiología, Biología Molecular y Neurociencias. IFIBYNE-UBA-CONICET, LFBM, Argentina.
[2.] Institute of Biomedical Research (BIOMED), Pontifical Catholic University of Argentina (UCA) and National Scientific and Technical Research Council (CONICET), C1107AFF Buenos Aires, Argentina.
[3.] Unidad de Estudios Agropecuarios (UDEA), INTA-CONICET, Córdoba, Argentina.
[4.] Universidad de Buenos Aires, Argentina.

Hind-paw formalin injection in rodents evaluates the pain response. The response is biphasic, with an early phase (related to peripheral inflammatory mechanisms) in the first minutes and a late phase (related to central mechanisms) 30 minutes later. Acid-sensing ion channels (ASICs) regulate synaptic activity and play important roles in pain signalling. Psalmotoxin-1 (an inhibitor of constituted ASIC1a-channels) and antisense ASIC1a RNA injected in mice before the formalin test have been shown to affect responses in both phases. However, whether ASIC1 protein levels are affected remains controversial: most work focused on mRNA levels because protein levels proved difficult to detect. We quantified ASIC1a protein at different levels of the pain pathway in regions rich in ASIC1a channels: the anterior cingulated cortex (ACC) and spinal cord (SC) at individual lumbar (L) cord segments L3, L4 and L5; as well as at their corresponding dorsal root ganglia (DRG). Sampling was performed to differentiate ASIC1a protein amounts at the regions ipsilateral

(Ip) and contralateral (Con) to the injection. At all these regions we also quantified ASIC1a and ASIC1b mRNA. Our work shows that the biphasic response to formalin injection was accompanied by increased amounts of ASIC1 protein at the different regions involved in pain processing. This increase was detected at the Con ACC compared to the Ip of the same animal and the Con and Ip ACC of vehicle injected animals ($p<0.001$). At the SC, formalin injection increased the amount of ASIC1 protein in a gradient with highest protein amounts at L3 and lowest at L5, with the same gradient observed at the corresponding DRG ($p<0.01$). No significant changes in ASIC1 mRNA levels were detected. The mechanism of ASIC1 upregulation might be dependent on regulatory microRNAs ($p<0.05$). This work highlights the potential significance of ASIC signalling in pain processing, as well as potential therapeutic avenues targeting these channels.

425. (488) **CHANGES IN HISTONE MODIFICATIONS IN HYPOOSMOLAR-STRESSED ASTROCYTES: NEW INSIGHTS IN TISSUE RESPONSE TO EDEMA AFTER BRAIN INJURY**

Matías Monteverde Busso, Camila Vidos, Alberto Javier Ramos, Alejandro Villarreal
Contacto: avillarreal@conicet.gov.ar

Astrocytes respond to brain injury through a mechanism known as reactive astrogliosis involving transcriptional, phenotypic and functional changes. Astrocyte functional changes have high impact on brain injury outcome; however the epigenetic mechanisms regulating gene expression, such as histone modifications, remain obscure. We have recently shown that astrocytes exposed to pro-inflammatory signals increase the level of histone acetylation. However, to date, there is no available description on early epigenetic changes in injury-affected astrocytes. We hypothesize that hypo-osmolar stress promoted by early edema, *prime* astrocyte to become reactive during injury progression. In a model of brain cortical injury by pial disruption in adult male Wistar rats (Villarreal et al., 2011), we addressed the levels of H3K9ac in astrocyte nuclei at 1.5 and 3.5 hours post injury. We observed, using immunofluorescence, a significative higher number of astrocytes with lower levels of H3K9ac at 3.5 hours when compared to non-injured hemisphere. Also, the injury promoted an increase in GFAP and AQP4 immunoreactivity, which radially decreased at higher distances from the injury core, probably indicating astrocyte swelling in response to edema. *In vitro*, we exposed primary culture of astrocytes to hypotonic (20, 30 and 40% osm) culture medium to promote hypo-osmolar stress. We observed a statistically significant decrease in the levels of H3K9ac after 1 and 3 hours which were restored to control values 24h- after recovery in complete isotonic medium. A similar pattern was observed for H3K27ac suggesting a phenomenon of global histone deacetylation in response to stress. Our results strongly suggest that, astrocytes exposed to edema-like microenvironment are able to dramatically change the global levels of histone acetylation. During the recovery in histone acetylation levels, chromatin might be re-decorated but in a "*reactive epigenome"*.

426. (521) **SEX-DIFFERENCES IN NEURONAL AND MICROGLIAL CONTRIBUTION TO THE VALPROIC ACID RAT MODEL OF AUTISM SPECTRUM DISORDER**

Traetta ME*[1,2] , Codagnone MG*[1,2], Litvak ET[1], Zárate S[3], Uccelli NA[1], Malleville Corpa MJ[1], Reinés A[1,2]
1. Instituto de Biología Celular y Neurociencias "Prof. de Robertis"; IBCN, Universidad de Buenos Aires-CONICET
2. Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
3. Instituto de Investigaciones Biomédicas, Facultad de Medicina, Universidad de Buenos Aires
**equally contributed*

Despite a startling increase in prevalence, the neurobiology of autism spectrum disorder (ASD) remains unknown. A crosstalk between neurons and glia at the synapse has been hypothesized to underly ASD. Nonetheless, it is still unclear the role of sex-differences. Here, we examined sex-differences in neuronal and microglial contribution to the experimental model of ASD by prenatal exposure to valproic acid (VPA-450 mg/kg). In the juvenile period, at days 30-35, female VPA showed a distinctive reduction in play and non-play social behaviors and increased stereotypical activities in the absence of an exploratory deficit. Then, brain immunohistochemistry was performed at day 35. In medial prefrontal cortex (mPFC), synaptophysin (SYN) increased in male and female VPA animals but the ratio between neuronal cell adhesion molecule (NCAM) and its polysialylated form (PSA-NCAM) increased in males and decreased in females of the VPA group. Regardless of sex, microgliosis was found in this region of VPA animals. In the hippocampus, both male and female VPA showed reduced SYN immunostaining and increased NCAM/PSA-NCAM balance, but females had preserved PSA-NCAM levels. Hippocampal microgliosis was only detected in female VPA rats. In culture, female VPA neurons isolated from cortex showed similar dendritic trees or synaptic puncta to control neurons. Male but not female VPA hippocampal neurons showed resistance to glutamate-induced synaptic remodelling. While cortical microglia isolated from male VPA showed a pro-inflammatory profile and resistance to phagocytic stimuli, female VPA cortical microglia matched controls. Hippocampal microglia from both sexes were able to respond to pro-inflammatory and phagocytic stimuli. Overall, prenatal exposure to VPA induced female distinctive postnatal behavioral impairments and sex-dependent effects on neuron and microglia patterns. These results open new avenues to study the neurobiological basis of ASD.

427. (555) **SPHINGOLIPIDS, EMERGING MEDIATORS IN PROLIFERATIVE RETINOPATHIES?**

Camila Torlaschi[1], Gabriela Gutiérrez Jofré[1], María Sol Pérez[1], Tamara Soto[1], Paola Scodelaro[2], Nora P. Rotstein[1], M. Victoria Simón[1].
1. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Depto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur-CONICET. Bahía Blanca, Buenos Aires, Argentina.
2. Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Depto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur-CONICET. Bahía Blanca, Buenos Aires, Argentina.

Müller glial cells (MGC) and retinal pigment epithelium (RPE) cells are crucial for preserving retina homeostasis but their reactive response contributes to the progress of retina proliferative diseases, as diabetic retinopathy. We demonstrated that sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) regulate MGC and RPE cell migration. We now investigated whether they regulate viability and fibrotic and inflammatory changes in these cells. Incubation of RPE cell cultures with 5 μM S1P or 10 μM C1P for 24 h increased mRNA levels of IL-6 and IL-8, inflammatory interleukins, and α-smooth muscle actin (α-SMA), an epithelial mesenchymal transition marker. C1P-induced migration of RPE cells was not affected by inhibiting C1P endogenous synthesis with NVP-231 (NVP), a ceramide kinase inhibitor, but was markedly reduced when S1P synthesis was blocked with an inhibitor of sphingosine kinase 1 (SphK1), the enzyme involved in S1P synthesis. Interestingly, C1P addition enhanced SphK1 transcription. These results imply S1P and C1P promote RPE cell migration, pro-inflammatory and pro-fibrotic changes, and S1P endogenous synthesis is essential for RPE cell migration. To evaluate the role of C1P in MGC and RPE cells in an *in vitro* model of high glucose (HG)-induced damage, we pre-treated primary MGC cultures, obtained from rat retinas, and D407 cells, a RPE cell line, with NVP or its vehicle, and then exposed them to HG (30 mM), normal glucose (NG, 5 mM) or an osmotic control (25 mM Mannitol + NG) for 24-72 h. NVP pre-treatment induced morphological changes both in MGC and RPE cells exposed to HG and affected their viability, decreasing the amount of cell nuclei, compared to NG and Mannitol-treated cultures, suggesting C1P synthesis is required to protect MGC and RPE cells from the oxidative stress induced by HG. As a whole, these results suggest S1P and C1P play multiple roles in proliferative retinopathies, promoting inflammatory changes but also preventing cell death.

428. (565) **RETINAL DEGENERATION PROMOTED BY EXCESS OF LIGHT: MECHANISM OF CELL DEATH AND NEUROINFLAMMATORY RESPONSE**

Manuel G. Bruera, María M. Benedetto, Alicia L. Degano and María A. Contin.
Depto. de Química Biológica Ranwel Caputto, CIQUIBIC (CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

The overexposure to artificial lights may be one of the many factors that can induce the interruption of retinal homeostasis, promoting the injury of this tissue by photoreceptor cell (PhC) death that results in retinal degeneration (RD). Previously, we have established an animal model of light-induced RD in Wistar rats through constant exposure to LED light (200 lux). Using this model, we demonstrated that, after 5 days of exposure, the retina suffers important functional and structural changes including PhC death, opsins re-localization and increased oxidative stress. In order to elucidate the different molecular mechanisms leading to RD promoted by light excess, the aim of this work was to study the glial and inflammatory response and cell death proteins expression in animals exposed from 2 to 8 days of constant LED light. Retinas were processed either for WB, Q-PCR or IHC; and evaluated the expression of glial markers (GFAP and Iba1), inflammatory cytokines (TNFα, IL-6, CX3CR1, CCL2) and proteins involved in death cell processes (BAX, BCL-2, CASP8, TNFR1, RIPK3 and TLR4). After 6 days of exposure (LL6), we observed a significant increase in GFAP expression ($p<0.001$), the total number of iba1+ microglial cells ($p<0.01$) and the number of these activated cells (amoeboid shaped) in the PhC layer ($p<0.01$) compared to control animals. At the same time-point, we observed increases in TNFα and IL-6 ($p<0.05$), CX3CR1 ($p<0.01$) and CCL2 ($p<0.001$) mRNA expression. These results suggest that constant exposure to LED light promotes glial activation indicating a neuroinflammatory response to light damage. On the other hand, there were no changes in the mRNA expression of pro-apoptotic proteins BAX and CASP8 at the times studied, but were changes in the anti-apoptotic protein BCL-2 (LL2, $p<0.05$), necroptosis protein RIPK3 (LL6, $p<0.01$) and receptors TNFR1 and TLR4 (LL2, $p<0.05$) respect to controls animals; indicating that necroptosis may be the principal mechanism of PhC death.

429. (569) **EXTRACELLULAR MATRIX PRETREATMENT IMPROVES MORPHOLOGY AND FUNCTIONALITY IN MÜLLER GLIAL CELLS IN A RETINAL DEGENERATION MOUSE MODEL**

Harmonie Vallese-Maurizi[1,2,3], Georgina Pamela Coló[1,4], Solange Viera[1,2,3], Luis Politi[1,3], Olga Lorena German[1,2,3]
[1]Instituto de Investigaciones Bioquímicas (INIBIBB), Depto. de Biología, Bioquímica y Farmacia (DBBYF), Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina, INIBIBB, DBBYF, UNS- CONICET, Bahía Blanca, Argentina [2] Laboratorio de Neurovirología, [3]Laboratorio de Neurobiología, [4]Laboratorio de Biología del Cáncer

Müller glial cells (MGC) are retina specialized cells, which promote photoreceptors (PHR) survival. We demonstrated that they are stem cells, whose regenerative potential and lamellipodia are decreased in the retinal degeneration mouse *(rd1)*, compared to the wild type (wt). This suggests that the extracellular matrix (ECM) could be altered in *rd1*, affecting *rd1* MGC morphology and functionality, and interfering with substrate adhesion and lamellipodia extensions, hence leading to an excessive number of neurons interacting with MGC. The aim of this work was to study *rd1* ECM protein expression and localization, and determine whether ECM pretreatment could restore the *rd1* MGC morphology and functionality. Using mixed neuro-glial cultures obtained from postnatal 2 days *rd1* and *wt* mice retinas, we analyzed by immunocytochemistry, osteonectin and fibronectin (FN) expression and focal adhesions (FAs) stained with paxillin. Also, *rd1* mixed neuro-glial cultures were seeded on culture dishes previously treated or not with ECM-enriched conditioned medium (ECM-CM) obtained from Schwanomma (RN22) cell line, to analyze *rd1* MGC morphology, FAs, proliferation and PHR survival (using BrdU and DAPI, respectively). Our results showed a decrease in osteonectin expression and both number and length of FAs along with an increase in fibrillary FN expression in *rd1* MGC, when compared to the *wt* condition. Also, FAs showed cortical locations and mature shape in *rd1* compared to the *wt*. Noteworthy, ECM-CM pretreatment restored *rd1* FAs and MGC lamellipodia extensions, hence decreasing the ratio in number of neurons growing on MGC. This ECM pretreatment, also promoted *rd1* MGC proliferation and decreased PHR death. As a whole, these results suggest that *rd1* neuro-glial cultures present alterations in EMC protein synthesis and/or secretion, and that ECM supplementation improves MGC morphology and functionality.

430. (622) **MITOCHONDRIAL-ASSOCIATED ENDOPLASMIC RETICULUM MEMBRANES IN AMYLOID-β INDUCED NEURON DAMAGE**

Valentina Saud[1], Roxana Mayra Gorojod[1], Flavia Eugenia Saravia[2], Mónica Lidia Kotler, Soledad Porte Alcon
[1] Laboratorio de Disfunción Celular en Enfermedades Neurodegenerativas y Nanomedicina, Depto Química Biológica, FCEN-UBA; IQUIBICEN-CONICET. [2] Laboratorio de Neurobiología del Envejecimiento, Depto Química Biológica, FCEN-UBA; IBYME-CONICET

Alzheimer´s disease (AD) is the most common type of dementia. The progressive neuronal degeneration—beginning in the hippocampus—is accompanied by Amyloid-ss peptides (Ass) aggregation in amyloid plaques. The accumulation of insoluble protein aggregates has been associated with autophagy impairment and neuronal cell death. The association between the membranes of endoplasmic reticulum (ER) and mitochondria (M) (MAMs) are crucial for calcium homeostasis, lipid synthesis and autophagy, among other biological functions. MAMs alterations are associated with different pathologies, including neurodegenerative disease. Moreover, it has been proposed that the metabolic disturbances seen in AD are consequence of an altered ER-M communication. Our goal is to study the link between MAMs alterations and autophagy dysregulation as the leading causes of hippocampal neuronal loss induced by β-amyloid. HT22 hippocampal cells were exposed for 24 h to a sub-toxic concentration (Crystal Violet assay) of fibrillar Ass peptides (2 μM Assf). Confocal microscopy analysis of GFP-Sec61β (ER marker) and DsRed2Mito (M marker) revealed that exposure to Assf reduces ER-M contacts (M1, $p<0.05$; M2 $p<0.05$). In addition, Assf altered the mitochondrial morphology increasing the proportion of cells with short/fragmented mitochondria. Next, we evaluated lipid droplets (LD) formation (Red Nile staining). HT22 cells exposed to Assf exhibits a reduction in the number of LD per cell ($p<0.001$), unaffecting LD diameter. Finally, we analyzed autophagy induction (Immunocytochemistry with anti-LC3). Exposure to Assf increased LC3 punctate (5.6-fold, $p<0.001$). However, after Bafilomycin A1 treatment, LC3 levels augmented roughly a 17%, pointing that Assf may impair the autophagic flux. Overall, our findings suggest that Ass accumulation affects ER-M communication and MAMs-regulated functions in the absence of cell death, indicating that MAMs alteration may be an early event in neurodegeneration.

431. (810) **STUDY OF LRP1 IN CELLULAR INFLAMMATORY COMPONENT DURING CHOROIDAL NEOVASCULARIZATION**

Albana Tovo[1], Paula Virginia Subirada[2], María Victoria Vaglienti[1], José Domingo Luna Pinto[3], María Cecilia Sánchez[1], Gustavo Alberto Chiabrando[4], Pablo Federico Barcelona[1].
[1]Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, UNC. CIBICI-CONICET, Córdoba, Argentina.
[2]Instituto Ferreyra, INIMEC-CONICET-UNC, Córdoba, Argentina.
[3]Departamento de Vítreo-Retina, Centro Privado de Ojos Romagosa, Fundación VER, Córdoba, Argentina.
[4]Centro de Investigación en Medicina Traslacional Severo Raúl Amuchástegui, IUCBC-CONICET, Córdoba, Argentina.

Age-related macular degeneration (AMD) in its Choroidal Neovascularization (CNV) stage is the leading cause of vision loss among adults. Mononuclear phagocytic cells (MPC), such as resident mi-

croglia and monocyte-derived macrophages, collaborate in establish a chronic inflammatory state that can lead to the onset of CNV. The multi-ligand receptor Low Density Lipoprotein Receptor-Related Protein 1 (LRP1), is a ligand-dependent anti-inflammatory factor expressed in the cell inflammatory component, including macrophages and microglia. However, the role of LRP1 in CNV is not established yet. In the present study we aim to characterize the LRP1 levels and localization during CNV progression in a mouse model of CNV. C57BL/6 adult mice were treated with four spots of argon green laser photocoagulation per eye. At 1, 4, 7, 14 and 21 days after laser, mice were sacrificed and choroid tissue was processed by Western Blot to study LRP1 protein levels. At 4 days after laser, TNFα and LRP1 transcript levels were analyzed by qRT PCR and LRP1 expression in peripheral blood monocytes and MPC from choroids tissue were evaluated by FACS assays. All experiments were compared with control animals without laser. We could observe on choroid tissue from CNV animals increased levels of LRP1 at 1 and 4 days after laser. At 4 days after laser, the mRNA levels of pro-inflammatory cytokine, TNFα, were increased. In addition, the number of peripheral blood monocytes and their LRP1 expression were increased in CNV mice respect to control. However, LRP1 mRNA levels was unmodified on choroid. In conclusion, we observed increased LRP1 levels in early stages of CNV progression associated with enhanced pro inflammatory cytokine expressions, which was reverted after 4 days. These results suggest that the increased levels of LRP1 in CNV is due to MPC infiltration from circulating monocytes and resident microglial cells. Further studies are needed to determinate the role of LRP1 on these cells during CNV.

432. (837) **α2-MACROGLOBULIN/ LRP-1 SYSTEM EXPRESSION IN A NON-PROLIFERATIVE RETINOPATHY INDUCED BY METABOLIC SYNDROME IN MICE**

Paz, María Constanza[1,2,3], Vaglienti, María Victoria[1,2], Barcelona, Pablo[1,2], Sánchez, María Cecilia[1,2].
[1]Centro de Investigación en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, [2]Dpto. de Bioquímica Clínica, Facultad de Ciencias Químicas, UNC, [3]Dpto. de Farmacología, Facultad de Ciencias Químicas, UNC

Metabolic Syndrome (MetSyn) is a complex disorder of metabolism that affects different organs including the retina, triggering retinopathy. This disease presents, in addition to vascular alterations, a progressive neuronal degeneration. In this sense, α2-Macroglobulin (α2-M) has been proposed as a mediator of neurotoxicity, through their specific receptor, LRP-1. The purpose of this work was to evaluate gene and protein expression of α2-M/ LRP-1 system in retinas of mouse model of MetSyn that exhibits features of early stage (non-proliferative) of retinopathy, such as neuronal impairment and mild vascular alterations. C57BL/6 (WT) and Apolipoprotein E knockout (ApoE KO) mice fed with normal diet (ND) or 10% w/v fructose diet (FD) in drinking water from 2 months of age were used. After 3 months of diet, retinal extracts were obtained for Western blot, qRT-PCR and retinal cryosections for immunofluorescence analysis. Data was statistically analyzed by two-way ANOVA and Bonferroni post-hoc ($p \leq 0.05$). All experimental procedures will be carried out following the standards of The Association for Research in Vision and Ophthalmology (ARVO) and the ethics committee of FCQ- UNC. The results showed α2-M mRNA expression in mice retina and a significant increase of α2-M protein expression in retinal extracts of ApoE KO mice with both diets [WT ND vs ApoE KO FD ($p< 0,0001$) vs ApoE KO FD ($p< 0,039$)], demonstrating that their expression is increased in mice with metabolic alterations. However, LRP-1 protein expression showed no differences between the experimental groups. Immunofluorescence results showed α2-M and LRP-1 protein expression, principally in the inner nuclear layer and in the inner limiting membrane of mouse retina. These results are more than encouraging and strongly support the study of the role of α2-M/ LRP-1 system in early stage of retinopathy induced by MetSyn.

433. (881) **PROTECTIVE EFFECT OF NITRO-OLEIC ACIDS IN NEOVASCULARIZATION AND NEURODEGENERATION IN OXYGEN-INDUCED RETINOPATHY MOUSE MODEL**

Vaglienti María Victoria[1,2], Subirada Paula Virginia[3], Paz Constanza[1,2], Bonacci Gustavo[1,2], Sánchez María Cecilia[1,2].
[1]Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina, [2]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina, [3]Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina.

Inflammation, oxidative and nitrosative stress are involved in Neovascular Retinopathies (NR). Nitro-fatty acids are important electrophilic signaling mediators with anti-inflammatory, antioxidant and cytoprotective properties (Keap1/Nrf2 pathway). Hence, our goal was to evaluate the effect of Nitro-oleic acid (NO_2-OA) in a validated oxygen-induced retinopathy mouse model (OIR). Briefly, C57BL/6 mice were exposed to 75% O_2 from P7 to P12, after that they were brought to room air (RA) for additional five (P17) days or nine days (P26). Age-match mice in RA were used as controls. Some OIR mice were i.o. injected at P12 with 5 μM of NO_2-OA or vehicle and i.p. at P14, P17, P20, P23 with 15 mg/Kg of NO_2-OA or vehicle. At P17 or P26 mice were sacrificed. Some eyes were fixed to obtain retinal whole mount for microscopy or cryosection and other retinas were used to analyze protein expression by Western blot or RT-PCR. The retinal functionality was measured by scotopic electroretinography (ERG). Amplitudes and latencies of a- and b-waves from scotopic ERG were recorded at P17 y P26. The whole mount showed that NO_2-OA induced the vascular regrowth and decrease the pathological neovascularization and vaso-obliteration at P17. Interestingly, RT-PCR revealed a significant increase in VEGF levels in OIR mice respect to RA mice, but not difference was found between NO_2-OA treatment and vehicle. In addition, Western blot of neural retinas showed significant changes in proteins involved in neurotoxicity and glial stress such as GS and GFAP at P17 and P26 in OIR mice treated with NO_2-OA respect to vehicle. No differences in ERG signals were observed between mice injected with vehicle or NO_2-OA at P17. However, NO_2-OA prevented the decrease in b-wave amplitude at P26. Correlating with a decrease in the caspase 3 total level at P26 OIR NO_2-OA prevented this decrease. Overall, these findings suggest that NO_2-OA Have multiple benefits for retinal cells in NR.

434. (887) **CHRONIC THYROID HORMONE EXCESS INDUCES ANGIOGENESIS AND NEURONAL DEATH IN THE ADULT ZEBRAFISH RETINA**

Pablo A. Iomini, Leandro Rocco, Claudio A.Bejarano, Ramón O. Bernabeu, María Paula Faillace
Universidad de Buenos Aires, Facultad de Medicina, IFIBIO-Houssay (CONICET-UBA), Buenos Aires, Argentina.

Adult zebrafish are capable of regenerating organs and tissues over their life span including brain, the spinal cord and retina. So, zebrafish are suitable experimental vertebrate models to identifysignalling systems that control cell genesis and differentiation in the nervous system. Central nervous system development and growth depend on thyroid hormones (TH)regulating cell proliferation, differentiation and patterning. TH signaling controlsmetabolism andcell growth throughout lifetime. So, we aimed to examine whether TH disequilibrium affected adult retina growth and regeneration. To this end, ouabain (20 μM) was intraocularly injected provoking a medium severity retina injury. Then, control orinjured zebrafish were treated with high doses of thyroxine (T_4)(or vehicle) diluted in fish tank water over 8-, 25- and 60-day periods. After a week, zebrafish lost melanin in their skin melanocytes which is a sign of hyperthyroidism. ANOVA andBonferroni tests were applied to analyze significant changes in cell numbers quantified on retinal layers and relative amounts of different gene mRNA levels were analyzed by RT-quantitative PCR. Cell death was qualitatively assessed by TUNEL and activated Caspase-3. Endothelial vascular cells were detected by lectin *Lycopersicum esculentum* (tomato) in the inner retina and choroid layers. Chronic treatmentwith T4 caused a significant reduction of photoreceptors, interneurons, and ganglion cells 25 and 60 days post injury. T_4 treatment also caused significant reductions in specific

retina cell markermRNA levels: PKC, GFAP, CNG3, CNG1, Thy1, and Conexin26. T_4 treatment increased vascular endothelial growth factor receptor mRNA levels, apoptosis, gliosisand angiogenesis. Sustained high levels of T_4induced proangiogenic and proapoptotic mechanismsthat abrogatedretina regeneration. T_4excessinduced morphological changesin the zebrafish retina that emulate neurodegenerative retinopathies.

NEUROSCIENCES II

Thursday, November 17, 14-15:30 hr
Chairs: Cecilia Sanchez - Ana de Paul - Analia Reines - Alejandro Villlarreal

435. (66) **INSULIN RESISTANCE AND GLIAL ACTIVATION AS CONCURRENT EVENTS IN EXPERIMENTAL ALZHEIMER'S DISEASE**

Melisa Bentivegna[1,2], Amal Gregosa[1,2], Ángeles Vinuesa[1,2], Carlos Pomilio[1,2], María Bonaventura[1], Jessica Presa[1,2], Nicolás González Pérez[1,2], Melina Bellotto[1,2], Victoria Lux[1], Flavia Saravia[1,2], Juan Beauquis[1,2]
1. Instituto de Biología y Medicina Experimental (IBYME)-CONICET, Buenos Aires, Argentina.
2. Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the leading cause of dementia. Besides amyloid beta (Aβ) and tau accumulation, inflammation and insulin resistance are common findings in AD brains. Astrocytes regulate these processes maintaining brain homeostasis and coordinating the inflammatory response. In the present work, our objectives were 1) to study the metabolic and inflammatory status of the PDAPP-J20 transgenic (TG) mouse, model of AD, and 2) to evaluate astroglial activation and insulin signaling upon Aβ exposure. We hypothesized that in AD, astrocytes adopt a phenotype that promotes inflammation and lose homeostatic functions. Evaluation in the open-field test showed an anxious-like behavior in TG mice. Insulin signaling measured by the pAkt/Akt ratio was impaired in the hippocampus ($p<0.05$) but not in the hypothalamus or the liver of TG, suggesting a specific deregulation in a region highly affected by amyloid pathology. Also, hippocampal insulin receptor levels (immunoblot) were decreased ($p<0.05$). Immunolabeling for insulin receptors showed a tendency to decrease in GFAP+ astrocytes, that also displayed increased GFAP and S100b labelling ($p<0.05$), suggesting proinflammatory reactivity. Then, we evaluated the effect of AB on astrocytes in vitro. Astrocytes exposed to Aβ showed increased NFkB nuclear translocation, decreased area ($p<0.05$) and size of mitochondria ($p<0.001$) and decreased AKT phosphorylation ($p<0.05$), suggesting inflammatory activation, mitochondrial alterations and impaired insulin signaling, respectively. Our results suggest that hippocampal insulin resistance and glial reactivity are concurrent events in experimental AD. Accordingly, astrocytes exposed to Aβ in vitro adopt a pro-inflammatory phenotype associated with faulty insulin signaling and mitochondrial morphological defects. The study of these interrelated phenomena could help to understand AD pathophysiology.

436. (79) **REGULATION OF $GABA_A$ RECEPTORS BY PROLONGED EXPOSURE OF CORTICAL NEURONS TO BENZODIAZEPINES IS MEDIATED BY A CALCIUM SIGNALING PATHWAY**

L. Carolina González Gómez, Sara Sanz Blasco, Nelsy B. Medina, María Clara Gravielle
Instituto de Investigaciones Farmacológicas (ININFA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

The clinical use of benzodiazepines has been limited by the development of tolerance to most of their pharmacological effects. In previous studies, we demonstrated that the prolonged exposure of rat cortical neurons to diazepam (DZ) produces a transcriptional repression of the $GABA_A$ receptor α1 subunit gene and this effect is prevented in the presence of nifedipine, an inhibitor of L-type voltage gated calcium channels (L-VGCC). The aim of this work was to investigate the signaling cascade triggered by the prolonged treatment with DZ that results in the regulation of the $GABA_A$ receptor. To this end, we exposed rat primary neuronal cultures to DZ (1 μM) for 48h in the presence or absence of different inhibitors. Results from this study indicated that the DZ-induced decrease in the mRNA and protein levels of the α1 subunit was inhibited in the presence of 1 mM EGTA ($p<0.05$, one-way ANOVA and Tukey post-hoc test). This suggests that the DZ-induced down-regulation of the α1 subunit depends on the calcium entrance from the extracellular space. Previous reports indicated that the α1 subunit gene can be transcriptionally repressed by the action of cAMP response element-binding protein (CREB) and inducible cAMP early repressor (ICER). Since the phosphorylation of CREB and the induction of ICER can be triggered by an increase in intracellular calcium concentration, we tested whether the DZ treatment can induce the activation of these two transcription factors. We observed that the DZ exposure induced a time-dependent increase in the phosphorylated levels of CREB and in the mRNA levels of ICER ($p<0.05$). Taken together, our results suggest that sustained stimulation of $GABA_A$ receptors by benzodiazepines activates an intracellular signaling cascade involving the stimulation of calcium influx through L-VGCCs. This effect results in the activation of two transcription factors, CREB and ICER, leading to the transcriptional down-regulation of the $GABA_A$ receptor α1 subunit gene.

437. (174) **SCHIZOPHRENIA-LIKE NEUROADAPTIVE CHANGES INDUCED BY KETAMINE INVOLVE ANGIOTENSIN II**

Victoria Belén Occhieppo[1], Osvaldo Martín Basmadjian[1], Gustavo Baiardi[2] and Claudia Bregonzio[1]
[1]Departamento de Farmacología Otto Orsingher, Facultad de Ciencias Químicas, Instituto de Farmacología Experimental Córdoba (IFEC-CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina
[2]Laboratorio de Neurofarmacología, (IIBYT-CONICET), Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina

Schizophrenia is a chronic disease affecting 1% worldwide population, of which 30% are refractory to the available treatments: thus, searching for new pharmacological targets is imperative. Ketamine administration is a validated preclinical model that recreates the behavioral and neurochemical features of the pathology, including the parvalbumin-expressing interneurons dysfunction. Angiotensin II, through AT_1 receptors (AT_1-R), modulates the dopaminergic and GABAergic neurotransmission. We evaluated the AT_1-R role in the long-term neuronal activation and behavioral alterations induced by repeated ketamine administration. Adult male Wistar rats received AT_1-R antagonist candesartan/vehicle (days 1-10) and ketamine/saline (days 6-10). After 14 days of drug-free, neuronal activation and behavioral analysis were performed. Locomotor activity, social interaction and novel object recognition tests were assessed at basal conditions or after ketamine challenge. Immunostaining for c-Fos, GAD67 and parvalbumin were assessed after ketamine challenge in cingulate, insular, piriform, perirhinal, and entorhinal cortices, striatum, and hippocampus. We found that ketamine-induced long-lasting schizophrenia-like behavioral alterations, and regional-dependent neuronal activation changes, involving the GABAergic neurotransmission system and the parvalbumin-expressing interneurons, were AT_1-R-dependent. Our results add new evidence to the wide spectrum of action of ketamine and strengthen the AT_1-R involvement in endurable alterations induced by psychostimulants administration, as well as their role in the development of psychiatric pathologies.

438. (269) **REGULATION OF NEUROSTEROIDOGENIC ENZYMES AFTER TESTOSTERONE TREATMENT IN THE SPINAL CORD OF THE WOBBLER MOUSE, A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

Esperante Iván[1], Meyer María[1], Banzán Carolina[1], Lima Analia.[1], Roig Paulina[1], Guennoun Rachida[3], De Nicola Alejandro Federico[1,2b] and Gonzalez Deniselle María Claudia[1,2a]

1. Instituto de Biología y Medicina Experimental (IBYME), CONICET
2. a: Dto. de Ciencias Fisiológicas, b: Dto de Bioquímica Humana, Facultad de Medicina, UBA
3. U1195 INSERM and University Paris Sud: "Neuroprotective, neuroregenerative and remyelinating small molecules"

Progressive degeneration of upper and lower motoneurons characterizes amyotrophic lateral sclerosis (ALS) which triggers muscle weakness and motor impairment. The Wobbler (WR) mouse, a model of ALS, displays a selective loss of motoneurons, astrocytosis and microgliosis in cervical spinal cord (CSC). Clinically, WRs develop forelimb muscle atrophy and gait disturbances. Previous work has shown that testosterone (T) treatment to WRs reduces microgliosis and astrogliosis, and increases ChAT+ motoneurons and oligodendrocytes in CSC. T binds to androgen (AR), estrogen and Sigma1 receptors (S1R) after bioconversion into several metabolites. Now, we investigated the effects of T on mitochondrial steroidogenic enzymes. T were implanted s.c. in 10 mm silastic tubes for 2 months to male WRs or controls (CTL). WRs or controls without T treatment received empty silastic tubes. T treatment increased seminal vesicles ($p<0.01$: WR+T vs WR and CTL+T vs CTL) and biceps weights in WRs ($p<0.05$ vs WR). In the ventral horn of the CSC, we found: 1) a reduction in the immunoreactivity (IR) of the enzyme that metabolizes cholesterol into pregnenolone, CYP11A1, in WR motoneurons ($p<0.05$ vs control), which showed similar IR in WR+T (NS vs WR); 2) a trend to high levels of pregnenolone (ng/g) in WRs ($p=0.05$ vs control), but low concentration in WR+T ($p<0.05$ vs WR); 3) a decrease in S1R+ neurons, a molecule located in mitochondrial membrane and regulator of steroidogenesis, in WRs ($p<0.001$ vs control), but an up-regulation in WR+T ($p<0.05$ vs WR). Similarly, MnSOD showed a reduction in WRs ($p<0.05$ vs CTL) and significantly increased IR in WR+T ($p<0.01$ vs. WR). None of these parameters were modified in CTL+T vs CTL. Considering that mitochondria is a target of oxidative stress in degenerating motoneurons, the effects of T on neurosteroidogenic enzymes in WR CSC may depend on an antioxidant effect of this androgen improving mitochondrial function.

439. (324) **PLASTICITY OF CORTICO-STRIATAL NEURONS OF THE ANTERIOR CINGULATE CORTEX DURING EXPERIMENTAL NEUROPATHIC PAIN**

María Jesús Trujillo, Constanza Ilarraz, Jesica Unger, Fernando Kasanetz
Grupo de Neurociencia de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Bernardo Houssay, Facultad de Medicina, UBA - CONICET, Buenos Aires, Argentina.

Neuropathic pain (NP) is a common and debilitating neurological condition. The elevated pain sensitivity results from a series adaptations at all levels of the nociceptive system. While much attention have been devoted to peripheral and spinal cord mechanisms, less is known about the processes taken place in brain areas involved in pain perceptions. In particular, the limbic system is believed to participate the persistence of pathological pain. A key region for this is the Anterior Cingulate Cortex (ACC), which is essential for affective connotation of pain and is hyperactive in patients suffering from pathological pain. In preclinical models, neuronal plasticity in the ACC mediates the nociceptive sensitization associated to NP.
The ACC projects massively into the dorsal medial striatum giving a direct pathway for the propagation of abnormal activity towards the limbic system. However, it is unknown whether cortico-striatal ACC neurons (ACC-CS) are affected during NP. Here we used a rodent model of NP induced by a sciatic nerve lesion and combined neuronal tracing and ex-vivo electrophysiology to study the neuronal plasticity of ACC-CS during pathological pain. Intrinsic membrane excitability was unchanged in NP animals compared to sham controls. Conversely, the maximal synaptic depolarizations induced by trains of afferent stimuli were higher during NP (sham: 9.77 ± 1.14 mv (n = 21); NP: 12.83 ± 1.00 mV (n=26), T-test: p = 0.04). This was not due to changes in dendritic integration since kinetics and temporal summation of post-synaptic potentials were conserved after sciatic nerve lesions. Finally, the greater synaptic strength in NP mice was associated with an increased train-evoked firing of action potentials (2way ANOVA, p=0.04, stim intensity vs firing curve). Taken together, our results show that NP affected information flow through ACC-CS. Future work will deepen on the molecular mechanisms behind this and on the consequences downstream on striatal neurons.

440. (380) **EFFECT OF SUBLETHAL COPPER OVERLOAD ON CHOLESTEROL *DE NOVO* SYNTHESIS IN UNDIFFERENTIATED NEURONAL CELLS. POSSIBLE ASSOCIATION WITH ALZHEIMER´S LIKE DISEASE ONSET**

Zubillaga Marlene1, Creo Victoria1,2, Rosa Diana3, Catanesi Cecilia2, Arnal Nathalie1.
1. Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), (CONICET- UNLP), Laboratorio de Neurociencia. Calle 60 y 120, CP 1900 La Plata, Argentina.
2. Instituto Multidisciplinario de Biología Celular (IMBICE) (CONICET-UNLP-CIC), La Plata, Buenos Aires, Argentina.
3. Laboratorio de Nutrición Mineral, Fac. Cs Veterinarias (UNLP). Calle 60 CP 1900 La Plata, Argentina.

Despite copper (Cu) is an essential trace metal for cells, it can induce harmful effects as it participates in the Fenton reaction. Involuntary exposure to Cu overload is much common than expected and has been linked with neurodegeneration, particularly with Alzheimer's disease (AD) evidenced by a positive correlation between free Cu in plasma and the severity of the disease. It has been suggested that Cu imbalance alters cholesterol (Chol) homeostasis and that high membrane Chol promotes the amyloidogenic processing of the amyloid precursor protein (APP) secreting the β-amyloid (Aβ) peptide. Despite the wide knowledge on the effects of Cu in mature brain metabolism, the consequence of its overload on immature neurons remains unknown. Therefore, we used an undifferentiated human neuroblastoma cell line (SH-SY5Y) to analyze the effect of sub-lethal concentrations of Cu on: 1- The *de novo* Chol synthesis and membrane distribution; 2- APP levels in cells and its distribution in membrane rafts; 3- The levels of Aβ in the culture medium. Our results demonstrated that Cu increases ROS and favors Chol *de novo* synthesis in both ROS-dependent and independent manner. Also, at least part of these effects was due to the activation of 3-hydroxy-3-methyl glutharyl CoA reductase (HMGCR). In addition, Cu increases Chol/PL ratio at the cellular membranes, specifically Chol content in membrane rafts. We found no changes in total APP cell levels, however, its presence in membrane rafts increases, with the consequent increase of Aβ in culture medium. We conclude that Cu overload favors Chol *de novo* synthesis in both ROS-dependent and independent manner, being at least in part, responsible of the high Chol levels found in the cell membrane, and membrane rafts. These may promote the redistribution of APP into the rafts favoring the amyloidogenic processing of this protein and increasing the levels of Aβ.

441. (402) **ANDROGEN ACTION ON ASTROGLIAL CELLS IN THE MOTOR CORTEX AND INTERNAL CAPSULE IN A MOUSE MODEL OF MOTONEURON DISEASE**

Banzán Carolina[1], Meyer María[1], Esperante Iván[1], Lima Analia.[1], Roig Paulina[1], De Nicola Alejandro Federico[1,2b] and Gonzalez Deniselle María Claudia[1,2a]
1. Instituto de Biología y Medicina Experimental (IBYME), CONICET
2. a: Dto de Ciencias Fisiológicas, b: Dto de Bioquímica Humana, Facultad de Medicina, UBA

Wobbler (WR) mouse, a model for human amyotrophic lateral sclerosis (ALS), shows motoneuron degeneration with increased astrogliosis and microgliosis in the spinal cord and motor cortex. In this work, we studied testosterone (T) effects on brain glial cells from male WRs and controls. Therefore, a silastic tube containing T was implanted s.c. in WRs at symptomatic stage for 2 months. We analyzed the number of glial fibrillary acidic protein (GFAP)+ astrocytes and glutamine synthetase (GS)+ cells/unit area in motor cortex and internal capsule from male controls, WRs and WR+T. Previous data showed that a group of WR astrocytes are GS negative in the spinal cord, enzyme necessary to detoxify glutamate into glutamine. We found a 2-fold increase in the number of GFAP+ cells/area in

internal capsule ($p<0.05$) and motor cortex from WRs vs controls ($p<0.05$). However, this parameter was not modulated in WR+T. We also studied GFAP+ cells in hippocampus, a region not affected by motoneuron degeneration. GFAP+ astrogliosis was also shown in hippocampal regions (CA1, CA2, CA3 and dentate gyrus) of WRs vs control ($p<0.05$), but without a T effect. Regarding GS immunoreactivity (IR), it showed significant group differences by ANOVA in both regions. We found lower number of GS+ cells/area in the motor cortex ($p<0.001$) and internal capsule ($p<0.01$) from WRs vs. control. After receiving T treatment, WR mice showed significant greater levels of GS+ cells in both regions ($p<0.05$-motor cortex; $p<0.01$-internal capsule) vs. untreated WRs. Colocalization studies between GFAP and GS showed that both markers colocalized in brain regions. In summary, T reversed low GS-IR in motor cortex and internal capsule from WRs, without regulating the number of GFAP+ cells. Since T effect on GS in both WR's brain regions is important for glutamate detoxification, neuroprotective effect of T may extend from the spinal cord to brain motor systems.

442. (416) **NUTRIENT RESTRICTION PREVENTS ASTROGLIAL AUTOPHAGY BLOCKAGE INDUCED BY AMYLOID BETA**

Amal Gregosa, Melisa Bentivegna, Melina Bellotto, Ángeles Vinuesa, Carlos Pomilio, Jessica Presa, Nicolás González Pérez, Flavia Saravia, Juan Beauquis.
IBYME – CONICET.
QB- FCEN- UBA.

Nutrient restriction is associated with increased lifespan and prevention of chronic diseases in various organisms. Previously, we reported that dietary restriction (DR) decreased hippocampal amyloid load, increased neurogenesis, and improved cognitive performance in mice modeling Alzheimer's disease (PDAPP-J20 strain, TG). Astroglial autophagy was studied as a potential mechanism involved in amyloid beta (AB) clearance. We found increased LC3 and p62 labeling in hippocampal astrocytes, suggesting blocked autophagy in TG mice. In vitro results showed that astroglial AB content was decreased after NR normalized LC3 levels. In the present study, our aim was 1) to evaluate in depth how astroglial autophagy is modulated in mice along a DR period (5 days with 60% of the habitual food intake) and 2) to analyze the autophagic flux in an in vitro model exposed to AB 1-42 peptides and nutrient restriction (NR). Adult C57BL/6 mice showed increased ketonemia after DR ($p<0.01$ vs. baseline), evidencing a fasting-like condition. Rab 7 protein levels in the hippocampus (immunoblot) showed a tendency to increase in animals exposed to DR ($p=0.0508$). These results support possible induced autophagy by DR period. In vitro, we exposed the astrocytic C6 rat cell line to AB 1-42 peptides and incubated them in NR or control media (2% vs. 10% FBS). We performed a Cyto ID assay to evaluate autophagosome levels and found increased fluorescence in AB-exposed cells compared with control (CTL vs AB, $p<0.05$). Interestingly, this effect was prevented in AB-exposed cells incubated under NR, suggesting a normalization of the autophagic flux. In conclusion, a 40% DR modulates hippocampal autophagy in adult mice, indicating a potential pathway for DR-associated increased AB clearance. Our in vitro results suggest that NR prevents the AB-associated blockage of the astroglial autophagic flux. We plan to perform mechanistic studies to determine which point of the autophagy pathway is modulated by NR.

443. (473) **PARTICIPATION OF NITRIC OXIDE SIGNALING WITHIN THE MEDIAL PREFRONTAL CORTEX IN THE EXPRESSION OF COCAINE SENSITIZATION**

Ponce Beti Maria Fernanda and Pérez Mariela Fernanda.
Departamento de Farmacología Otto Orsingher, Instituto de Farmacología Experimental de Córdoba (IFEC-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

The medial prefrontal cortex (mPFC), a brain region that regulates cognitive functions and reward motivated behaviors, is implicated in the neuropathological mechanisms of drug addiction. Nitric oxide (NO) is a neurotransmitter that plays a major role in initiating and maintaining the behavioral effects of psychostimulant drugs, such as cocaine (COC) sensitization (SENS). In fact, acute COC administration increases NO release in the mPFC, which was prevented by nitric oxide synthase type-1 enzyme (NOS-1) inhibition by systemic administration of a selective inhibitor (7-nitroindazole, 7NI). Furthermore, systemic 7NI injection during repeated COC administration prevented the persistent increase in membrane excitability of mPFC pyramidal neurons and long term potentiation in hippocampus (HP) after short-term withdrawal. However little is known about the contribution of NO signaling within the mPFC to the expression of COC SENS. Objective: to determine whether intra mPFC NOS-1 inhibition after COC SENS reverses its expression. Material and methods: mPFC cannulated male Wistar rats (42 days old) received five daily COC injections (i.p) and locomotor activity was monitored on days 1 and 5 to evaluate development of COC SENS. On day 6, animals received intra-mPFC administration of 7NI (0.1 µmol/µl; 0.5 µl/side) or DMSO. On day 7, rats received an i.p. challenge of SAL or COC and locomotor activity was measured. Results: sensitized rats showed a significant increase in locomotor activity on day 5 compared to day 1 within the group ($p<0.05$ Two-Way RM-ANOVA), but no differences were found in locomotor activity on day 7 compared to day 5 after 7NI intra-mPFC administration ($p>0.05$, preliminary results). Conclusions: it seems that NOS-1 inhibition within m-PFC after COC SENS is not able to reverse its expression, as it was previously observed after intra-HP administration. Probably, NO signaling within the mPFC does not play a fundamental role in the expression of COC SENS.

444. (475) **CHARACTERIZATION OF NON-DISABLING SENSORIMOTOR DEFICITS INDUCED BY MILD TRAUMATIC BRAIN INJURY IN RATS**

Sofía de la Fuente and Mariela Fernanda Pérez
Departamento de Farmacología Otto Orsingher, Instituto de Farmacología Experimental de Córdoba (IFEC-CONICET), Facultad de Ciencias Quimicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Traumatic brain injury (TBI) causes many neuropathological manifestations including cognitive, emotional, motor and psychological deficits, probably related to early neuroinflammatory processes. At cellular level, axons of catecholaminergic neurons are particularly vulnerable to primary and secondary injury mechanisms. Then, alterations in dopaminergic neurotransmission, mainly at striatum and the limbic system, could be involved in the mechanisms that underlie sensorimotor deficits induced by TBI. In fact, administration of dopamine-acting drugs in TBI patients improved symptoms related to the executive and cognitive function. We have previously shown that mild TBI (mTBI) generates cognitive deficits coincidently with increased levels of oxidative stress (OS) biomarkers (protein-AOPP and lipid-MDA peroxidation), right after mTBI that lasted over a 7 days (d) in brain areas mainly related to these functions. However, little is known about mTBI-induced sensorimotor consequences nor OS in brain structures related to these effects. Objective: to characterize motor and sensory deficits as well as AOPP and MDA levels in striatum after mTBI. Materials and methods: mTBI was induced in anesthetized adult male Wistar rats and 7 d after were tested in grip strength (GS), hot plate (HP) or amphetamine (0.5 mg/kg)-induced locomotor activity (LA). Other groups were sacrificed 60 min, 24 h or 7 d after mTBI to determine AOPP or MDA levels. Results: preliminary results indicate that mTBI induced significant decrease in time to fall in GS, increased the latency in HP ($p<0.05$ unpaired t-test) and reduced LA (RM-ANOVA). AOPP and MDA determinations are still in progress. Conclusions: mTBI induced sensorimotor deficits in animals with apparent normal motor performance. These deficits may be related to early neuroinflammatory processes that could alter neuronal functioning in brain structures related to cognitive, sensorimotor and psychiatric disorders described in TBI patients.

445. (527) **DECREASED PROTEIN EXPRESSION AND TRAFFICKING UNDERLIES IMPAIRED MITOCHONDRIAL DNA REPAIR PATHWAY IN THE HIPPOCAMPUS OF OVARIAN HORMONE-DEPRIVED RATS**

Micaela Avaca[1], Ricardo Gredilla[2], Ivana Villa[1], Analía Rei-

nés[3], Tinna Stevnsner[4], Sandra Zárate[1]
[1]Instituto de Investigaciones Biomédicas, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina , [2]Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, Madrid, [3]Instituto de Biología Celular y Neurociencias 'Prof. E. De Robertis', Facultad de Medicina and Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [4]Department of Molecular Biology and Genetics, Aarhus University, Denmark

Mitochondria dysfunction and oxidative stress critically affect the brain due to its high demand of energy and low antioxidant capacity. Oxidative damage to mitochondrial DNA is repaired by Base Excision Repair (BER) pathway through several steps comprising lesion-specific glycosylases and endonucleases. Ovarian hormone loss is related to brain mitochondrial dysfunction and oxidative stress. The aim of this work was to assess if hormone deprivation affects protein expression and/or mitochondrial localization of BER enzymes to explain previous results regarding differential activity of such enzymes in the hippocampus (Hp) and cerebral cortex (Cc).
To this aim, adult rats were ovariectomized (OVX) or sham-operated (SHAM). After 12 weeks, we obtained cytosolic, mitochondrial and nuclear proteins from total tissue homogenates from the Hp. They were analysed by WB using antibodies for BER glycosylases NEIL1 and 2, which remove oxidized bases, and AP endonuclease1 (APE1). Both total and mitochondrial NEIL2 was lower in the Hp of OVX rats ($p<0,05$; Student´s t test) with no differences in cytosolic or nuclear fractions, rendering lower NEIL2mito/total ratio. Conversely, NEIL1 showed no changes in any of the fractions. APE1 was lower in all the fractions in the Hp of OVX rats ($p<0,05$, Student´s t test), but no differences were found in APE1mito/total ratio. Our results show that OVX decreases some BER protein levels in the Hp, which correlates with previously observed lower ARNm levels and activity of such enzymes in this brain region. Lower NEIL2mito/total ratio suggests impared mitochondrial trafficking of this enzyme in the Hp of OVX rats. Our results suggest that the Hp has low capability of responding to mtDNA oxidative damage after ovarian hormone deprivation through impared NEIL2 expression and mitochondrial localization. Further studies in Cc will determine if this mechanism underlies regional differential action of hormonal status on brain mitochondrial BER pathway.

446. (587) **GAIT IN PEOPLE WITH PARKINSON'S DISEASE: COMPARISON BETWEEN COMMUNITY ENVIRONMENT AND DUAL TASK PERFORMANCE**

Hernán Lattini[1], Rodrigo Juarez[1], Ignacio Primo[1], Santiago Cueto[2], María Eugenia Ferri[1], Conrado Borgatello[3], Silvana Rosso[3], Manuel Rodríguez[1].
[1]Departamento de Rehabilitación. Fundación Rosarina de Neuro-Rehabilitación (FRN). Rosario, Argentina. [2]Instituto Universitario Italiano. Rosario, Argentina. [3]Laboratorio de Toxicología Experimental (LATOEX), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina.

Gait disorders in people with Parkinson's disease (PD) may be exacerbated by environmental demands and aggravated with others simultaneous tasks. The aims of this work were to analyze the community environment walking cost of PD patients and correlate with dual tasks performed in a clinical setting. Patients expressed their agreement through an informed consent by Biomedical Research Ethics Committee (IRB), FRN. PD patients (n = 27; age average 68.3 ± 9; disease length 11.3 years; L-dopa ON, Hoehn & Yahr stages 1 - 3) without cognitive impairment, were tested using 10 Meter Walk Test (10MWT). 10MWT was applied under three conditions: 1) in a gym while the patient simultaneously performed a cognitive task -CT- (reciting week days in reverse), 2) in a gym while the patient simultaneously performed a motor task -MT- (carry a tray with a glass) and 3) in the community environment -CE- (on the sidewalk). Data were analyzed comparing different groups (mean ± SD) by ANOVA and then were correlated. Results 10MWT cost (% relative to baseline) showed statistical differences between CE -5.7 ± 13.08 vs CT -18.48 ± 11.61 (***$p<0.001$) and CT vs MT -11.84 ± 11.76 (**$p<0.01$). Correlations results showed CE vs CT r = 0.884 (****$p<0.0001$) and CE vs MT r = 0.871 (****$p<0.0001$). CE cost was minor than those observed in patients while performing cognitive tasks in controlled environment. This difference could be explained by some PD patients were more stimulated by the environment to walk. Moreover the CE would not demand more attentional resources than CT. CE cost did no show statistical difference respect to MT cost, likely due to in both groups the sensory-motor resources are involved in the execution of the activity. We found strong positive correlations between groups; whereby the gait cost could be proportionally influenced by both the environment and the type of task. Finally, the study of PD patients´ gait in a CE is necessary to understand the impact on daily life of PD.

447. 636. **RECOVERY OF ASTROCYTE-VASCULAR COMMUNICATION IN THE HIPPOCAMPUS OF MICE WITH CEREBRAL AMYLOID ANGIOPATHY FOLLOWING TREATMENT WITH THE GLYCAN-BINDING PROTEIN GALECTIN-1**

Jessica Presa1,2 Carlos Pomilio1,2 Camila Oses1, Amal Gregosa1,2, Melisa Bentivegna1,2, Melina Bellotto1,2, Nicolas Gonzalez Perez1,2, Angeles Vinuesa1,2, Juan Beauquis1,2, Valeria Levi1, Mariano Soiza Reilly3, Gabriel A. Rabinovich2 Flavia Saravia1,2
1 Depto Quimica Biologica FCEN UBA
2 IBYME CONICET
3 IFIBYNE UBA-CONICET

Cerebral amyloid angiopathy (CAA) is caused by amyloid perivascular deposition. In Alzheimer's Disease (AD), CAA is found in arteries, arterioles and capillaries, being detrimental to their function and correlating with disease progression. Clinical interventions would benefit from restoring vascular changes. Galectins, a family of β-galactoside-binding proteins, play key roles in regulating immune, neuronal and vascular circuits. The neuroprotective effects of Galectin-1 (Gal1) in a model of CNS autoimmune inflammation, led us to explore its potential role in AD. Tg PDAPPJ20 mice-CAA/AD model- were treated with recombinant Gal1 (rGal1; three weekly i.p. 9 injections; 100 ug). Vehicle-treated 12 months-old Tg-mice showed large amyloid deposition around vessels on the hilus of the hippocampus, a highly vascularized region affected early during AD. Notably, Tg mice treated with rGal1 (Tg-Gal1) exhibited a 35% decrease in amyloid deposition around vessels compared to Tg-Veh ($p<0.05$) without vascular alterations. Clearance of ss-amyloid from the brain interstitium occurred mostly through the blood-brain barrier (BBB) and required astrocytic-vessel interaction, as the end feet wrapped around the vasculature contributed to vasomotion and fluid movement. While Tg-Veh mice showed a decrease in astrocyte feet-vessel contact compared to NTg-Veh mice, Tg-Gal1 mice showed marked recovery of the ensheathing of the vasculature, assessed by lectin staining and GFAP IF analysis. Also, recovery of the specific endfeet protein Aquaporin-4 in Tg-Gal1 ($p<0.02$ vs Tg-Veh) was verified by quantitative analysis of AQP4-lectin associations using array tomography. To investigate the mechanistic basis of this effect, we assessed BBB integrity with Evans blue. Tg-Gal1 mice showed lower vascular permeability to this dye compared to Tg-Veh mice ($p<0.05$). Our results support a central role for Gal1 in restoring BBB properties and endothelium-astrocytes communication in the hippocampus of AD mice.

448. (711) **NEUREGULIN-1 MODULATES LPS-INDUCED MICROGLIAL ACTIVATION**

Julia Ticchi, Andrea De Laurentiis, Fernando Correa.
Centro de Estudios Farmacológicos y Botánicos, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (UBA/CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

Microglial cells are one of the main effectors of the innate immune response in the CNS. Excessive microglial activation leads to CNS damage due to excessive production of pro-inflammatory mediators. Several studies have revealed that neuregulins (NRGs) are involved in brain function and psychiatric disorders. It has also been demon-

strated that microglia express NRGs, and their levels are markedly increased in activated microglia. Furthermore, it has been shown that Rac1, a downstream mediator of the Nrg1 signaling pathway, plays a central role in the inflammatory response and microglial neurotoxicity in the CNS. Objectives: To study the possible effects of Nrg1 on BV2 microglial cells in a model of inflammation induced by bacterial lipopolysaccharide (LPS) and to characterize the signaling pathways involved in the modulation of the microglial response.
Material and Methods: Murine microglial cell line BV2 cultures were treated with LPS and/or Nrg1. Next, proteins were collected to determine Rac1 activation optimal concentration /exposure time to LPS, as well as the optimal concentration of Nrg1. Total proteins were collected for signaling pathway analysis and supernatants were also collected to study cytokine production. Results: We determined that the optimal concentration of LPS, which was 100 ng/ml ($p<0.05$) and the optimal time of treatment, which was 15 minutes ($p<0.05$) for Rac1 activation. Furthermore, we established that the optimal concentration of Nrg1 in the activation of Rac1 in the microglia cell line BV2 is 50 ng/ml ($p<0.05$). We observed that Nrg1 interferes with the LPS-induced increased production of IL-6 ($p <0.05$). Preliminary results show that the *in vitro* treatment with Nrg1 enhances the LPS-induced activation of Rac1 and ERK1/2 MAPK signaling pathway.
Conclusion: In the cell line BV2, Nrg1 modulates LPS-induced microglia activation by downregulating IL-6 production, possibly by targeting Rac-1 and ERK1/2 MAPK signaling pathways.

449. (819) **PRODROMAL TIMELINE OF A PARKINSON'S DISEASE MODEL: ANXIETY-LIKE BEHAVIOR PRECEDES COGNITIVE AND MOTOR IMPAIRMENT**

Leandro Gabriel Champarini, Macarena Lorena Herrera, Matías Javega Cometto, Aracely Naranjo Viteri, Rosana Crespo, Gastón Diego Calfa, Claudia Beatriz Hereñú
Instituto de Farmacología Experimental Córdoba (IFEC-CONICET) - Departamento de Farmacología. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Parkinson's disease results from a progressive dopaminergic neuronal loss, characterized by multiple motor and non-motor symptoms. Currently, its diagnosis is based on motor deficits, but there is also a variety of previous and coexisting non-motor symptoms that result from dysfunction of interconnected systems. We aimed to determine, in a rat model of neurotoxicity, the progression of deficits on cognitive tasks and anxiety-like behavior before the onset of motor impairment. Animals, at day 0, were injected with 6-OHDA or with vehicle solution into the dorsolateral striatum (experimental or control groups, respectively). Independent groups of rats were tested only once in a behavioral task after 1, 2 and 3 weeks post lesion (Contextual fear conditioning, Y-maze, elevated plus maze, light-dark box test and locomotor activity test). We observed anxiety-like behavior 2 and 3 weeks post lesion. We observed working memory impairment in 6-OHDA rats after 3 weeks of neurodegeneration, without motor alterations. These results could be associated with a partial lesion of the nigrostriatal DA system as we found a decrease in tyrosine hydroxylase immunoreactivity in substantia nigra, ventral tegmental area, and striatum. We concluded that a single bilateral infusion of 6OHDA induced anxiety-like behavior before cognitive alterations that preceded locomotor deficits in the employed dopamine-depleted animal model.

450. (869) **EVALUATION OF DRUG SEEKING BEHAVIOUR ON NICOTINE PLACE PREFERENCE IN ZEBRAFISH**

L. Rocco, A. Pisera-Fuster, M.P. Faillace, R. Bernabeu.
Department of Physiology and Institute of Physiology and Biophysics (IFIBIO-Houssay, UBA-CONICET), School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

Seeking of drugs is commonly evaluated in a specific environment for assessing drug preference. However, cognitive strategies involved in drug seeking are mostly unknown. To assess the strength of environmental cues that can be associated with nicotine in the zebrafish brain reward circuitry, we have designed herein a modified conditioned place preference (CPP) paradigm. This task was devised to identify salient environmental cues relevant for strong nicotine–environment association and drug seeking induction. During test sessions, background colors of the CPP tank chambers were shifted and preference for colors associated to nicotine was assessed. We have compared several tank designs and different compartment colors. Our findings indicated that zebrafish seeking behavior was strongly dependent on compartment color shades. Combination of red and yellow environments, which were preferred and avoided compartments, respectively, was the most effective design presenting the highest CPP-score. Interestingly, animals that stayed for longer periods in the environment conditioned to nicotine during a first testing interval were also able to follow the background color shade conditioned to nicotine to the other compartment immediately after background colors were relocated between compartments. During a second testing period, zebrafish also stayed for longer periods in the colored compartment paired to nicotine during conditioning. These findings suggest that under salient environmental conditions, zebrafish voluntarily followed a shifting visual cue previously associated with nicotine delivery. Furthermore, our findings indicate that zebrafish exhibit spatial associative learning and memory, which generates a repertoire of conspicuous locomotor behaviors induced by nicotine preference in the CPP task.

451. (908) **MATERNAL POLYPHENOL SUPPLEMENTATION AS A PREVENTIVE STRATEGY OF PERINATAL BRAIN INJURIES**

Joana Antonela Asensio[1,2], Victoria Muscia Saez[2], Rodrigo Damián García[2], Alicia Mabel Seltzer[1], Marcela Vazquez Prieto[2].
1) Laboratorio de Lesiones Perinatales, Instituto de Histología y Embriología de Mendoza (IHEM) - CONICET, Mendoza. 2) Laboratorio de Nutrición y Fisiopatología de la Obesidad, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo; Instituto de Medicina y Biología Experimental de Cuyo (IMBECU) – CONICET, Mendoza.

Injuries to the developing nervous system during the fetal period constitute the main cause for motor and intellectual disabilities. Intrauterine growth restriction (IUGR) is a condition in which a fetus fails to achieve its genetically determined growth potential and, therefore, the fetus is born with malnutrition and neurological impairment. This pathology is primarily associated with an impaired supply of nutrients and oxygen and a deteriorated antioxidant defense system. There is increasing evidence supporting that polyphenols target multiple inflammatory components and lead to anti-inflammatory mechanisms. The aim was to investigate the protective effect of maternal dietary supplementation with grape pomace extract (GPE), rich in polyphenols, on the alterations in the physical, neurological and motor development of the offspring of spontaneously hypertensive rats (SHR) as a model of IUGR. Wistar Kyoto were used as normotensive controls. Female rats (6 rat/group) were fed during 12 w either: control diet (SHR) or control diet supplemented with 300 mg GPE/kg body weight (SHR+GPE). GPE was administered 6 w prior, during and 3 w after pregnancy. Pups were evaluated from week 9 to 12 for physical, neurological and motor milestones through a battery of tests. Our results indicate that SHR pups born from GPE-fed mothers presented an improvement in weight curve compared to SHR control pups. Eye opening, ear unfolding and auditory startle milestones appeared earlier in SHR+GPE pups compared to SHR. In addition, systolic and diastolic blood pressure showed a significant decrease in SHR+GPE pups at 35 postnatal days. Overall, maternal dietary intervention with bioactive compounds could be a promising strategy for the prevention of perinatal brain injuries and the developmental delay observed in SHR pups. PICT 2018-03056.

452. (915) **DIFFERENCE IN COGNITIVE PERFORMANCE IN MIDDLE AGED FEMALE AND MALE MCGILL-R- THY1-HAPP TRANSGENIC RAT MODEL OF ALZHEIMER DISEASE**

MV Oberholzer#1, NC Colettis#1, M Habif#1, MJ Russo2, A. Josiowicz1, RF Allegri2,
AC Cuello3, DA Jerusalinsky*1
*#same contribution. *corresponding author*

1LaNyN, IBCN, Fac. de Medicina, UBA; 2FLENI y Fac. de Medicina; 3Dept Pharmacol. & Therapeutics, McGill Univ. Montreal, Canada

While characterizing learning and memory in heterozygous McGill-R-Thy1-APP transgenic (Tg) Wistar rat model of Alzheimer disease (AD) with a transgene of human amyloid precursor protein-751 (APP751) with *Swedish* and *Indiana* mutations of familial AD (Leon et al., 2010), we observed differences between females and males. Open field: was used to assess spontaneous exploration and habituation to the environment. 12 month-old (mo) female and male wt and Tg rats showed similar performances in the 1st OF session. 24 h later, exploratory parameters were significantly lower in Tg and wt rats, indicating that they recognized and habituated to the environment, consolidating a long-term memory (LTM). Novel object recognition assesses rat's capacity to discriminate objects. 1h after training with two identical objects (A) and (A') rats were presented with (A) and a novel (B). Rats spent significantly longer exploring (B) than (A), expressing a short- term memory (STM). 24h later, they were presented with (A) and a novel (C). Wt rats spent longer exploring (C) than (A), denoting LTM formation, while there were not significant differences for Tg rats, regardless of sex. 24 h later, rats were tested for object location. (A) remained whereas (A') was moved to a new location. Only wt females denoted spatial discrimination and LTM of the previous configuration, while wt males and Tg rats did not.
Inhibitory avoidance: Each rat was left in a light compartment; when entering a dark one, it gets a mild foot-shock; latency to enter was recorded. 24 h later, latencies were significantly higher for wt females and males, and Tg females, though not for Tg males that did not form/express this associative LTM. 14 days later, latency was significantly higher for wt females, though not for wt males nor for Tg rats. Our results showed a sexual dimorphism in associative memory at middle age, emphasizing the relevance of sex while assessing cognitive functions.

NEUROSCIENCES III

Saturday, November 19, 9-10:30 hr
Chairs: Claudia Bregonzio - Flavia Saravia - Alicia R. Rossi

453. (65) **ALTERATIONS OF THE EFFERENT SYSTEM IN OUTER HAIR CELLS OF A MOUSE MODEL OF HEARING LOSS**

Ezequiel Rías[1,2], Santiago Simone[1,3], Camila Carignano[1,2], Sofía Stupniki[1,2], Marcela Vera[1,2], Guillermo Spitzmaul[1,2], Leonardo Dionisio[1,2].
[1]Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), CONICET-UNS. [2]Departamento de Biología, Bioquímica y Farmacia, UNS. [3] Departamento de Ciencias de la Salud, UNS.

KCNQ4 is a voltage-gated potassium channel responsible for extruding K^+ from the outer hair cells (OHC) after sound stimulation. OHC excitability is under the control of the efferent pathway mediated by the Medial Olivocochlear (MOC) system. In response to overstimulation, this system activates the nicotinic acetylcholine receptor (nAChR) α9α10, which triggers calcium-dependent K^+ channels (BK and SK2) activation, increasing K^+ cell permeability, and helping KCNQ4 to restore the membrane potential. KCNQ4 absence leads to intracellular accumulation of K^+ and chronic depolarization that may damage hair cells, causing hearing loss. We hypothesized that the lack of KCNQ4 in mice (KO) affects the organization and function of the MOC system, impacting the hearing process. Using confocal imaging, we evaluated the MOC terminals contacting OHC at two ages: when the auditory system is finishing maturation (2 weeks old (W)) and when it is fully developed (4W). At the mature age, the MOC terminals are located exclusively in the basal domain of OHC in wild-type (WT) animals. At 2W, both genotypes showed the same percentage (~50%) of synaptic contacts located in the lateral domain. Later on, terminals were relocated to the basal membrane in WT while ~32% of them remain in the lateral domain in KO animals at 4W. Moreover, we detected a decrease in the number of synaptic contacts per OHC in 4W KO mice. The volume of the synaptic terminals did not change among genotypes at any age. On the other hand, we analyzed by qPCR the gene expression of the postsynaptic efferent components located in the MOC synapse. The mRNA expression of α10 decreased ~3.5-fold with no changes in the α9 subunit, while BK and SK2 mRNA decreased ~8-fold in 4W KO animals. These results demonstrate that chronic depolarization of OHC impairs the maturation process of the efferent synaptic innervation and the expression of its components in OHC, altering cell function and contributing to hearing detriment.

454. (99) **PROGESTERONE MODULATES GLIAL CELL REACTIVITY AND Maintains THE MEMBRANE expression OF NEURONAL COTRANsPORTER KCC2 IN INJURED SPINAL CORD: IMPLICATIONS FOR THE DEVELOPMENT OF SPASTICITY**

Sol Ferreyra[1], Mariana Rey[2], Florencia Labombarda[3,4], Alberto Yorio[5], Héctor Coirini[2], Susana Gonzalez [1,3]
[1]Laboratorio de Nocicepción y Dolor Neuropático, IBYME-CONICET; [2]Laboratorio de Neurobiología, IBYME-CONICET; [3]Facultad de Medicina, UBA; [4]Laboratorio de Bioquímica Neuroendócrina, IBYME-CONICET; [5]Laboratorio de Biología del Comportamiento, IBYME-CONICET

Spinal cord injury (SCI) triggers spinal glia reactivity that contributes to decreased expression/activity of KCC2-a neuronal transporter involved in chloride homeostasis-thus promoting the development of spasticity, a common and difficult-to-treat disorder. We have shown that progesterone (PG), a neuroactive steroid, improves functional outcomes and prevents injury-induced neuropathic pain. Here we studied whether PG may modulate acute neuroinflammatory events related to KCC2 dysfunction and long-term alterations in the frequency-dependent depression of Hoffman (H) reflex, a tool to assess spasticity. Male rats (SD) were subjected to spinal transection at T13 level and received daily PG (16 mg/kg sc, n=18) or vehicule (SCI, sc n=18). Uninjured rats were used as control (C, n=18). Lumbar spinal cord was obtained 3 days post-injury. The mRNA levels of astrocytic (Cx43, S100A10) and microglial (iNOS, Arg1, CD206, TGF-β) markers and KCC2 were determined by real time RT-PCR. KCC2 protein was evaluated in membrane fractions by Western blot. The amplitud of the H wave (μvolts), achieved by electrical stimulation of the tibial nerve, was calculated at 1, 4 and 8 Hz to determine frequency depression of H-reflex. PG decreased the injury-induced mRNA expression of iNOS ($p<0.01$ vs SCI) and Cx43 ($p<0.05$ vs SCI), associated with neurotoxic events, maintained the high levels of Arg1 ($p<0.001$ vs C), CD206 and TGF-β ($p<0.05$ vs C), and increased S100A10 ($p<0.05$ vs C, $p<0.05$ vs SCI) all related to protective glial phenotypes. PG preserved the protein expression of KCC2 at the plasma membrane ($p<0.01$ vs SCI), but did not change its mRNA levels. Also, PG restored rate-dependent depression of H-reflex (at 4 and 8Hz, $p<0.001$ vs. SCI), which was impaired in SCI animals ($p<0.001$ vs C). Our findings add new evidence to support the use of progesterone-based therapies and open novel translational perspectives for treating SCI-induced spasticity (PICT 2152; PIP 266).

455. (118) **BRAIN IRRIGATION IN THE SOUTH AMERICAN PLAINS VIZCACHA. SPECIES SPECIFIC MORPHOLOGICAL PECULIARITIES SUPPORT THE IMPORTANCE OF EXPANDING THE STUDY OF BRAIN ANATOMY AMONG SPECIES**

Alejandro Raúl Schmidt[1,3], Pablo Ignacio Felipe Inserra[1,3], Mariela Giacchino[1], Sergio Ferraris[2], Fernando Lange[2], Julia Halperin[1,3], Alfredo Daniel Vitullo[1,3], Verónica Berta Dorfman[1,3].
1 Centro de Estudios Biomédicos Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides, Ciudad Autónoma de Buenos Aires, Argentina. 2 Centro de Ciencias Veterinarias (CCV), Universidad Maimónides, Ciudad Autónoma de Buenos Aires, Argentina. 3 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

The neurovascular flow supplies the brain with oxygenated blood

and nutrients to satisfy its metabolism, and the circle of Willis (CoW) provides more than 80% of the flow required. It serves as a safety system in case of arterial occlusion. Considering that cerebral irrigation participates in different neuropathological processes, different animal models are explored to study *in situ* the neurovasculature distribution and function. The aim of the present work was to characterize the irrigation topology (distribution and number of the arteries) of the CoW of the vizcacha, *Lagostomus maximus*, and to compare it with other hystricomorphs and myomorphs species. Six adult vizcachas were studied using encephalic vascular cast with latex and angiography. A caudo-rostral flow direction was determined, beginning in the spinal and vertebral arteries, and converging in the basilar artery that irrigates the caudal region of the CoW. Both caudal communicating arteries are connected by the rostral communicating artery shaping the CoW. From this, the rostral cerebral arteries irrigate the frontal lobes, while the blood flow from the carotid system supplies the hypothalamus and the lateral lobes. High anatomical similarity in the CoW with small particularities, that escape of the convergent evolution exerted on the structure, was determined among study species. The topology observed in vizcacha could be considered as a morphological possibility that can be found throughout the phylogeny, not an evolutionary adaptation per se. The topological peculiarities of the CoW such as the absence of the caudal or rostral communicating artery, compared among the evaluated related species, supports the need for a revision of its classic function as a security system. Finally, this work supports the importance of expanding our understanding of brain anatomy among species which specific peculiarities may contribute to a better understanding of the functional neuroanatomy. Grants: FCFF, PIP036.

456. (121) **NEUROPATHOLOGY AND COGNITIVE DYSFUNCTION IN A RAT MODEL FOR METABOLIC SYNDROME**

Santiago Ronchetti [1], Florencia Labombarda [1,2], Paulina Roig [1], Alejandro F. De Nicola [1,2], Luciana Pietranera [1,2]

1 Laboratorio de Bioquimica Neuroendocrina, Instituto de Biología y Medicina Experimental (IBYME)-CONICET

2 Depto de Bioquimica Humana. Facultad de Medicina UBA

Metabolic Syndrome (MS) is the medical term for the combination of at least three of the following factors: obesity, hyperlipidemia, hyperglycemia, insulin resistance and hypertension. The spontaneously hypertensive rat (SHR) is an accepted animal model for the study of human MS that reveals all the features of the syndrome when fed high-fat, high-carbohydrate diets. The intake of high-fat diets in rats has been shown to produce brain neuropathology. In humans, MS has been shown to increase the risk of cognitive impairment, dementia, and Alzheimer's disease. In order to characterize the neuropathology and cognitive dysfunction of MS we fed SHR with a high-fat diet (4520 Kcal/kg) along with a 20% sucrose solution to drink. We also evaluated SHR and Wistar-Kyoto (WKY) fed with standard diet. At the end of the experiment SM rats displayed a significant increase in body weight, BMI and AC/TC ratio compared to SHR rats fed a normal diet. We also found increased in fasting glucose levels and in the OGTT in SM rats. Blood pressure was significantly higher in both SHR and SM rats when compared to WKY. SM rats present high blood triglyceride levels We also measured the expression of IBA1+ microglia in the prefrontal cortex (PFC) and the hippocampus (HC) by immunohistochemistry and classified microglia according to its morphology. We found that while ramified microglia predominated in normotensive rats, SM rats presented an increased proportion of the hypertrophied phenotype. Furthermore, we evaluated hippocampal dependent memory using the novel object recognition test (NOR) and found SM rats performed poorly when compared to WKY In conclusion, SHR rats fed a high-fat and high-sucrose diet developed all the characteristics of MS together with neuropathological alterations and cognitive impairments. These results present this as an interesting model for the study of treatments to alleviate neuropathological and cognitive alterations associated with MS

457. (198) **EFFECT OF EARLY LIFE STRESS ON THE VISUAL SYSTEM IN ADULT MICE**

Ruth E. Rosenstein[1], Hernán H. Dieguez[1], Nathaly Bernal Aguirre, Damian Dorfman[1], Juan S. Calanni[1]

[1]Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine/CEFyBO, University of Buenos Aires/CONICET,

Early life stress is defined as a period of severe and/or chronic trauma, as well as environmental/social deprivation or neglect in pre/postnatal stage. Presently, the impact of ELS on the visual system in the adult stage is unknown. Using an animal model of maternal separation with early weaning (MSEW), which mimics early life neglect, we analyzed the long-term ELS consequences in the visual system. Mice were separated from the dams for 2 h at postnatal days (PNDs) 2-5, for 3 h at PNDs 7-9, for 4 h at PNDs 10-13, for 6 h at PNDs 14-16, and weaned at PND17. At the end of each separation period, mothers were subjected to movement restriction for 10 min. Control pups were left undisturbed from PND0, and weaned at PND21. At PND 65-70, MSEW did not affect the body weight or the electroretinogram a- and b-wave amplitude, but significantly decreased scotopic threshold response (STR, $P<0.01$), photopic negative response (PhNR, $P<0.01$), and visual evoked potential amplitude ($P<0.01$). MSEW did no change the thickness of retinal layers, but significantly decreased synaptophysin content (assessed by immunohistochemistry and Western blot, $P<0.05$), and Brn3a(+) retinal ganglion cell (RGC) number (assessed by immunohistochemistry and Western blot, $P<0.01$). Moreover, MSEW significantly increased Iba-1(+) area, and Iba-1(+) cell soma size (by immunohistochemistry, $P<0.01$), consistently with an increased numbers of amoeboid microglial cells. MSEW increased plasmatic corticosterone levels at PND10 ($P<0.01$). Mifepristone (injected every 3 days between PNDs 4-16) significantly prevented the effect of MSEW on STR ($P<0.01$), and PhNR amplitude ($P<0.01$), and preserved RGC Brn3a(+) cell number, Iba-1(+) area, and Iba-1(+) cell soma size ($P<0.01$). These results suggest that retinal alterations might be included among the childhood adversity-induced threats to life quality, which could be prevented by an early pharmacological intervention with mifepristone.

458. (213) **SPATIAL MEMORY AND SOCIAL OLFACTORY IMPAIRMENT REVEAL PREMATURE AGING EVOKED BY PERINATAL PROTEIN MALNUTRITION**

Nadina Ferroni[1], Camila Mimura[1], Eliana Rozowykwiat[1], Leandro Urrutia[2], Germán Falasco[2] Eduardo T. Cánepa[1] and Silvina V. Sonzogni[1]

[1] Laboratorio de Neuroepigenética, Departamento de Química Biológica, Universidad de Buenos Aires, and IQUIBICEN Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad de Buenos Aires, Argentina

[2] Centro de Imágenes Moleculares, Fleni, Escobar, Buenos Aires, Argentina.

Early-life adversity, like protein malnutrition, increases the vulnerability to develop long-term effects on brain structure and function. The aim of this work is to study if perinatal protein malnutrition (PM) leads the occurrence of premature aging in a murine model and the molecular mechanisms involved. Mice dams were fed with normal (NP, casein-20%) or low protein diet (LP, casein-8%) during gestation and lactation. Female offspring were evaluated at the ages of 2, 7 and 12 months (M) in cognitive and olfactory skills. Also, positron emission tomography (PET), cellular senescence, and altered gene expression were studied as potential causes of accelerated aging in the hippocampus (HC) and olfactory bulb (OB). The statistical analysis was performed by general linear model (GLM), the fixed effects were Diet, Age and Diet*Age interaction and T-tests. p-values lower than 0.05 were considered statistically different. We found that PM impairs spatial memory and that coincide with altered glucose metabolism and sirt7 upregulation in the HC. When senescence was evaluated, we observed a higher senescence-associated β-galactosidase activity and p21 expression in LP-12M-old mice. We also found alterations in hippocampal neurogenesis in LP 12M-old mice, they show a higher number of newborn neurons that cannot end the maturation process. Regarding the olfactory system, the social-odor discrimination was impaired along life in LP mice which coincides with an increase in glucose metabolism. In the OB, the senescence marker p21 was upregulated in LP mice and Sirt2 and Sirt7 were

downregulated. Also, LP mice showed a higher number of newborn neurons in the subventricular zone at 2M which then recovers to normal values. Together, our results show that perinatal PM causes long-term impairment in cognitive and olfactory skills through an accelerated senescence phenotype, an increased glucose metabolism and altered sirtuins expression in the HC and OB.

459. (231) **BEHAVIORAL, COGNITIVE AND HISTOPATHOLOGICAL ASPECTS OF PATIENTS WITH DRUG-RESISTANT TEMPORAL LOBE EPILEPSY WHO UNDERWENT EPILEPSY SURGERY**

Micaela Sanzo[1], Fausto Calella[1], Lucas Orlando Quiiroles[1], Mats Snijders[1], Patricia Solís[3], Hector Konopka[2], Pablo Seoane[2,3], Silvia Oddo[2,3], Laura Ruth Guelman[4], Luciana D´Alessio[1,2].

1. Universidad de Buenos Aires, Facultad de Medicina, IBCN-CONICET

2. Universidad de Buenos Aires, Centro de Epilepsia, Hospital Ramos Mejía

3. Hospital del Cruce, ENYS-CONICET

4. Universidad de Buenos Aires, Facultad de Medicina, CEFYBO-CONICET

Introduction: The histopathological compromise of the temporal pole in patients with resistant temporal lobe epilepsy (TLE) and hippocampal sclerosis (HS) may be associated with behavioral and cognitive symptoms. The aim of this study was to analyze histological markers and to correlate with clinical, cognitive and behavioral variables. Methods: Histological samples of temporal cortex (pole), obtained from patients with resistant TLE who underwent standard temporal lobectomy, were included. Samples were studied with immunohistochemistry (immunoperoxidase) with antibodies Anti-NeuN, Anti-GFAP and Anti-Calbindina (CB). In addition, the clinical records of the presurgical protocol were reviewed, including clinical and complementary studies (VEEG, MRI), cognitive studies (Rey Complex Figure, Rey Auditory Verbal Learning), behavioral assessment (SCID I - DSM IV, depression Beck Inventory, EJE V). Nonparametric statistic tests were performed. Results: Twenty-five patients with resistant TLE and HS were included. Five of them presented a temporal pole cortex compromise in the MRI (*blurring* or HS plus; focal cortical dysplasia IIIa*)*. Eight posmortem samples, matched by age and sex without pathology, were included. We found a higher density of CB+ neurons ratio in layer II of patients with *blurring* compared to controls (p=0,047, Kruskal Wallis). In patients with TLE (n=25), the density of glia nuclei showed a significant correlation with the epilepsy time duration (r= - 0,48, p=0.03). In addition, the neuronal density negatively correlated with the global functionality scale (Axis V of DSM IV) (r= - 0,41; p=0,014) (Spearman). Conclusion: In this exploratory study a higher density of CB+ neurons was associated with MRI blurring. Clinical and behavioral symptoms correlated with histopathological changes of the temporal pole. Further studies should be made to confirm these preliminary findings.

460. (369) **MODULATION OF ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR BY CANNABIDIOL**

Juan Facundo Chrestia, María del Carmen Esandi, Cecilia Bouzat

Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Departamento de Biología, Bioquímica y Farmacia (DBByF), Universidad Nacional del Sur-Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET).

Cannabidiol (CBD), an important terpenoid compound from marijuana with no psychoactive effects, has become of great pharmaceutical interest for several health conditions. As CBD is a multitarget drug, there is a need to establish the molecular mechanisms by which CBD may exert therapeutic as well as adverse effects. The α7 nicotinic acetylcholine receptor is a cation-permeable ACh-gated channel present in the nervous system and in non-neuronal cells. It is involved in different pathological conditions, including neurological and neurodegenerative disorders, inflammation, and cancer. The aim of this work is to study α7 as one of the therapeutic targets of CBD in order to determine the mechanism by which the phytocannabinoid exerts its effect on the receptor. Wild-type and mutant α7 receptors were expressed in mammalian cells. To evaluate receptor ionotropic function, high-resolution single channel recordings in cell-attached and inside-out patch configurations were used. Changes in intracellular calcium levels were measured by confocal microscopy. We here reveal that CBD modulates α7 ionotropic and metabotropic functions. CBD leads to a concentration-dependent decrease of α7 single-channel activity with an IC_{50} of 0.54 ± 0.13 μM. The inhibition of α7 activity, which takes place through a membrane pathway, is neither mediated by receptor phosphorylation nor overcome by positive allosteric modulators and is compatible with CBD stabilization of resting or desensitized α7 conformational states. At the cellular level, CBD inhibits the increase in intracellular calcium triggered by α7 activation, thus decreasing cell calcium responses. The modulation of α7 is of pharmacological relevance and should be considered in the evaluation of CBD potential therapeutic uses. Thus, our study provides novel molecular information of CBD multiple actions and targets, which is required to set the basis for prospective applications in human health.

461. (404) **THE VOLATILE HALOGENATED ANESTHETIC SEVOFLURANE INHIBITS MICROTUBULE-GENERATED ELECTRICAL OSCILLATIONS IN THE HONEYBEE BRAIN**

Brenda C. Gutierrez, Horacio F. Cantiello, and María del Rocío Cantero

Laboratorio de Canales Iónicos, Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD, CONICET-UNSE), Santiago del Estero, Argentina (4206).

Microtubules (MTs) are highly conserved cytoskeleton structures associated with information transfer within neuronal processes. Recent electrophysiological studies demonstrated that different assemblies of brain MTs generate highly synchronous electrical oscillations (Cantero et al. *Sci Rep* 2016, 2018). To further explore the MT electrical activity of the brain, we applied the patch clamp technique on MT sheets and brains obtained from the honeybee (*Apis mellifera*), as recently reported (Gutierrez et al. *Front Mol Neurosci* 2021). High resistance seal patches of MT sheets showed electrical oscillations that linearly depended on the holding potential between ±200 mV and had an average conductance of 9.2 ± 0.3 nS (n = 14). To observe these oscillations in the context of the brain, we further explored local field potentials (LFP) in the Triton X-permeabilized whole honeybee brain, unmasking spontaneous oscillations after but not before tissue permeabilization. In both preparations, the frequency domain spectral analysis of time records indicated at least two fundamental peaks at ~38 Hz and ~93 Hz. To evaluate the effect of halogenated ether anesthetics on the electrical properties of MTs, we tested the inhalational anesthetic sevoflurane used in the clinic. The addition of sevoflurane (12.5% v/v) had an inhibitory effect on high seal patches of bee brain MT sheets, reducing electrical oscillations by 69.4 ± 16.1% (n = 7). Whole brain electrical oscillations were also reversibly inhibited by the addition of sevoflurane (n = 3). The present data indicate that the electrical activity of MTs provides a novel signaling mechanism in the honeybee brain that targets volatile anesthetics and may be implicated in the brainwave oscillations observed in the insect brain.

462. (498) **PERSISTENT ASTROGLIAL DNA METHYLATION AND DOWNREGULATION OF HOMEOSTATIC GENES IN A LITHIUM- PILOCARPINE MODEL OF TEMPORAL LOBE EPILEPSY (TLE)**

Gomez DD1, Rossi A1, Villarreal A1, D´Alessio L, Ramos AJ1

1. IBCN UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires

Retrospective studies in TLE patients have shown the common feature of an initial precipitating injury (IPE) in early childhood, usually complex febrile with Status Epilepticus (SE). The IPE is usually followed by a silent period of years until chronic epilepsy emerges. We have previously shown extensive neuroinflammation and reactive astrogliosis in this silent period in the Li-pilocarpine rodent model of TLE (Rossi et al, 2013; 2017). Here, we studied if extensive astrogliosis induce epigenetic alterations in DNA methylation and homeo-

static genes expression. Adult male Wistar rats (220-240 g) were treated with 127 mg/kg LiCl and 18 h later they received 30 mg/kg pilocarpine ip. Status Epilepticus (SE, IPE) were allowed to persist for 30 min and then seizures were stopped by 10 mg/kg diazepam. At different times during the latency period (7, 21 or 35 DPSE, days post SE), the abundance of 5 methyl Cytosine (5mC) in the astroglial DNA and the expression of several astroglial homeostatic genes (Kir4.1, aquaporin 4 (AQP4), glutamine synthetase (GS), In addition, we analyzed by RT-qPCR the changes in the expression of DNA methyl transferases DNMT1 and DNMT3a. Level of astroglial 5mC was also studied in hippocampal sections of surgical resections of TLE patients. We observed a global hypermethylation in astrocytes, accompanied by increased expression of DNMT1/DNMT3a and a reduction in the expression of GS, Kir4.1 and AQP4 at 7, 21 and persisted until 35 DPSE. Reactive astrogliosis and increased 5mC in astrocytes were also observed in the hippocampal sections of TLE patients. We conclude that astrocytes show persistent downregulation of homeostatic genes during the latency period that follows an experimental IPE. This correlates with increased 5mC in the DNA that may be responsible for the homeostatic gene repression. Supported by grants: PICT 2017-2203; UBACYT; PIP CONICET 479

463. (586) **MODULATORY EFFECT OF DIAZEPAM ON CHRONIC EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: NEUROINFLAMATION AND BEHAVIORAL STUDIES**

María C. Fabio[a,b]; María Inés Zalosnik[a]; Carmela Felippa Ambort[a] and Alicia L. Degano[a]

[a]Departamento de Química Biológica "Ranwel Caputto", Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina; [b] Instituto de Investigación Medica Mercedes y Martin Ferreyra, Cordoba, Córdoba, Argentina.

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease that mimics many of the pathological features of multiple sclerosis (MS). The aim of the present study was to analyze the effects of Diazepam (Dz) on EAE clinical signs, neuropathology and behavior in a chronic model of the disease. 9-weeks old mice were immunized with MOG35-55 peptide (EAE group) or adjuvant alone (CFA group) and pertussis toxin. Beginning the day of the clinical onset (9-12 dpi), CFA-Dz and EAE-Dz groups were injected i.p. with Dz (2 or 4 mg/kg) every 48 hs. After the acute period (26-30dpi), mice were exposed to a battery of behavioral tests in order to assess motor skills, anxiety, and cognitive deficits. Neuropathology and inflammatory markers (Iba-1, GFAP, TNFα) were analyzed in spinal cord and hippocampus by IHC and real-Time RT-PCR. Experimental designs were factorial 2 [Condition (EAE vs CFA)] x 2 [Treatment (Saline vs Dz)]. All data was analyzed using two-way ANOVA, followed by Tukey test, when appropriate. We found that 2 mg/kg Dz significantly ameliorated clinical signs of the disease ($p<0,05$; EAE-Dz vs EAE) and cellular infiltration in spinal cord (*$p<0,05$; EAE-Dz vs EAE). Chronic administration of Dz did not affect locomotor activity and anxiety behavior in any group. Cognitive deficits persisted in both EAE and EAE-Dz mice in chronic stages, while EAE-Dz mice showed a significant reduction of gliosis and inflammatory markers in the hippocampus (Iba-1, GFAP, TNF; $p<0,05$; EAE-Dz vs EAE). Interestingly, the expression of the benzodiazepine peripheral receptor TSPO, was lower in hippocampus from both Dz-treated groups ($p<0.01$; CFA/EAE vs CFA-Dz/EAE-Dz), suggesting TSPO might play a role in the effects induced by Dz. Our present results show that, at the dose and schedule used here, Dz modulates clinical EAE without generating side effects in animal behavior. These results may have important implications for new therapeutic applications in MS.

464. (615) **TRANSFERRIN-LOADED EXTRACELLULAR VESICLES INTRANASALLY ADMINISTERED INDUCE REMYELINATION IN CUPRIZONE-DEMYELINATED MICE**

Mattera VS [1], Correale JD [2] ,Pasquini JM [1].

1 Departamento de Química Biológica e Instituto de Química y Fisicoquímica Biológicas (IQUIFIB). Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires y CONICET. 2.Instituto de Química y Fisicoquímica Biológica (IQUIFIB) y FLENI. Buenos Aires, Argentina.

Extracellular vesicles (EVs) are involved in diverse cellular functions, playing a major role in cell-to-cell communication not only in physiological conditions but also under pathological scenarios. In this sense, these particles are powerful mediators of information representing a promising therapeutic strategy. Oligodendrocytes (OLs) are specialized myelinating glial cells, which are mainly damaged in chronic demyelinating diseases such as multiple sclerosis (MS). Most myelin-forming OLs are developed from oligodendrocyte precursor cells (OPCs) in the Central Nervous System (CNS) and the glycoprotein transferrin (Tf) plays a critical role in iron homeostasis and delivery within the brain and has pro-differentiating effects on oligodendroglial cells both in *vitro* and *in vivo* when is intracranially administered. These data led us to search for a less invasive and controlled technique to deliver Tf to the CNS. In previous work, we were able to successfully isolate and load EVs from mouse and human plasma with Tf (EVTf) through the binding to its receptor TfTR1, contained in the outer membrane of the EV. In the present study, EVs were isolated by size-exclusion chromatography, loaded with Tf, labeled with a lipophilic dye, or with the fluorescent cargo Tf texas red. The current study is focused to evaluate if Tf-loaded EVs can enter the OPC cell, the pathways involved in such process, and its intracellular fate by co-localization assays accessed by confocal microscopy. Furthermore, we studied the promaturating effect of EVTf in vitro and it's in vivo remyelinating effect through intranasal (IN) administration in CPZ-demyelinated mice, as assessed through different cell markers and myelin staining. Additionally, we evaluated the potential cargo protection given by the transport in EVs. The results demonstrated that EVTf increases the OLG differentiation in culture, as well as reduced the demyelination induced by CPZ in the CNS.

465. (640) **COGNITIVE AND MOTOR DYSFUNCTION IN zQ175 HUNTINGTON´S DISEASE MICE**

Julieta Bruno, Federico López Couselo, Diego Rivas, Mateo Palmieri, Julieta Saba, Lila Carniglia, Daniela Durand, Mercedes Lasaga, Carla Caruso.

INBIOMED, Instituto de Investigaciones Biomédicas UBA-CONICET. Facultad de Medicina, UBA. CABA, Argentina.

Huntington's Disease is a neurodegenerative genetic disorder caused by CAG repeat expansion in the huntingtin gene that generates motor, cognitive and psyquiatric symptoms in humans. In this work, our objective was to evaluate sex differences in cognitive and motor function of zQ175 knock-in (HD) and wild type (WT) mice at 4 or 8 months (M) of age. We tested their behavior by using the open field test, the gait analysis, and the novel object recognition (NOR) test. Results: In the open field test, HD female mice traveled less distance, moved more slowly ($p<0.001$) and expressed an anxiety-like behavior ($p<0.01$) compared to WT mice at 4 and 8M. Only HD male mice of 8M showed a decrease in the distance traveled ($p<0.01$) and velocity ($p<0.001$). Footprint analysis showed that HD female mice at 4M decreased their hindpaw ($p<0.01$) and forepaw stride length ($p<0.05$). At 8M they showed an increase in their hindpaw and forepaw stride length ($p<0.05$), and a reduction in their forepaw base ($p<0.05$). In contrast, HD male mice of 4M exhibit no differences in gait analysis but at 8 M showed an increase in the stride length and base forepaw ($p<0.05$). Finally, during NOR, HD mice showed a minor discrimination index at both ages than WT mice ($p<0.05$). Although total exploration time of the objects between HD and WT were similar, mice of 8M tend to decrease the total exploration time compared to mice of 4M. Conclusion: HD mice show cognitive and motor dysfunction. HD female mice present an earlier deterioration of motor coordination, whereas HD male exhibit later impairment compared to HD female mice.

466. (737) **PROCESSES LENGTHENING AND NEURONAL ADHERENCE ARE DEREGULATED IN CDK5-DEFICIENT HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS**

Mucci Sofía*, Rodriguez-Varela MS*, Allio CP*, Isaja L*, Fe-

rriol-Lafoulliere SL*, Sevlever GE*, Scassa ME* and Romorini L*.
** Laboratorio de Investigaciones Aplicadas a Neurociencias (LIAN), Instituto de Neurociencias (INEU-FLENI-CONICET), Escobar, Buenos Aires, Argentina.*

CDK5 is an essential molecule involved in neuronal survival and homeostasis, thus making the study of the consequences of CDK5 deficiency in a human neuronal model relevant. For this reason, we aim to analyze the differentially expressed genes in WT and KO-CDK5 (CRISPR/Cas9 edited) H9 human embryonic stem cells (hESCs)-derived neurons. Initially, we differentiated WT and KO-CDK5 H9 cell lines into neurons using a defined medium and validated their phenotype by immunostaining with neuronal *lineage-defining markers* (TUJ-1, MAP2, MAP5). Next, an RNA-seq analysis (n=3) was performed with RNA isolated from WT and KO-CDK5 hESCs-derived neurons. We found 920 differentially expressed genes between WT and KO-CDK5 hESCs-derived neurons by using the DESeq2 package in R Studio (alpha = 0.01). Through a GO analysis of biological processes, we found that among the differentially expressed genes 137 participate in neurogenesis and neuronal differentiation. Next, we proceed to the validation of 5 genes found differentially expressed by RNA-seq analysis by RT-qPCR. Then, we studied processes lengthening in WT and KO-CDK5 H9 hESCs-derived neurons. We found that KO-CDK5 neurons showed a marked increase in the length of their processes with respect to the WT counterparts. In addition, the adherence of KO-CDK5 deficient neurospheres to Geltrex pre-coated plates was remarkably reduced compared to WT neurospheres (10.40%±2.95 vs. 78.12%±7.37, respectively). In conclusion, although CDK5 deficiency did not impair neural differentiation of hESCs, its disruption altered the transcriptome of hESCs-derived neurons, reduced process lengthening and decreased cell adhesion.

467. (826) **NEUROANATOMICAL ANALYSIS OF NEURONAL SETS THAT CO-EXPRESS THE GROWTH HORMONE SECRETAGOGUE RECEPTOR AND THE CANNABINOID TYPE1 RECEPTOR IN THE MOUSE BRAIN**

Camila Saenz1, Gimena Fernandez1, Nicolas DeFrancesco1, Kenneth Mackie2, Mario Perello1.
1CONICET- Neurophysiology Lab, IMBICE, La Plata Argentina, 2Department of Psychological & Brain Sciences and the Gill Center, Indiana University, Bloomington, Indiana 47405.

The growth hormone secretagogue receptor (GHSR) is a G-protein coupled receptor (GPCR) highly expressed in the brain. GHSR mediates the effects of two hormones: ghrelin and liver-expressed antimicrobial peptide 2. Notably, GHSR acts via ligand-independent mechanisms, which include constitutive activity and allosteric modulation of other GPCRs. The cannabinoid receptor type 1 (CB1R) is also a GPCR highly expressed in the brain. CB1R is activated by endogenous endocannabinoids (i.e., anandamide) as well as phytocannabinoids, such as tetrahydrocannabinol (THC) the principal psychoactive cannabinoid of cannabis. Interestingly, GHSR and CB1R expression have been observed within many of the same brain nuclei, suggesting that these GPCR may act on common neuronal sets to mediate its neurobiological effects. Here, we explored the extent of this putative GHSR and CB1 interaction in the brain of male mice. To map GHSR distribution, we used two complementary approaches: 1) a fluorescent variant of ghrelin (Fr-ghrelin) and 2) a mutant mouse in which GHSR promoter drives the expression of GFP (GHSR-eGFP mice). In both cases, the presence of CB1R was visualized using a validated anti-CB1R antibody. Using the Fr-ghrelin labeling together with CB1R immunolabeling, we found that cells containing both GHSR and CB1R are mainly located in the hippocampus area, where GHSR cells were also positive for CB1R representing the 45,46 ± 7,28 % of all GHSR cells ($p<0.05$, one sample t-test). In brain sections of GHSR-eGFP mice inmunostained with CB1R antibody, we found cells containing both GHSR and CB1R are mainly located in the hippocampus posterior area, where GHSR cells were also positive for CB1R representing the 40,08 ± 13,33 % of all GHSR cells ($p= 0.095$, one sample t-test). In contrast, simultaneous presence of GHSR and CB1R was not observed elsewhere in the brain. Thus, we started to elucidate some of the neuronal populations where GHSR and CB1 may directly act.

468. (924) **APOE4 HETEROLOGOUS EXPRESSION, PURIFICATION UNDER NON-DENATURING CONDITIONS AND EFFECTS ON THE MITOCHONDRIAL NETWORK OF A NEURONAL CLONAL CELL LINE**

Ezequiel Serrano[1], Gabriel Valdivieso[2], Pablo G. Sanz[1], Dr. Guillermo Luis Taminelli[3]and Francisco J. Barrantes[1]
[1]Laboratory of Molecular Neurobiology and [2]Laboratory of Cell & Molec. Biol. Lab. [3] Laboratorio de Biotecnología de la Reproducción Animal, BIOMED UCA-CONICET, Buenos Aires, Argentina.

Apolipoprotein E (ApoE) is a major risk factor for Alzheimer disease. The *APOE4* allele increases the risk up to 15 times in homozygous carriers. To learn about the effects of ApoE4 on neuronal cells, we purified the recombinant protein expressed in the *E. Coli* BL21 strain. Soluble ApoE4 was purified by affinity chromatography using a Ni-NTA resin, followed by the release of the His-Trx tag with 3C-protease and size exclusion chromatography. A yield of ~10 mg/L was obtained with a purity of 95%, as analyzed by SDS polyacrylamide gel electrophoresis. The structural integrity and biological function of the purified ApoE4 was analyzed by circular dichroism and a DMPC assay. To analyze the possible effect of ApoE4 on mitochondrial function, samples of the clonal cell line CNh were incubated with 17 μg/mL of recombinant ApoE4 for 24 h. Mitochondria were labeled with MitoTracker Orange and imaged in vivo. Mitochondrial morphology was analyzed with MiNA and MicroP. Reactive oxygen species (ROS) and mitochondrial membrane potential (Ψm) were studied with the fluorescent probes DCFH-DA and TMRE, respectively. Both MiNa and MicroP analyses showed that ApoE4 modulates the mitochondrial network, with an increase in mitochondrial fusion and proliferation. The Ψm decreased upon treatment of CNh cells with ApoE4 ($p<0.05$), while ROS production showed an increase ($p<0.05$). Higher concentrations of ApoE4 (68 μg/mL) also produced an increment in ROS production ($p<0.001$) and reversed the ApoE-induced decrease of Ψm ($p>0.05$). In conclusion, ApoE4 increases mitochondrial proliferation and fusion, induces ROS and a disbalance of Ψm.

NEUROSCIENCES IV

Saturday, November 19, 14-15:30 hr
Chairs: Damian Dorfman - Juan Beauquis

469. (102) **WIRED CHOLESTEROL METABOLISM IS RELATED WITH FERROPTOSIS LEADING TO PARKINSONISM-LIKE DISORDERS**

Maniscalchi, A.*[1]; Benzi Juncos, O.N.[1,2]; Conde, M.C.[1,2]; Fermento, E.[1,2]; Funk, M.I.[1,2]; Curino, A.[1,2]; Facchinetti, N.P.[1,2]; Alza, N.P.[1,3] Salvador, G.A.[1,2].
[1]Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur (UNS), Consejo Nacional de Investigaciones Científicas y Técnicas. [2]Departamento de Biología, Bioquímica y Farmacia, UNS. [3]Departamento de Química, UNS

Ferroptosis is a novel type of oxidative and non-apoptotic cell death associated with neurodegenerative disorders. Previously, we have established an in vivo model of iron overload (C57BL/6 mice treated with iron-sucrose) that triggers ferroptosis in the nervous system related with markers of neurodegeneration. Our aim was to characterize lipid metabolism alterations in mice midbrain of this experimental model. We found that midbrain lipid content was altered by iron overload. Cholesterol and diacylglycerol levels were increased ($p<0.001$), while their acylated forms diminished ($p<0.001$). Neutral lipid changes were associated with elevated hydrolysis catalyzed by mono- di- and triacylglycerol lipases ($p<0.001$). A reduced activity of acylation enzymes, such as acyl-CoA, lysophospholipid and monoacylglycerol acyltransferases was also observed ($p<0.001$). Furthermore, SREBP-1 and filipin staining was increased by iron treatment ($p<0.05$). Besides motor skill impairment ($p<0.001$), iron-treated

mice presented a decreased recognition memory (p<0.001). To ascertain the role of neuronal-glia communication in cholesterol metabolism, we exposed N27 dopaminergic neurons and C6 astrocytes to iron -overload. Astrocytes showed the highest levels of cholesterol accumulation upon treatment (p<0.05). Our findings indicate that impaired cholesterol and triacylglycerol metabolism are biomarkers of midbrain neurodegeneration triggered by ferroptosis.

470. (150) **ROLE OF CERAMIDE SYNTHESIS IN GLIAL ACTIVATION AND COMMUNICATION AFTER A LIPOTOXIC STIMULUS**

Melina Bellotto1,2, Melisa Bentivegna1,2, Amal Gregosa1,2, Carlos Pomilio1,2, Nicolás González Pérez1,2, Jessica Presa1,2, Flavia Saravia1,2, Juan Beauquis1,2, Ángeles Vinuesa1,2.

1 Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina. 2 Departamento de Química Biológica, FCEN, UBA.

Saturated fatty acids (SFA) are basic components of western-style diets. High systemic and cerebral levels promote chronic inflammation and metabolic disorders. This scenario could lead to brain dysfunction through multiple pathways and be a risk factor for neurodegenerative diseases. Previously, we have shown that a high-fat diet (HFD) induces central and peripheral inflammation, cognitive impairment and hippocampal glial activation, in young and adult mice (Vinuesa et al. 2016, 2019). One of the mediators involved in these pathways are ceramides, lipidic molecules that, besides their physiological roles, could induce inflammation. In the present work, we aimed to study 1) the impact of palmitate (PA), one of the most abundant SFA in western-style diets, on glial activation and communication and 2) the role of ceramide synthesis mediating the effects of PA. Incubation of microglia (BV2 mouse cell line) with 0.5 µM PA induced NFkB p65 nuclear translocation (p<0.0001) and increased expression of IL1β (p<0.05), suggesting the adoption of a proinflammatory or M1 phenotype. Treatment of microglia with Cambinol, an inhibitor of ceramide synthesis, ameliorated microglial activation. Treatment of C6 rat astrocytes cell line with PA failed to induce IL1β expression. However, conditioned media (CM) from PA-exposed microglia did (p<0.001), and this effect was absent when microglia were pretreated with Cambinol. Isolation of exosomes from PA-microglia CM exerted the same effect, suggesting a relevant function for these extracellular vesicles. Our results suggest a role for glial ceramide synthesis mediating the induction and propagation of inflammation succeeding lipotoxicity. In present and future experiments, we aim to determine the role of ceramide synthesis in glial-neuronal communication and the promotion of neurodegeneration. References Vinuesa, A. et al. (2016). *Psychoneuroendocrinology*, *72*, 22–33. Vinuesa, A. et al. (2019). *Molecular neurobiology*, *56*(7), 5075–5094.

471. (160) **CORRELATION BETWEEN BIOMARKERS OF OXIDATIVE STRESS AND DEGREE OF COGNITIVE IMPAIRMENT IN PATIENTS WITH LEUKOARAIOSIS AND PROBABLE ALZHEIMER'S DISEASE**

Alejandra Cimato[1,3], Margarita Martínez Sarrasague[1,3], Fabiana Lairion[2,3], Christian Saporito Magriñá[2,3], Jorge Serra[3], Enrique Marschoff[4], Liliana Oudkerk[5], Graciela Ada Bianchi[5], Raúl Dominguez[5], Marisa Gabriela Repetto[2,3]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Física, Buenos Aires, Argentina, [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Química General e Inorgánica, Buenos Aires, Argentina, [3]Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL, UBA-CONICET), Buenos Aires, Argentina, [4]Instituto Antártico Argentino, Buenos Aires, Argentina, [5]Universidad de Buenos Aires, Hospital Sirio-Libanés, Buenos Aires, Argentina

Oxidative stress (OS) and oxidative damage (OD) are associated to the etiopathogenesis of Leukoaraiosis (L) and Alzheimer disease (A). The aim of this work was to evaluate whether OS markers in the blood of patients with L and A correlate with the degree of cognitive impairment (CI). Methods: OS biomarkers: carbonylated proteins (CO), oxidized lipids (TBARS), superoxide dismutase (SOD), catalase (CA), total (GSHt), reduced (GSH), and oxidized (GSSG) glutathione (by spectrophotometry), and IL-6 (by ELISA) were determined in the blood of elderly patients (75±6 years): 17 with a diagnosis of L (magnetic resonance imaging, brain computed tomography and Fasekas (F) cognitive test), 16 with A (neurological and cognitive tests: Clinical Dementia Rating (CDR)) and 17 healthy subjects (controls, C). The correlation between parameters was evaluated by Spearman coefficient (r). Results: In L, the advancement of CI correlates with age (r:0.38). CO increased in plasma from L (F2) and A (CDR2), SOD increased in L and A (p<0.01) and CA in L (F3) (p<0.05) without correlation with CI. GSHt in erythrocytes was lower in L and A compared to C (40% and 92% respectively). In A, IL-6 was higher than in C (p<0.01) and also than L F2 (p<0.01). In L, CO and IL-6 correlate positively and moderately with the degree of brain lesions (r:0.27 and 0.55, respectively). In A, CO (r:0.46), total erythrocyte proteins (r:0.35), and TBARS (r:0.40) correlate moderately and negatively with CI. GSHt decreases with CI in both pathologies (rL: 0.66, rA: 0.87). GSSG in L (r: 0.97), and GSH/GSSG in A (r: 0.89) would be good parameters to evaluate the OS that accompanies the progression of the CI. Conclusion: In L, there would be a reversible response to oxidative stress with control of redox homeostasis; while in A, the chronic oxidative processes and OD would be irreversible and with loss of redox homeostasis (oxidative distress), that worsens with the increase in DI and the progression of the disease.

472. (165) **NEURODEGENERATIVE EFFECTS OF THE CYANOTOXIN β-N-METHYLAMINO-L-ALANINE (BMAA) ON RETINAL CELLS**

Tamara B. Soto[1,2], Paula E. Tenconi[1,2], Melina V. Mateos[1,2], Luis E. Politi[1], Olga L. German[1,2]

[1]Instituto de Investigaciones Bioquímicas (INIBIBB); [2]Depto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina

The chronic intake of the non-proteic aminoacid BMAA, a cyanotoxin released by many cyanobacteria occurring in most dams and water resources, was linked with the development of neurodegenerative diseases. We previously, demonstrated that BMAA generates cell death in amacrine and photoreceptor neurons (PHRs) and that NMDA receptors induce amacrine cell death through β-carbamate production. We here investigated whether BMAA is mis-incorporated into polypeptide chains of retinal neurons, replacing serine, thus triggering neurodegenerative processes. Also, we investigated the neuroprotective role of RXRs receptors, and the effects of BMAA on a retinal pigment epithelial cell line (ARPE-19). We incubated pure neuronal cultures obtained from newborn rat retinas with 0,4 μM BMAA, and ARPE-19 cells with 0,4, 1 and 10 μM of BMAA during 3 days. We then evaluated cell death and apoptosis by Trypan Blue, TUNEL assays, and DAPI staining; mitochondrial activity by Mitotracker and MTT assays; ROS levels by using the probe H2-DCF-DA and cytoskeleton integrity by immunocytochemical methods. In pure neuronal cultures, pre-treatment with serine (25 μM) or HX630 (RXR agonist, 1 μM), prevented the increase of fragmented nuclei and apoptosis induced by BMAA, both in amacrine neurons and PHRs. In addition, preliminary results showed that BMAA induced ROS increase, which was reduced in presence of HX630.

In the ARPE-19 cells, 10 μM BMAA induced cell death and decreased mitochondrial activity. Noteworthy, at different sub-lethal concentrations, BMAA induced alterations in mitochondrial morphology and distribution. These results suggest that BMAA induces subcellular changes affecting viability in both neurons and ARPE-19 cells, confirming BMAA as a threat to human health by inducing neurodegenerative damages. Furthermore, RXR receptor activation and serine supplementation exert a protective effect against BMAA toxicity in retinal neurons.

473. (189) **REGULATION OF SYNAPTOSOMAL 2-ARACHIDONOYLGLYCEROL METABOLISM BY TETRAHYDROCANNABINOL DURING AGING**

Sabrina Rosicler Salas, Ana Clara Pascual, Pablo Gustavo

Milano, Susana Juana Pasquaré
Laboratorio de Cannabinología. INIBIBB-UNS-CONICET. Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Buenos Aires, Argentina.
e-mail: srosicler@inibibb-conicet.gob.ar

The endocannabinoid 2-arachidonoylglycerol (2-AG) is known for its neuroprotective role. During aging, 2-AG metabolism in rat cerebral cortex (CC) synaptosomes (Syn) is dysregulated, resulting in a decrease in its availability, which could lead to synaptic dysfunction. On the other hand, 2-AG metabolism was shown to be modulated by ligands of its own receptors. Delta-9-tetrahydrocannabinol (THC) is a partial cannabinoid receptor agonist present in *Cannabis* sp. extract (CE), which is widely used in Medicinal Cannabis (MC). Our previous results indicated that THC-enriched CE (1 µM THC) restored 2-AG hydrolysis in aged CC Syn. The purpose of this study was to evaluate if THC could modulate 2-AG synthesis. To this end, THC-enriched CE were obtained from *Cannabis Y Griega* female flowers, previously grinded and heated at 115 °C for 40 min. Extractions were performed by adding 5% (w/v) ethanol, vortexed, sonicated and agitated. The mixture was centrifuged and the supernatant was evaporated under a stream of N_2. THC, cannabidiol (CBD) and cannabinol (CBN) were quantified by HPLC. CC Syn from adult (4-6 months) and aged (24-26 months) rats were isolated by differential centrifugation and purified in Ficoll gradients. 2-AG synthesis, by lysophospholipid phosphohydrolase (LPAase) and by diacylglycerol lipase (DAGL), was assessed by incubating Syn with THC-enriched CE (1 µM THC) or 1 µM pure THC, and the respective radiolabeled substrate, simultaneously. Results showed that either CE or pure THC increased LPAase activity in adult Syn ($p<0.05$). However, pure THC caused a slight decrease in the activity only in aged Syn ($p<0.05$). On the other hand, DAGL activity was not modified by THC either in adult or in aged Syn ($p>0.05$). In conclusion, THC-enriched CE could attenuate aged synaptosomal 2-AG deficit, while pure THC could potentiate this loss. These findings highlight the importance of using the whole-plant CE in the success of MC therapy.

474. (330) **THE LIMITS OF THE BRAIN "IMMUNE-PRIVILEGE": PARTICIPATION OF THE PERIPHERAL IMMUNE SYSTEM IN EPILEPSY**
Alicia R. Rossi, Dante Gomez Cuautle; Florencia Rodríguez, Ingrid Mailing, Luca Sapia, Alberto Javier Ramos.
IBCN UBA-CONICET, Facultad de Medicina, UBA.

Childhood febrile seizures is a classical clinical finding in the retrospective studies of Temporal Lobe Epilepsy (TLE) adult patients. The period between febrile seizures and the chronic TLE is called latency period where the processes that leads to epileptogenesis have place. Using an hyperthermic seizure model (HS) in Wistar rats, we previously demonstrated that exposed male-HS had moderate reactive gliosis with an atypical astrocytic distribution at 15 days post HS (DPHS), have lower convulsive threshold, a significant increase in Iba-1+ microglia while females do not. Both sexes showed an activation of the spleen white pulp, evidenced by an intense disorganization of Malpighi corpuscles. Having this in mind we extended our study to analyze the response of the peripheral immune system, and the possibility of behavioural changes in adult HS animals. Rat pups (10 days old) were placed in a glass chamber, and their core temperature was raised (39-42°C). Seizures onset was monitored behaviourally. At 15DPHS, blood was obtained by intracardiac puncture and processed for flow cytometry. At 15 and 35DPHS rats were fixed, brains removed and processed for immunohistochemistry. Other group of animals was examined at 50DPHS with Barnes Maze in order to evaluate possible alterations in spatial memory. We observed that moderate reactive astrogliosis persists in males at 35DPHS, in peripheral blood an increase in the population of CD4 vs CD8 lymphocytes was observed without changes in the total population of T-lymphocytes (CD3+). Barnes Maze assay did not show statistically significant alterations in spatial memory. We conclude that peripheral immune system reacts to the HS-induced seizures, and probably the mobilized CD4+ cells are directed towards the CNS. While gross CNS alterations and behavioural alterations were not observed, subtle reactive gliosis persist probably related to altered wiring in these developing brains. Supported by: UBACYT, PIP479, PICT2017-2203.

475. (352) **METFORMIN PROMOTES AUTOPHAGY IN GLIAL CELLS AND REDUCES NEUROINFLAMMATION IN EXPERIMENTAL MODELS OF TYPE 2 DIABETES MELLITUS**
Nicolás Gabriel González Pérez, Carlos Pomilio, Ángeles Vinuesa, Melina Belloto, Melisa Bentivegna, Amal Gregosa, Jessica Presa, Juan Beauquis, Flavia Saravia

Drug repositioning is a strategy for identifying new uses for approved safe drugs that are outside the scope of the original medical indication. Pathological chronic activation of the brain immune response and autophagy impairment frequently occur in association with aging and is exacerbated in age-related diseases, like type 2 diabetes (T2D) and Alzheimer's disease. Here, we tested the potential therapeutic role of metformin –the first-line drug used for treatment in T2D– in reducing neuroinflammation and restoring autophagic flux in astrocytes and microglia. Our preliminary results employing cell lines showed no effect of metformin on viability; astrocytic cells exposed to 0,2 mM metformin during 2 h showed an increased immunoreactivity for the autophagy marker LC3; while microglial cells exposed 30 min to this concentration exhibited increased autophagy, evaluated by western blot against LC3 and the autphagy-specific substract p62 ($p<0.05$). Moreover, in microglia, metformin treatment reduced the expression of the proinflammatory cytokine IL-1β induced by palmitate ($p<0.05$) a well known metabolic insult frequently present in western diet together with a reduction in phagocytic activity assessed by latex beads incorporation ($p<0.001$). Employing an in vivo model of T2D by high fat diet administration, we found that treatment of metformin 4% p/v, i.p, 3 times per week during 3 weeks, decreased microglial size in the hippocampus as an indicator of neuroinflammatory response in comparison to T2D mice treated with vehicle ($p<0.05$). Taken together, our results suggest that metformin is capable of modulating glial response by promoting autophagy and reducing neuroinflammatory reaction in experimental models of T2D. Considering drug repositioning, metformin emerges as a therapeutic approach in diseases without effective cure where neuroinflammation play a central role, like neurodegenerative diseases.

476. (382) **PROCESSES LENGTHENING AND NEURONAL ADHERENCE ARE DEREGULATED IN CDK5-DEFICIENT HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS**
Mucci Sofía*, Rodriguez-Varela MS*, Allio CP*, Isaja L*, Ferriol-Lafoulliere SL*, Sevlever GE*, Scassa ME* and Romorini L*.
** Instituto de Neurociencias (INEU-FLENI-CONICET)*

CDK5 is an essential molecule involved in neuronal survival and homeostasis, thus making the study of the consequences of CDK5 deficiency in a human neuronal model relevant. For this reason, we aim to analyze the differentially expressed genes in WT and KO-CDK5 (CRISPR/Cas9 edited) H9 human embryonic stem cells (hESCs)-derived neurons. Initially, we differentiated WT and KO-CDK5 H9 cell lines into neurons using a defined medium and validated their phenotype by immunostaining with neuronal *lineage-defining markers* (TUJ-1, MAP2, MAP5). Next, an RNA-seq analysis (n=3) was performed with RNA isolated from WT and KO-CDK5 hESCs-derived neurons. We found 920 differentially expressed genes between WT and KO-CDK5 hESCs-derived neurons by using the DESeq2 package in R Studio (alpha = 0.01). Through a GO analysis of biological processes, we found that among the differentially expressed genes 137 participate in neurogenesis and neuronal differentiation. Next, we proceed to the validation of 5 genes found differentially expressed by RNA-seq analysis by RT-qPCR. Then, we studied processes lengthening in WT and KO-CDK5 H9 hESCs-derived neurons. We found that KO-CDK5 neurons showed a marked increase in the length of their processes with respect to the WT counterparts. In addition, the adherence of KO-CDK5 deficient neurospheres to Geltrex pre-coated plates was remarkably reduced compared to WT neurospheres (10.40%±2.95 vs. 78.12%±7.37, respectively). In conclusion, although CDK5 deficiency did not impair

neural differentiation of hESCs, its disruption altered the transcriptome of hESCs-derived neurons, reduced process lengthening and decreased cell adhesion.

477. (476) **TOPOGRAPHIC DISTRIBUTION AND EXPRESSION OF MRP4 (ABCC4) IN THE CENTRAL NERVOUS SYSTEM (CNS) OF NORMAL RATS**

Julieta Beatriz Allegro[1], Ana Clara Romeo[1]; Carolina Inés Ghanem[1,2].

1. Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET), Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.

2. Cátedra de Fisiopatología. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.

Previously, it has been described that Mrp4 protein (Abcc4), a member of the Adenosine Binding Cassette transporters (ABC), is expressed in the blood-brain barrier (BBB) and the choroid plexus, mainly located in the endothelial cells of the capillaries. However, until now there were no systematic and comparative studies of its expression among different structures of the central nervous system (CNS). Therefore, the present work aimed to study the different levels and patterns of Mrp4 expression in discrete areas of the CNS. For this purpose, male control Wistar rats were anesthetized, and the brain and cerebellum were removed. Proteins isolated from the nucleus accumbens (NAc), dorsal striatum (DStr), non-motor cortex (Cx), and cerebellum (Cer) were processed to determine Mrp4 expression by Western blotting. The results were expressed as fold of change in the specific area compared to Mrp4 expression in the total brain homogenate. Another, set of rats was intracardially perfused with paraformaldehyde solution. Brains were removed and Mrp4 was detected in coronal sections by immunohistochemistry. The results showed that Mrp4 expression differs among areas: the highest expression (3,5 fold) was detected in Cer, the expression in NAc was significantly increased by 1,5 fold, while the expression in Dstr was similar to the complete brain expression of the protein. On the contrary, Mrp4 expression in Cx was significantly decreased by 0,5 fold. Additionally, brain immunohistochemistry showed the typical pattern of expression within capillaries sections in NAc and DStr. However, Mrp4 was not only expressed within the capillaries of the Cx but also in cellular nuclei. In conclusion, the results indicate that Mrp4 expression varies in magnitude and pattern among the SNC. This suggests that a deeper study to characterize the nuclear pattern of expression is mandatory.

478. (491) **SELECTIVE PURKINJE NEURONAL DAMAGE BY ACUTE ACETAMINOPHEN (APAP) INTOXICATION IS ASSOCIATED WITH OXIDATIVE STRESS AND IN THE CEREBELLUM OF RATS**

María Belén Vigo[1], Lisandro Orbea[1], Julieta Beatriz Allegro[1], Ana Clara Romeo[1], José E Manautou[2]; Carolina Inés Ghanem[1,3].

1. Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET), Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.

2. Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, United States

3. Cátedra de Fisiopatología. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.

Previously, we have demonstrated that APAP overdosing, which does not result in acute liver failure (ALF), reduces locomotor activity (~50%) and dopaminergic markers. Furthermore, decreased neuronal processes and astrogliosis in brain areas controlling locomotion are also observed. Therefore, the aim of the present work was to evaluate the effect of acute APAP intoxication on the structure and the principal constituent cells of the cerebellum, the main region of the central nervous system governing motor coordination and learning. For this purpose, male Wistar rats were dosed with APAP (1g/kg; i.p., n=5) or vehicle (n=5). 24h later, the cerebellum was processed to examine histopathological changes and the presence of edema. Also, the protein expression of markers of astrocyte structure (Glial fibrillary protein; Gfap), and neuronal structure (Neurofilament heavy; NF200) was determined by Western blotting, and oxidative stress status was also determined indirectly by analysis of nuclear translocation of Nrf2, a master regulator of antioxidant responses. Histological cerebellar tissue analysis by hematoxylin and eosin staining revealed no discernible ultrastructural changes or edema (also confirmed by tissue water content). However, a significantly decreased by 24.5% in the number of Purkinje neurons in the APAP treated group ($p<0.01$) was detected. Also, APAP increased Nrf2 nuclear translocation by 41% compared to the vehicle control group ($p<0.01$). In contrast, no significant changes in GFAP, SOX 10, and NF200 were observed. In conclusion, the results indicate that APAP acute intoxication in the absence of ALF, produces a selective decrease in Purkinje cell density in the cerebellum, possibly due to oxidative stress, most likely due to a direct toxic effect of APAP in the brain.

479. (518) **STUDY OF NEUROPROTECTIVE FACTORS MEDIATED BY OMEGA 3 AND COGNITIVE STIMULATION AGAINST AMNESIC MILD COGNITIVE IMPAIRMENT IN PATIENTS RESIDENTS OF THE CITY OF CÓRDOBA-ARGENTINA**

Castro Zamparella, Tatiana; Cáceres, Magdalena; Balaszczuk, Verónica

Instituto de Investigaciones Psicológicas IIPsi-Unidad Ejecutora del CONICET, Facultad de Psicología - Universidad Nacional de Córdoba.

General description: In older adults, neurodegenerative disease is very prevalent. In Latin American with low economic resources, public health proposals can be based on preventive and educational actions, promoting brain protection factors. Background shows that a diet based on foods rich in essential fatty acids Omega 3 (ω3) contributes to the prevention of neurological clinical diseases. **Goal:** Explore neuroprotective factors mediated by dietary supplementation (ω3) and cognitive stimulation that could delay amnesic-type cognitive decline and thus, the diagnosis of dementia. Material and Method: Non-probabilistic sample of adults between 60-80 years of age, both sexes, from Córdoba City, Argentina. N=14 divisions in Healthy Control Group (S) and Cognitive Impairment (DC). Pre-post evaluation neurocognitive battery and blood lipid profile were made. Treatment with ω3 capsules (1 g) for 24 weeks and cognitive stimulation workshops for 24 weeks. To then exchange and complete 12 months with the other treatment. Results: The groups S:ω3 and S:EC didn´t present changes pre-post treatment. In the DC:ω3 group, maintenance of cognitive function is observed, as in the DC:EC group. Although the performance in the DC groups is lower due to the cognitive condition of the participants, no pre-post treatment changes are observed. The DC:ω3 and DC:EC groups are statistically different from the S:ω3 group. Discussion and conclusions: In clinical practice, it is expected that an older adult with cognitive failures compatible with MCI get worse their performance from one year to the next if the conditions and habits are not modified. The data obtained so far showed a tendency that the intervention sustained for 12 months at the level of external stimulation of cognitive functions and abilities and the adequate nutritional contribution provided by ω3 sustains cognitive performance. Therefore, health trends are focused on prevention and thus provide quality of life to the adult population.

480. (556) **INCREASED IL-17 PLASMA LEVELS ASSOCIATED WITH MORE ACTIVATED CIRCULATING CD4⁺ LYMPHOCYTES IN PATIENTS WITH MOOD DISORDERS**

Tifner V[1], Arena AR[1], Grendas LN[1,3], Penna MB[1,3], Hunter F[1,4], Olaviaga A[1,5], Prokopez C[1,6], Armesto AR[1], Alvarez RI[3], Chiapella LC[7], Carrera Silva EA[2*], Daray FM[1*], Errasti AE[1*]

**Co-senior authors*

1. Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires

2. Instituto de Medicina Experimental, IMEX-CONICET- Academia Nacional de Medicina

3. Hospital General de Agudos Dr. Teodoro Álvarez

4. Centro de Salud Mental N° 3 Dr. Arturo Ameghino

5. Hospital General de Agudos Dr. Cosme Argerich

6. Hospital Neuropsiquiátrico Braulio A. Moyano
7. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, CONICET.

During the last years, emerging evidence associated Mood Disorders (MD) with increased inflammatory status suggesting a role for the innate and adaptive immune system in the developing and maintaining of MD. This study is part of a larger ongoing observational, cross-sectional study and aimed to determine the activation status of immune circulating cells in patients with MD coursing a clinical episode of active depression (AD) or during no clinically active depression (NAD). The specific aim of the present work is to answer if MD patients show a differential immune T cell profile analyzing the frequency of CD4+ and CD8+ T cells, the activation status of those CD4+ lymphocytes and their associated cytokines profiling. Patients were evaluated by psychiatrists using the International Psychiatry Interview MINI to diagnose MD and the Hamilton Depression Rating Scale (HADRS) to define AD (N=21) and NAD status (N=21). MD sample was 26% male and 74% female with 18-55 year age range and matched with a healthy control group (HC) by age and gender (N=23). Blood samples were obtained and directly stained and analyzed by flow cytometry. Additionally, a panel of 16 plasma cytokines was measured. IRB approved the study, and each participant gives the written consent. Our results show that AD patients have an increased CD4/CD8 ratio (2.57) vs. NAD (1.98) and HC (1.99) and increased frequencies of CD3+CD4+CD69+ and CD3+CD4+PD1+ circulating lymphocytes compared with patients with NAD ($p<0.01$). Furthermore, the canonical Th17 cytokine, IL-17, was also increased in the plasma of patients with AD in comparison to HC ($p<0.01$), as well as IL-8, IL-18 and sTREM2 ($p<0.05$). These results clearly show that the T lymphocyte compartment of patients with MD exhibits an unbalanced inflammatory condition. Altogether, the adaptive immune response is playing a key role in the physiopathology of MD patients.

481. (705) **CEREBROSPINAL FLUID FROM NEWLY DIAGNOSED RELAPSING-REMITTING MULTIPLE SCLEROSIS PATIENTS INDUCES GLUTAMATE-MEDIATED TOXICITY IN PRIMARY HIPPOCAMPAL NEURONAL CULTURES**

Samanta Armonelli[1], Giuliana Simoni[1], Alejandro Vilcaes[1], Liliana Patrucco[2], Edgardo Cristiano[2], Mariano Bisbal[3], Juan I. Rojas[2], Alicia L Degano[1], Pablo H. H. Lopez[1].
[1] Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET-UNC), Argentina.
[2] Centro de Esclerosis Múltiple de Buenos Aires-CEMBA, Hospital Italiano de Buenos Aires, Argentina.
[3] Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Argentina.

Glutamate (Glu) toxicity has been postulated as a factor contributing to neurodegeneration in Multiple Sclerosis (MS), and is currently under study as a biomarker for neurodegeneration. We recently described a correlation between high Glu concentration in cerebrospinal fluid (CSF) and different imaging parameters of neural atrophy in newly diagnosed Relapsing-Remitting MS (RRMS) patients. The aim of this work was to demonstrate a direct neurotoxic effect of Glu in the CSF from MS patients on rat primary hippocampal neuronal (HN) cultures. CSF samples from 18 newly diagnosed RRMS patients free of treatment and 18 control subjects were collected, and their toxicity tested by acute exposure on HN cultures previously grown for 8 days in vitro, then cells were further cultured for an additional 24h. The HN axodendritic network was visualized by immunofluorescence using the neuron-specific marker β-3 tubulin. Glu-mediated neurotoxicity in CSF was identified by pre-treatment of cultures with MK-801, a non-competitive NMDA receptor antagonist; or pre-treatment of CSF with the enzyme Glu dehydrogenase (GDH), which converts Glu in α-ketoglutarate. Acute exposure of CSF from control subjects to HN did not induce significant neurotoxicity when compared to control (untreated) cultures. On the contrary, exposure of CSF from RRMS to HN resulted in a robust axodendritic network loss (t-test; $p<0,0001$; mean 40 ± 0,06 %). Pre-treatment with MK-801 fully protected cultures from RRMS-derived CSF neurotoxicity ($p=0,004$, n=6, two way ANOVA). Similarly, GDH-mediated degradation of Glu present in CSF from RRMS prevented neurotoxicity ($p=0,0008$; n=6). Overall, our results confirm that Glu present in CSF from RRMS patients exerts axodendritic damage in HN. This data support the role of Glu as a biomarker to predict and monitor disease progression of RRMS patients, and identify a therapeutic target to prevent neurodegeneration associated with clinical progression.

482. (715) **STUDY OF THE BIOCHEMICAL, BIOPHYSICAL, AND BEHAVIORAL EFFECTS OF OMEGA-3 FATTY ACIDS ON NORMOTENSIVE AND HYPERTENSIVE RATS**

M. Lucrecia Longarzo[1], Franco JC. Dolcetti[1], Sofía Cervellini[1], Milagros Sisti[1], Romina Vázquez[1], M. Celeste Villa- Abrille[2], M. Laura Fanani[3], M. José Bellini[1] and Sabina M. Maté[1].
[(1)] Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP); UNLP, La Plata, Argentina
[(2)] Centro de Investigaciones Cardiovasculares de La Plata (CIC); UNLP, La Plata, Argentina
[(3)] Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), UNC, Córdoba, Argentina

Consumption of omega-3 polyunsaturated fatty acids (PUFAs) - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - leads to their incorporation into biological membranes, modifying membrane properties, and thereby affecting signal transduction and cellular function, ultimately benefiting patients with neurodegenerative diseases. In this work, we investigated the effects of early nutritional supplementation with EPA and DHA on cognitive behavior, anxiety, and activity in normotensive (Wistar) and spontaneously hypertensive rats (SHR), and the possible biochemical and biophysical mechanisms underlying the observed effects. After weaning, the animals received orally EPA and DHA for 16 weeks (200 mg/kg body mass/day). The open field test, anxiety behavior test, and a cognitive test were performed the day before the sacrifice. On sacrifice day, plasma and cerebral cortex samples were isolated, and total lipids were extracted. The total free fatty acid composition in plasma was analyzed and the properties of Langmuir monolayers of total lipids from the cerebral cortex were studied. Naive SHR rats showed increased levels of rearing activity that were partially ameliorated by dietary supplementation with PUFAs; the treatment also decreased anxiety behavior assessed by the Marble Burying Test. PUFAs supplementation led to a significant increase in the levels of EPA, DHA, and docosapentaenoic acid in plasma and reduced the total percentage of omega-6 fatty acids. Anti-inflammatory lipid mediators derived from PUFAs (resolvins) were increased in the plasma of SHR-supplemented rats compared to the untreated group. Finally, an altered phase miscibility behavior with stabilization of liquid lipid domains were registered in lipid monolayers from the cerebral cortex of SHR-treated compared to SHR-control rats. These results suggest that a diet supplemented with PUFAs from an early age in SHR modifies the biophysical properties of cerebral cortex membranes and may help to prevent the chronic inflammation associated with neurodegenerative diseases and improve associated behavioral disorders.

483. (758) **PATERNAL ETHANOL INTAKE ALTERS OFFSPRING ANXIETY-LIKE BEHAVIOUR IN A SEX DEPENDENT MANNER**

Gotfryd Lucila[1], Cambiasso Maite[1], Stinson Marcelo Gabriel[2], Birolo Sol[3], Fesser Estefanía Aylén, Cánepa Eduardo[2], Calvo Juan Carlos[1], Fontana Vanina Andrea[1].
[1] Instituto de Biología y Medicina Experimental (IBYME-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.[2] Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN-CONICET) Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.
[3] Departamento de Química Biológica de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,Ciudad Autónoma de Buenos Aires, Argentina.

Previously, we observed that paternal ethanol intake affects both reproductive biology and reflexes acquisition in male offspring. Aim:- To evaluate the effects of male ethanol consumption on offspring

behaviour and its correlation with the expression levels of immediate early genes (IEGs). Methods:CF-1 male mice were exposed (treated group,T) or not (control group,C) to 15% (v/v) ethanol in drinking water *ad libitum* for 12 days. C and T males were mated with non-treated CF-1 females (1:1). Behavioural tests (Open Field or OF, Elevated Plus Maze or EPM) were performed on their offspring. Total RNA was extracted from the resected medial prefrontal cortexes (mPFC) of the offspring in order to evaluate IEGs expression (Npas4, EGR1, Arc, cFos) through RT-qPCR. Results: The male offspring of T presented an anxiety-like behaviour (less permanence time and entries to the central square,$p<0.05$) in the OF test. Female T pups also showed the same behaviour (less time in the open arms,$p<0.01$) but in the EPM test. Egr1 expression was higher in T offspring for both sexes ($p<0.05$). Offspring of C showed a positive correlation between expression levels of EGR1 and Npas4, Arc, and cFos for both sexes ($p<0.01$) while this was not observed in the T progeny. In male C offspring, time spent in the central square in the OF test negatively correlated with brain-to-body weight ratio ($p<0.05$), but this correlation was lost in the T descendants. In female C offspring, time spent in open arms in the EPM test positively correlated to the expression of Egr1 ($p<0.05$) and Arc ($p<0.05$) but not in T offspring. Conclusions:In the mPFC paternal ethanol intake disrupts the correlation between IEGs expression levels for both sexes. It also alters offspring´s anxiety-like behaviour in a sex dependent way. In male offspring, it could be related to a lower brain-to-body weight ratio while in female offspring, it could be associated with higher expression of Egr1 and its loss of correlation with other IEGs.

484. (822) **INVOLVEMENT OF TOLL LIKE RECEPTOR 4 (TLR4) IN THE EFFECT OF HIGH-FAT DIET AND/OR CHRONIC STRESS**

Sofía Quiroga, María Paula Marcone, María Rosa González Murano, María Agustina Vidal, Ana María Genaro*, Adriana Laura Burgueño*.
Instituto de Investigaciones Biomédicas (BIOMED)/UCA CONICET
** both authors contributed equally to the work*

High fat diet (HFD) or chronic stress (CS) lead to low-grade inflammation, promoting neuroinflammation and memory impairment in rodents. To analyze the role of the inflammasome NLRP3 in these alterations we compared the effect of HFD and CS in C57Bl/6J (WT) and TLR4 KO mice. We previously found that HFD in WT increases body weight and basal glycemia levels. These results were not observed in KO mice. The aim of this study was to analyze the effect of HFD and/or CS on cognition and gene expression in hippocampus from WT and KO mice. At 4 weeks of age, males received standard diet (SD) or HFD. After 12 weeks, they were exposed (or not) to CS for 8 weeks, resulting in 4 groups for each strain: 1) SD, 2) SD+CS, 3) HFD, and 4) HFD+CS. To assess cognitive performance, we conducted the Object location test (OLT). Hippocampal mRNA expression levels of PYCARD, NLRP3 (components of NLRP3 inflammasome) and NGF, BDNF and SIRT1 (as markers for cognitive deficits) were assess by qPCR. We found that OLT was altered in WT HFD or CS ($p<0.05$) whereas in KO mice only CS produced a decrease in the discrimination index (DI) ($p<0.01$). However, the DI in KO was higher than WT in all groups, denoting a better spatial memory. WT HFD showed higher levels of NLRP3 and PYCARD ($p<0,01$), also CS per se increased levels of both genes ($p<0,05$). In KO HFD and CS individually produced an increase in NLRP3 mRNA levels ($p<0,01$ and $p<0,05$ respectively). In KO NLRP3 showed higher levels of mRNA than in WT ($p<0,001$), while the opposite occurred with PYCARD ($p<0,001$). KO HFD+CS showed higher levels of BDNF ($p<0,05$). In KO SD+CS we observed lower levels of NGF ($p<0,01$) than KO SD. Also, SIRT1 levels were higher in KO SD and HFD+CS compared with WT mice ($p<0,05$). These results suggest the involvement of TLR4 in HFD and/or CS in the development of cognitive deficits in WT mice. Further analysis of protein expression will be necessary for a better understanding of the role of TLR4 in this animal model.

ONCOLOGY I *Thursday, November 17, 9-10:30 hr*

Chairs: Valeria Segatori - Ulises Orlando - Marcela Villaverde

485. (28) **TUMORIGENIC EFFECT MEDIATED BY FATTY ACID SYNTHASE IN A MURINE MAMMARY ADENOCARCINOMA MODELS FED WITH HIGH PALMITIC ACID AND FRUCTOSE DIET**

Tamara Mazo [1,2], Victoria Ferrero V, Neri Nelso Barotto[2], Julieta Don[1], Laura Yennerich[2], Erica Solla[1], Liliana Sosa[1], Valeria Rodríguez[1], Amado Quintar[1], Maria Eugenia Pasqualini[1,2]
[1] Institute of Health Sciences Research (INICSA-CONICET) – School of Medicine Sciences, National of Cordoba University, 5000 Córdoba, Argentina;
[2] Departament of Cell Biology, Histology and Embryology School of Medicine Sciences, National of Cordoba University, 5000 Córdoba, Argentina

Breast cancer (BC) is the first leading cause of mortality in women and is associated with genetic and epigenetic factors such as dietary compounds. The fatty acid synthase (FASN) is involved in de *novo* lipogenesis, catalyzing the synthesis of endogenous fatty acids. In early stages of carcinogenesis, the activation of FASN is mediated by hypoxia, which is induced by high concentrations of simple carbohydrates and fats. Its overexpression is associated with a poor prognosis, however, the dietary regulation of FASN in BC development is still unknown. The aim of this study was to evaluate the variation in dietary palmitic acid (PA) and fructose (Fr) on the regulation of FASN expression mediated by hypoxia in murine BC development. BALB/c mice (n=40) were divided in 4 dietary groups, CONTROL (6%corn oil+30%Fr), PCS (20%palm oil+15%Fr), PBA (20%corn oil+45%Fr) and PCS+PBA (20% palm oil+45%Fr). After 90 days mice were inoculated with murine breast adenocarcinoma LM3 cells ($1x10^6$cell). In this model we evaluated tumor volume (calimeter), lipid profile (gas chromatography, GC), FASN expression (Western Blot and immunohistochemistry) and tumor histology (H/E). In vitro model: cultured LM3 were treated with PA (40μM-50μM) and/or Fr (2.5μM) for 24hs. We evaluated viability (resazurin), apoptosis (Hoechst), lipid profile (GC), FASN expression. Three replicates were minimally performed by experiment and analyzed by ANOVA. The PCS presented the highest percentage of PA and the PBA, a high percentage of ω-6 PUFAs in membranes respect to the other groups. The PCS+PBA diet produced an increment in tumor growth, infiltration and necrosis. FASN expression was increased in this group as well as after PA and Fr (40/2.5uM) LM3 treatment ($p<0.05$).The PA and Fr (40/2.5uM) decreased LM3 apoptotic cells and PA 40μM increased cell viability ($p<0.05$). We demonstrated that diets high in PA and Fr induce tumor development in murine BC, mediated by an increment in FASN enzyme expression.

486. (83) **EVALUATION OF ANGIOTENSIN-(1-7) AS A NEW COMBINATION THERAPY TO REVERSE RESISTANCE TO VEGFR INHIBITORS IN TRIPLE NEGATIVE BREAST CANCER**

Agustina Carnevale[1], Pedro Salaberry[1], Thomas Walther[2], Edith Kordon[1], Albana Gattelli[1] and Carolina Schere-Levy[1].
[1]IFIBYNE-UBA-CONICET, University of Buenos Aires, Argentina; [2]University Medicine Greifswald, Greifswald, Germany.

In triple negative breast cancer (TNBC) the absence of targeted treatment and resistance to current therapies are crucial factors precluding improvements in mortality. Central in the mechanism of resistance is the activation of tyrosine kinase receptors, including VEGFR, the receptor for the main angiogenic factor, VEGF. VEGFR inhibitors as Axitinib (Ax) or Bevacizumab (Bev) succeed in reversing some cases but in long term, induce resistance and metastatic disease in other patients. Renin-angiotensin system has been implicated in multiple aspects of cancer progression through ACE/ AngiontensinII (AngII) pathway inducing angiogenesis and metastasis. Angiotensin 1-7 [Ang-(1-7)] is generated from AngII by ACE2 enzyme. Previously, we found that AngII promotes invasion of TNBC cell lines by enhancing VEGFR signaling, and Ang-(1-7) counteracts pro-tumorigenic actions of AngII. Our group also demonstrated in renal carcinoma cells that treatment with Ax or Bev decreased ACE2

expression, and the addition of Ang-(1-7) in an *in vivo* combination treatment with Ax generated additive suppression of renal tumor growth and improved survival outcomes. We determined by bioinformatic tools that both ACE and ACE2 are expressed in patients with TNBC. In this study, our aim is to evaluate *in vivo* the effects of combined therapy of Ax + Ang-(1-7) in the treatment of TNBC. As in renal cancer, we found that treatment with Ax or Bev reduces ACE2 expression ($p<0,05$) in two metastatic TNBC cell lines (MB-MDA-231, EO771) measured by qRT-PCR. In allograft mouse models, the treatment with Ax + Ang-(1-7) did not alter mammary tumor growth compared to Ax as a single agent. Importantly, the addition of Ang-(1-7) significantly inhibited the development of lung metastatic foci ($p<0,02$) and decreased VEGF expression in tumor tissue. Our results suggest that combined treatment with Ang-(1-7) reverses resistance to VEGFR inhibitors by reducing the metastatic index and improving therapy.

487. (85) **NEW BIOMARKERS AS PUTATIVE PREDICTORS OF BREAST CANCER PROGRESSION**

Marianela Sciacca[1,4], María del Pilar Carballo[2], Ezequiel Lacunza[3,4], Lina Marino[2], María Fernanda Roca[2], Martin Abba[3,4], Érica Rojas Bilbao[2], Ana María Eiján[1,4]and Catalina Lodillinsky[1,4].

[1]ResearchArea, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Department of Pathology, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina. [3]CINIBA, School of Medical Sciences, National University of La Plata, La Plata, Argentina. [4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Ductal carcinoma in situ (DCIS) in breast cancer is defined as a proliferation of epithelial neoplastic cells contained within the lumen of mammary ducts (Cowell, 2013). Currently, evidence suggests that *in situ* tumors are precursors of invasive tumors, but progression markers are not yet available. By using an intraductal xenograft model, we demonstrated that membrane-type 1 matrix metalloproteinase (MT1-MMP) is essential for the transition from *in situ* to invasive tumors (Lodillinsky, 2016; 2021). Subsequently, we completed an RNAseq analysis (Illumina HISeqTM, $p<0.05$, FDR <0.05) based on differential MT1-MMP expression (MT1-MMP^{HIGH} vs MT1-MMP^{low}) where we identified 47 differentially expressed genes. A Molecular Portrait of High-Grade DCIS was previously performed (Abba, 2015). When comparing their genetic profile against the MT1-MMP^{high} population, we found up-regulated SPARC, PTGS2, and CLCA2 only in the most aggressive group (DCIS-C1) and in the MT1-MMP^{high}cell population, highlighting them as promising targets in early breast cancer progression. SPARC expression was analyzed in a breast tumors cohort with *in situ* features obtained in the Pathology Department of the Institute of Oncology A. H. Roffo(n=60). We observed that SPARC expression was higher in tumors with worse prognosis and nuclear grade III. We also found a positive correlation between SPARC and MT1-MMP expression in 1217 breast cancer samples (from TCGA, p-value$< 2.2e^{-16}$), and an increase of SPARC expression in tumor and metastatic tissues compared to normal tissue. When SPARC is knocking down in LM38-LP cell line (qPCR,Crl vs siSPARC, **** $p<0.0001$,T-test), the MT1-MMP-dependent capacity of gelatin degradation is reduced (Crl vs siSPARC, T-test, p-value= 0,0216),demonstrating the pro-invasive role of SPARC. Our results suggest that SPARC should be regarded as a pro-tumoral marker of early breast cancer progression.

488. (86) **SUBPOPULATIONS OF STROMAL CELLS INDUCE DIFFERENTIAL EFFECTS ON STEM CELL STATES IN BREAST CANCER/ SUBPOPULATIONS OF STROMAL CELLS ALTER DIFFERENTIALLY MAMMOSPHERE FORMATION ABILITY OF BREAST CANCER CELLS.**

Osinalde TM, Giorello MB, Chasseing NA, Vellón L.

Within the tumor microenvironment, certain subpopulations of stromal cells are able to trigger aberrant tissue reparative processes that, in turn, favor tumor growth, including the acquisition/loss of cancer stem cell (CSC) states. Here, we addressed whether conditioned media from CD105+ CD34- and CD105- CD34- subpopulations of stromal cells non-associated to blood vessels (CD105+/CM and CD105-/CM, respectively) from breast cancer (BC) patients was able to affect mammosphere formation and migration in BC-derived MCF-7 cells. First, we tested MCF-7 cells mammosphere formation ability in the presence of 20% and 50% CM and did not observe significant differences in the amount or morphology of the mammospheres, thus selecting the 20% CM condition for further assays. Next, we assessed whether CD105+/CM and the CD105-/CM altered the frequency of stem cells, as measured by mammosphere formation and quantified by extreme limiting dilution assay (ELDA) and further statistical analysis with a specialized software (http://bioinf.wehi.edu.au/software/elda). We found that the CD105-/CM increased mammosphere frequency (1/19,4; CI=95% 1/39,8-1/9,6) when compared to the CD105+/CM (1/29,5; CI=95% 1/59,4-1/14,8) and the CM-control (1/21,2; CI=95% 1/43,4-1/10,5). Even though it is necessary to add conditioned media from more patients to the study, these results suggest that different subpopulations of stromal cells from the breast of BC patients differentially affect mammosphere formation and, hence, stem cells states in BC-derived epithelial cells.

489. (107) **FIBRONECTIN-BINDING INTEGRINS PARTICIPATE IN THE CONTROL OF GEF-H1 ACTIVATION AND ITS ROLE IN BREAST CANCER**

Lucía Fernández Chávez, Vicente Bermúdez, Exequiel Gonzalo Alonso, Karen Schweitzer, María Julia Ferronato, María Eugenia Fermento, Eliana Noelia Alonso, María Marta Facchinetti, Alejandro Carlos Curino and Georgina Pamela Coló.

Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-UNS-CONICET), Argentina.

Rho family of small GTPases plays a crucial role in several biological processes and cycles between on and off states. This switch is controlled by GEFs (Rho-activators) and GAPs (Rho-inhibitors). We have previously identified by Mass Spectrometry (MS) analysis that GEF-H1 is the major GEF that activates RhoA and we observed a significant GEF-H1 overexpression in human breast cancer biopsies compared with normal breast tissue. The aim of this work is to study the role of GEF-H1 depletion in tumor development and the mechanism of GEF-H1 fibronectin (FN)-binding integrins dependent activation. Using CRISPR/Cas9 technology, we generated GEF-H1-knock out (KO) cells in a murine invasive breast cancer cell line (LM3). GEF-H1-KO cells were implanted subcutaneously in the mammary fat pad of BALB/c mice, showing a significant decrease in tumor formation (n=14, $p<0.0004$), lung metastasis development (n=12, $p<0.0348$) and an increase in apoptosis (BAX staining n=12, $p<0.0001$) compared with mice inoculated with WT cancer cells. In order to study the mechanism of GEF-H1 activation, we used mouse fibroblasts that only express FN-binding integrins (pKO-α5β1 or -αvβ3). After immunostaining we observed that αvβ3 integrins triggers the dissociation of GEF-H1 from microtubules, leading to RhoA activation, stress fibers (SF) formation and focal adhesions (FA) maturation. The opposite effects were observed after GEF-H1 depletion, suggesting that αvβ3 integrins control RhoA activity thought GEF-H1 activation. After analyzing pKO-fibroblast MS-phosphoproteomics data and looking in several phospho-sites databases, combined with different kinase inhibitors assays, we found MARK2/3 as a candidate for GEF-H1 phosphorylation. Indeed, S151 was found as the main site involved in the regulation of GEF-H1 localization and activity. These findings indicate that activation of GEF-H1/RhoA is orchestrated in FN-adherent cells in an integrin-specific manner and promotes tumor and metastatic processes.

490. (131) **MODIFICATIONS OF THE TUMOR MICROENVIRONMENT (TME) DURING THE EARLY STAGES OF THE M-234p TRIPLE NEGATIVE MURINE MAMMARY ADENOCARCINOMA GROWTH**

Matías E. Fusini[1], Johan Pinzón Rivas[1], Viviana R. Rozados[1,2], O. Graciela Scharovsky[1,2,3], Leandro Mainetti[1,2], María José Rico[1,2]

[1]Instituto de Genética Experimental, Facultad de Ciencias

Médicas, Universidad Nacional de Rosario, [2]CONICET, [3]CIC-UNR.

The TME and its interaction with tumor cells are recognized as essential factors for tumors' progression. The complex nature of TME, integrated by different cell types, fibers and mediators allow different approaches to understand its role in cancer. Our aim was to study the variations in several TME components along the 1st week of tumor growth and to relate them to tumor proliferation level in early tumor stages. BALB/c mice were challenged s.c. with M-234p. On days 3, 5 and 7, groups of mice were sacrificed, tumors excised, fixed and paraffin included. TME components were histologically and immunohistochemically studied. The results obtained when comparing day 7 (N=6) *vs* day 3 (N=6) were: less N° of eosinophils/mm^2, P<0.01; higher % of collagen area, P<0.01; higher N° of αSMA$^+$ cells, P<0.01; higher N° of HIF-1α$^+$ cells, P<0.01; the lymphocyte infiltrate slightly increased from days 3 to 5 and then, decreased on day 7 (day 5 *vs* day7, P<0.01); the structure of blood vessels (BV) on day 3 was near normal with vascular permeability (VP +) and vascular congestion (VC +), while on day 7, BV showed a highly altered structure, with interrupted connective tissue sheet, few endothelial cells with intercellular gaps forming a discontinuous BV and lack of pericytes with increased VP (+++) and VC (+++). At the same time points, the N° of mitosis/mm^2 in tumor cells were higher on day 7 than on day 3, P<0.01. During the early phases of tumor growth take place several important modifications of TME which facilitate its progression and correlate with a concomitant increase in tumor proliferation, namely: decrease in eosinophils diminishing part of the innate immune response, increase in collagen area and αSMA$^+$ cells, indicating higher presence of cancer associated fibroblasts, increase in HIF-1α$^+$ cells together with a change of BV going from normal to abnormal. These results suggest several treatment interventions pointing to particular TME elements to be tested in the future.

491. (132) **ENVIRONMENTAL CONCENTRATIONS OF HEXACHLOROBENZENE REDUCE THE EFFICACY OF DOXORUBICIN AND PACLITAXEL TREATMENT ON TRIPLE NEGATIVE BREAST CANCER CELLS**

Yamila Sanchez[1], Abigail Vasquez[1], Gabino Rolandelli[1], Catalina Costas[1], Andrea Randi[2], Alejandro Español[1]

1 Laboratorio de Inmunofarmacología Tumoral, Centro de Estudios Farmacológicos y Botánicos, CONICET- Universidad de Buenos Aires

2 Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires

The contamination by hexachlorobenzene (HCB) is a relevant problem since it has been detected in human serum and breast milk. It activates Aril hydrocarbon Receptor (AhR), which is associated with tumor development. HCB induces proliferation in different types of breast tumor cells, but it is unknown its role in antitumor breast therapy. Triple negative (TN) subtype constitute 10% of breast tumors and present a bad prognosis due to the lack of specific therapeutic targets. They are treated with chemotherapeutic drugs such as paclitaxel (PX) and doxorubicin (DX). The aim of this work was to determine if HCB modulates the antitumor effect of PX and DX in the human TN breast cancer cell line MDA-MB231. We demonstrated by Western blot assays that these cells express AhR, and by MTT assays that HCB does not modify their viability in the range of 10^{-12}M-10^{-5}M (p>0.05). These cells are sensitive to PX and DX in a concentration-dependent manner (CI50PX:1.34x10^{-3}M CI-50DX:6.1x10^{-10}M). PX (10^{-7}M) exerts its effect by modulating Ras (89.8±5.5% <p0.001), p38MAPK (95.4±8.5% p<0.001) and NF-KB (45.0±4.8 p<0.01), and DX (10^{-7}M: 48.7±3.6%) does it by modulating ERK1/2 (30.5±3.9% p<0.01). The pre-treatment during long periods of time with HCB in relevant environmental concentrations ($10^{-8/-7}$M) shifts PX and DX concentration-response curves to the right indicating a reduction in the sensitivity to the antitumor therapy (p<0.001). The modulation of PX effect by HCB (96.8±7.1% p<0.001 vs PX) is mediated by NF-KB (69.9±2.1% p<0.001) and the modulation of DX effect (84.9±0.4% p<0.001 vs DX) is mediated by ERK1/2 (51.6±4.2% p<0.001 vs DX), since the preincubation with the selective inhibitors of these kinases reverses the effect of HCB. We conclude that, although HCB in relevant environmental concentrations does not modulate the proliferation *per se*, it exerts an inhibitory effect in PX and DX antitumor therapy in TN breast cancer cells with the participation of NF-KB and ERK1/2 kinases.

492. (139) **STUDY OF ANTITUMORAL ACTIVITY OF COMPOUNDS DERIVED FROM NATURAL PRODUCTS. EFFECT OF NAPHTOQUINONES ON BREAST CANCER PROGRESSION**

Luciana Cañonero[1], Aldana Schey[1], Lizeth Ariza Bareño[1], Andrés Bechis[1], Diego J. Britez Neira[1], Julián Fernandez[2], Juan Robirosa[2], Jorge Palermo[2], Laura B. Todaro[1], Alejandro J. Urtreger[1].

1- Área Investigación, Instituto de Oncología "A. H. Roffo", Facultad de Ciencias Médicas, Universidad de Buenos Aires, Buenos Aires, Argentina.

2- Laboratorio de Unidad de Microanálisis y Métodos Físicos en Química Orgánica (UMYMFOR) de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Breast cancer is a major public health problem, being the second leading cause of cancer death in women on developed countries. Conventional therapies such as surgery, chemotherapy and radiotherapy seem to have reached a plateau of therapeutic efficacy. Therefore, the study of naphthoquinones, a family of natural compounds (and semi-synthetic derived compounds) is proposed as an alternative for the treatment of these neoplasms. Naphthoquinones are a group of highly reactive organic species that present anti-inflammatory activities at very low toxicity rates and till nowadays their usefulness in oncology remains unexplored. In the present work, eleven 5-(N-indolyl)-1,4-naphthoquinones substituted with anilines at C-2 or C-3 were tested, employing normal and tumor derived mammary cell lines. The original naphthoquinone (N0) and 3 derivatives (named 4-pMT, 5-AnT and 6-AnC) showed a strong antiproliferative effect with an Inhibitory Concentrations 50 (IC50) of 5.8±0.2 μM, 15.25±2.5 μM, 24.7±1.9 μM and 9.9±1.2 μM respectively (MTS assays). Through flow cytometry we could determine that LM3 cells, treated with the N0 and 5-AnT compounds, increased the Sub-G0 and S fractions of the cell cycle, events compatible with the presence of apoptotic cells and with an impairment in cell replication respectively. These compounds also increased the expression of BAX and PARP two proapoptotic markers, analyzed by Western Blot. In contrast, in the normal cell line NMuMG, no significant alterations were observed in cell cycle stages or in the aforementioned apoptotic markers. Based on these results, we consider that naphthoquinones and their derivatives are promising compounds to continue their study in preclinical settings and may, in the future, become a new tool for the treatment of breast cancer.

493. (140) **TGF-β SIGNALING PATHWAY IS MODULATED BY GPC3 IN BREAST CANCER CELLS**

Maia Jazmín Martinez Gomez[1], Mariángeles Díaz[1], Magali Delgado Pastore[1], Ana Clara Lugones[1], Ramiro Perrotta[2], Stella Maris Ranuncolo[1], Mariana Salatino[2], María Giselle Peters[1].

[1]Área Investigación, Instituto de Oncología "Ángel H. Roffo"-UBA, [2]Laboratorio de Glicomedicina, IBYME-CONICET

We have previously shown that GPC3 can induce a reversal of the epithelial-mesenchymal transition (EMT) traversed by mammary tumor cells. Through EMT modulation, GPC3 inhibits the metastatic spread. Since the TGF-β pathway is a key regulator of EMT, we set out to analyze whether the effects of GPC3 on tumor progression are mediated by this pathway. We performed *in silico* studies using the TCGA database. In association with the impact of GPC3 on EMT, gene enrichment analysis showed that some signatures involved in the EMT inhibition are upregulated in patients with tumors with high GPC3 expression levels (FDR<0.05, NES=1.58). Moreover, the signature related to the activation of the TGF-β pathway was downregulated in this group of patients (FDR<0.05, NES=-1.51). To deepen

these findings, we conducted *in vitro* studies using breast cancer cells with the GPC3 expression modified. By WB we showed that the GPC3 overexpression in MDA-MB231 cells induced a reduction in Smad2/3 phosphorylation, while the GPC3 silencing in MCF-7 cells caused its increase. These results suggest that GPC3 inhibits the TGF-β pathway. To confirm this, we studied the Smad4 subcellular localization. Our WB showed lower levels of that molecule in the nuclear fraction of MDA-MB231-GPC3 cells, while Smad4 nuclear levels were higher in MCF7-sh GPC3 cells. To assess the TGF-β signaling inhibition role in the GPC3-induced cellular effects, we reversed this inhibition by treating MDA-MB231-GPC3 cells with recombinant TGF-β. When we analyzed cell morphology, we found that while GPC3-overexpressing MDA-MB231 cells exhibited an epithelial phenotype, the TGF-β pathway activation caused a recovery of the fibroblastic appearance of MDA-MB231 cells. This result suggests that the inhibition of TGF-β signaling is necessary for the GPC3 effect on the EMT process. In summary, our results highlight the central role of the TGF-β pathway on the effects of GPC3 in EMT completed by breast cancer cells.

494. (146) **LEVOGLUCOSENONE AND ITS DERIVATIVES: NEW ALTERNATIVES FOR BREAST CANCER TREATMENT**

Aldana M. Schey[1], Lizeth Ariza Bareño[1], Luciana Cañonero[1], Andrés Bechis[1], Diego Britez Neira[1], Florencia Cámpora[2], Beatriz E. Pava Gómez[2], Ariel M. Sarotti[2]. Alejandra Suarez[2], Rolando Spanevello[2], Marcela Villaverde[1], Laura B. Todaro[1], Alejandro J. Urtreger[1].

1- Área Investigación, Instituto de Oncología "A. H. Roffo", Universidad de Buenos Aires, Buenos Aires, Argentina.

2- Instituto de Química de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario-CONICET.

Breast cancer is a major public health problem, being the second leading cause of cancer death among women in developed countries. Levoglucosenone results from pyrolytic treatment of cellulose-containing materials, and it has been used for the synthesis of different biological compounds but its usefulness in oncology remains unexplored. In vitro, levoglucosenone (compound 1) and structurally related derivatives (compounds 2, 3 and 4) showed a strong antiproliferative effect associated with apoptosis induction. In vivo compounds 1 and 2 highly reduced tumor growth and lung dissemination. In this work we determined the effect of combining the compounds with cell metabolism modulators and evaluated the effect of new derivatives. Using the fluorophore TMRM we observed that the compounds induce the loss of mitochondrial membrane potential which could the responsible of the apoptosis induction. Moreover, as an energetic compensatory process, a 2-fold increase of glucose consumption and lactate production was detected ($p<0.05$). This result prompt us to investigate whether the combination with glycolysis inhibitors could increase the effect of the compounds on cell viability. Interestingly, the hexokinase inhibitor, 2-deoxyglucose, significantly enhanced levoglucosenone effect ($p<0.05$). Loewe curves from Combenefit software described this interaction as a synergistic effect ($p<0.01$). Recently, 27 new derivatives have been developed and explored on their in vitro biological activity. Only 3 of these compounds (58A, 59A and 37D) were capable of inducing cell death with IC50 values of 5,9±2; 5,4±3 and 7,2±4 uM for LM3 and 12,7±5; 4,5±2 and 21,9±6 uM for 4T1 cells. In most cases, these IC50 values were lower than those presented by compounds 1 to 4, being the in vivo study promising. Based on our results, we believe that our compounds could become an important alternative for breast cancer management, either as a single drug or enhancing the effect of pre-existing therapies.

495. (179) **ANTIPROLIFERATIVE EFFECT OF *COLEUS NEOCHILUS* EXTRACT ON HUMAN BREAST CANCER CELL LINES**

Carla Luciana Mayora Justel[1], María del Carmen Esandi[1,2], Carlos Villamil[2], Carolina Gomila[1], Gabriela Salvador[1,2], Cecilia Bouzat[1,2], Isabel Lüthy[3], Natalia Alza[1] and Ariana Bruzzone[1]

[1]Instituto de Investigaciones Bioquímicas Bahía Blanca-CONICET, [2]Departamento de Biología Bioquímica y Farmacias-UNS, [3]Instituto de Biología y Medicina Experimental-CONICET

The *Plectranthus* species have a rich diversity of ethnobotanical uses. Nevertheless, the most frequently use of these species is for their medicinal properties. This work focuses on *Coleus neochilus* (Schltr.) Codd, also known as "boldo rastrero" (BR), a specie closely akin to the genus *Plectranthus*, and its effect on human breast cancer cell lines. A crude ethanolic extract of leaves of BR was prepared. BR incubation decreased cell viability in MCF-7, MDA-MB-231 and T47D cancer cell lines. The extract concentration that caused a 50% inhibition in cell viability (IC_{50}) was lower for tumor cells than for non-tumor cells (about 45, 19, 26 vs 50 for MCF-7, MDA-MB-231, T47D and MCF-10A respectively). At 10 µg/ml, BR reduced cell proliferation in all tumor cells but not in non-tumor cells (i.e., 45% reduction in MCF-7, $p<0.001$). An additive effect of BR with therapeutic drugs was observed on reduction of cell viability (MCF-7 cells: BR: 14% reduction, 10 µM tamoxifen (Tx): 53% reduction, BR+Tx: 67% reduction; MDA-MB-231 cells: BR: 25% reduction, 10 nM paclitaxel (Px): 36% reduction, BR+Px: 53% reduction). Using a colorimetric assay, we detected a 24 % increase in caspase activity after 12 h ($p<0.05$), suggesting a BR-mediated apoptosis induction. Oxidative stress was not induced after BR exposure. From the original ethanolic extract, three different fractions were obtained by extraction with different solvents: chloroform, ethyl acetate, methanol-water. In MCF-7 and MDA-MB-231 cells, reduced cell viability was observed only after incubation with the chloroform fraction, indicating the presence of bioactive molecules responsible for the cytotoxic effect. In conclusion, these results demonstrate the antiproliferative properties of BR on breast cancer cells. Moreover, a new additive effect of clinical therapeutic drugs and BR in tumoral cells was described.

496. (212) **WNT PATHWAY DYSREGULATION AND ITS RELEVANCE IN ENDOCRINE RESISTANT BREAST CANCER MODELS**

Marcela Coianis[1], Virginia Figueroa[1], Gabriela Pataccini[1], Silvia Vanzulli[2], Claudia Lanari[1], Caroline A. Lamb[1].

1: Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina.

2: Patóloga independiente, Argentina

Endocrine therapy is the standard treatment for patients with luminal breast cancer. However, after treatment most patients develop hormone resistance, by mechanisms that may include dysregulation of growth factor signaling pathways. Fibroblast growth factor 2 (FGF2) consists of a secreted low molecular weight form and several nuclear high molecular weight forms (HMW-FGF2). We previously demonstrated that HMW-FGF2-overexpression in endocrine responsive T47D cell lines, induced hormone resistance, dysregulation of the WNT signaling pathway and an increase in androgen receptor (AR) expression. We hypothesize that FGF2 induces WNT pathway activation which, in turn, induces AR expression. The aim of this study was to evaluate the expression of downstream effectors of the WNT/β-catenin pathway in endocrine resistant breast cancer models to assess if targeting this pathway may be an effective treatment for these tumors. We used the endocrine resistant T47D-HMW-FGF2 and T47D-YB cell lines, the latter naturally expressing higher levels of HMW-FGF2 than the responsive T47D cells, to determine the expression of different WNT/β-catenin effectors compared with the parental cell lines growing *in vitro* and/or *in vivo*. T47D-HMW-FGF2 tumors expressed significantly higher levels of WNT4, DVL3 and AXIN1 while T47D-YB cells expressed higher DVL2 levels compared to control cells ($p<0.05$). Trends towards upregulation of other WNT family members were found in resistant compared to responsive cell lines. We found that blocking the WNT pathway with LGK974 or ICG001 inhibitors, reduced cell proliferation of T47D-HMW-FGF2 and T47D-YB cells ($p<0.05$). *In vivo*, we previously showed that LGK-treatment of T47D-HMW-FGF2 reduced tumor growth and, herein, we found a decrease in phosphorylated LRP6 and AR. Our results suggest that targeting the WNT and/or AR pathway may be an alternative therapy for endocrine-resistant breast carcinomas.

497. (226) **DECIPHERING THE CAUSES FOR A LATE FIRST MEDICAL CONSULTATION AMONG BREAST CANCER PATIENTS WHO ATTEND A SUBURBAN BUENOS AIRES HOSPITAL**

Marcela Coianis[1*], Leo Saldain[1*], María Victoria Grandoni[2], Cecilia Surdo[2], Javier Burruchaga[2], Paula Martínez Vázquez[2], Andrea Donati[2], Nancy De Mori[2], Pedro L. Casserly[2], Beatriz L. Kennel[2], Eunice Spengler[2], Claudia Lanari[1], Caroline A. Lamb[1].
**: equal contribution*
1: Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina.
2: Hospital Magdalena V. de Martínez, General Pacheco, Buenos Aires, Argentina

Breast cancer (BC) is still one of the main causes of cancer mortality in women worldwide. Delays in diagnosis and treatment impact on patient survival and thus, identifying women with higher risk of late presentation may allow designing preventive strategies. The aim of this study was to evaluate the time for first medical consultation among women with BC attending the *Magdalena V. de Martínez* hospital and to determine the causes that may influence this delay and its impact on cancer stage at diagnosis. Three hundred BC patients were interviewed using a self-reported questionnaire and socioeconomic and demographic variables were collected, namely highest education level completed, employment status and BC awareness. These answers were associated to patient clinical records, clinical staging, and tumor size, among others. The median age of our cohort was 58 years and the mean age of first childbirth was 22 years. Seventy one percent of the patients had incomplete primary or high school education and 77% of the tumors (231/300) were self-detected. Moreover, delay in the first medical visit was more than one month in 77% of the cases, being ignorance the most common cause of postponement. Even though 77% reported awareness of breast self-examination, only 22% of the patients practiced it. Regarding tumor size, 87% of the tumors were palpable (T1c-T4) and 47% of the patients had locally advanced or metastatic stages at their first visit. We found significant differences in the proportion of patients with incomplete school education and higher delays in first visit (*Fisher* p=0.017). Also, patient delays were associated with larger tumors (*Fisher* p=0.029) and advanced stage (lymph-node positive IIa to IV; *Fisher* p=0.0046). These results indicate that efforts should be aimed at early detection to reduce the stage at diagnosis which may impact on overall survival. A major drawback seems to be the difficulties in completing school studies in low-income households.

498. (237) **MECHANISM OF 2´-NITROFLAVONE AND SAFINGOL ANTITUMOR SYNERGISTIC COMBINATION IN MAMMARY CANCER CELLS**

Juan Manuel Anselmi Relats, Leonor Roguin, Mariel Marder, Julieta Marino, Viviana Blank
Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET), Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Sphingosine kinase 1 (SphK1), a lipid kinase overexpressed in some mammary tumor cells, regulates the balance between proapoptotic ceramides and prosurvival sphingosine-1-phosphate. Furthermore, SphK1 inhibitors prevent catabolism of ceramides, contributing to tumor cell death. It has been reported that certain flavonoids exert antitumor activity through an increment in ceramide levels. Previously, we demonstrated that the synthetic flavonoid 2´-nitroflavone (2NF) and the SphK1 inhibitor safingol synergistically inhibited cell proliferation and induced apoptosis in LM3 murine mammary tumor cells. In this work, we examined the modulation of JNK and ERK 1/2 MAPK transduction pathways. Results showed that after 1h of incubation with both compounds, the levels of p-JNK and p-ERK1/2 MAPKs were significantly increased (2,3 ± 0,3 fold, $p<0,05$; 1,5 ± 0,1 fold, $p<0,01$, respectively). Moreover, the antiproliferative effect of the combination was reverted when cells were preincubated with JNK SP600125 or ERK1/2 PD98059 inhibitors. Additionally, we studied the antitumor efficacy of 2NF combined with safingol in human mammary MDA-MB-453 cancer cells and we also found a synergistic reduction of cell growth after treatment with 5 μM 2NF and 0,625 μM safingol. Analysis by Compusyn software showed Combination Index values of 0,63 ± 0,08 (48 h) and 0,73 ± 0,06 (72 h). Ethidium bromide and acridine orange staining revealed that safingol potentiated the apoptosis induced by 2NF in these cells. When we evaluated the expression of Bax proapoptotic protein, results obtained by Western blot showed a 3,6 ± 0,4 fold increment after cell incubation with both 2NF and safingol for 24 h ($p<0.001$). Taken together, these results demonstrated that the combination of 2NF and safingol induced a synergistic antitumor effect in mammary cancer cells, probably mediated by the activation of JNK and ERK1/2 MAPKs pathways.

499. (246) **ROLE OF EXTRACELLULAR VESICLES IN BREAST CANCER MICROENVIRONMENT**

Maria Celeste Rodriguez-Baili, German Alejandro Gil
CIQUIBIC-CONICET. Quimica Biologica Ranwel Caputto, FCQ.UNC. Córdoba.
email:celeste.rodriguez@unc.edu.ar

Antiestrogen adjuvant treatments are first-line therapies in patients with estrogen receptor-positive (ER +) breast cancer. The treatment strategies need to be improved because most patients eventually become endocrine resistant and many others are initially refractory to these treatments. The tumor microenvironment, mainly macrophages, play an essential role in the development and progress of cancer; however, the molecular mechanisms underlying these effects remain poorly understood. Extracellular vesicles (EVs) secreted by almost all cells have been proposed as one of the main forms of cell-cell communication, being involved in proliferation, migration, endocrine resistance, invasion, drugs administration, among others. We proposed that EVs are one of the most important players in cell communication and could be one of the responsible for the endocrine resistance that we had observed in our previous work. When MCF7 cells were cultured with EVs coming from macrophages conditioned with TNF, the proliferation measured with Alamar Blue was significantly increased after 24 hours in presence of the EVs subtype 100K and 200K pellet (p value 0.0021 and 0.0019), on the other hand the MCF10A shown a significantly increase in presence of the EVs subtypes 10K and 200K (p value 0.016 and 0.006). We performed a wound healing assay and the combination of both subtypes of EVs 100K and 200K coming from TNF macrophages increased migration in MCF7 cells after 48 hours (p value 0.0327). All the results were obtained from three independent experiments, with Two-way ANOVA and Dunnet´s post test analysis, and suggest that EVs are involved in the proliferation and migration of mammary cells, and that this increase depends on the EV subtype. Preliminary results suggest that EVs from a stable MCF cell line expressing CD63-GFP (EVs-marker) can be uptake by macrophages and transform them into an M2-like profile that reveals the role of EVs in modulating the recipient cell phenotype.

500. (251) **EFFECT OF THE COMBINED TREATMENT OF THE NOVEL TELOMERASE INHIBITOR R1D2-10 WITH PACLITAXEL IN HUMAN BREAST CANCER CELL LINES**

Vilarullo Roman Nicolas, Mengual Gómez Diego Luis, Balcone Lara, Maggio Julian, Gomez Daniel Eduardo*, Armando Romina Gabriela*
Molecular Oncology Unit, Center of Molecular and Translational Oncology, Quilmes National University, Bernal B1876BXD
**Contributed equally*

Cellular immortality is one of the main features of cancer. Tumor cells have an unlimited replicative potential, principally due to telomerase activity, which acts adding TTAGGG repetitions at the end of chromosomes in each cell division. For this activity it is necessary the assembly of many components, where the most relevant are the catalityc retrotranscriptase (hTERT), the RNA template (hTR) and diskeryn (DKC1). In our previous work we developed and evaluated a novel inhibitor of telomerase assembly, selecting as target the interaction between hTR-DKC1. Briefly, we performed a Dock-

ing-Based Virtual Screening against PUA domain of DKC1 and selected R1D2-10 compound, which showed in vitro inhibitory effect on telomerase activity, caused telomere shortening and induction of senescence and apoptosis. Based on these results, this work aims to evaluate the effect of R1D2-10 treatment in three breast cancer cell lines: MDA MB 231, MDA MB 468 and MCF-7. Regarding cell proliferation, we observed an inhibitory effect in the three lines, obtaining a IC50 value of 9,52 μM to MDA MB 231, 9,79 μM to MDA MB 468 and 5,95 μM to MCF-7. Based on this, we defined a non-cytotoxicity concentration of 2 μM to evaluate telomerase activity. We carried out a RQ-TRAP assay, and found that R1D2-10 treatment for 48 h caused a telomerase inhibition of 57,3% in MDA MB 231, 30,8% in MDA MB 468 and 64,7% in MCF-7 in comparison to control (Unpaired T-Test, P<0,05). Given these results, we decided to explore the effect of R1D2-10 combined with Paclitaxel, a conventional chemotherapeutic agent employed in breast cancer, using a cell proliferation assay. Results were analyzed by Compusyn Software, confirming a synergistic effect of these drugs in the aforementioned cell lines (CI<0,8). The obtained results set the basis for continuing with preclinical evaluation of R1D2-10, aiming to reveal its potential clinical use for breast cancer treatment.

501. (266) **EFFECTS OF 2'NITROFLAVONE ON THE EGF SIGNALING PATHWAY AND CELL PROLIFERATION IN BREAST CANCER CELLS**

Julieta R Cebrón[1,2], Mariana A Bojorge[1,2], Mariel Marder[1,2], Johanna G Miquet[1,2], Lorena González[1,2].
[1] Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (UBA). [2] Instituto de Química y Fisicoquímica Biológica (IQUIFIB), UBA/CONICET.

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor associated with tumorigenesis of several tissues and has been proposed to be involved in the molecular mechanism of action of flavonoids. For that reason, flavonoids are proposed to be combined with drugs that target this receptor. 2'nitroflavone (2'NF) is a synthetic flavone obtained in our institute that has previously demonstrated to produce apoptosis in breast cancer cells and affect the expression of receptors related to EGFR activity. With the aim of investigating if a combinatory therapy involving 2'NF and EGFR inhibitors would be a possible effective treatment for breast cancer, the present study analyzes if this flavone modulates the EGF signaling pathway and cell proliferation activity. MDA-MB-231 and MCF-7 breast cancer cells were treated with 2NF at a concentration of 20 μM and 10 μM respectively or vehicle for 1 and 48 h. Afterward, cells were stimulated with EGF (25 ng/ml) for 10 min. The protein content and phosphorylation at activating residues of EGFR, Akt and Erk were assessed by immunoblotting. Besides, the effect on cell proliferation was determined by hexosaminidase assay. For this determination MDA-MB-231 and MCF-7 cells were seeded and treated with 2'NF (20 μM and 10 μM respectively) or vehicle and EGF (25 ng/ml) for 48 h. Results showed a reduction in EGFR phosphorylation induced by EGF after 48 h of treatment with 2'NF in both cell lines ($p<0.05$); while in the case of Akt there is a diminution in its phosphorylation after 1 ($p<0.05$) and 48 h ($p<0.01$). In the case of Erk phosphorylation there is a decrease in MCF-7 ($p<0.01$) but an increase in MDA-MB-231 ($p<0.05$). As regards EGF-induced proliferation, a decrease was observed in both cell lines ($p<0.0001$) when cells were treated with 2'NF. In conclusion, 2'NF demonstrated to have effects on EGF signaling pathway and cell proliferation activity which could justify a combinatory therapy involving 2'NF and EGFR inhibitors.

502. (294) **EFFECT OF INHIBITION OF THE WNT PATHWAY IN BASAL-LIKE MAMMARY TUMOR CELLS**

Lucía P. Petriz Otaño[1], Carla M. Felcher[2], M. Jimena Mosna[1], Federico J. Garde[1], Marcelo G. Stinson[1], Daniela Di Bella[3], Edith C. Kordon[2], Abel L. Carcagno[1]
[1]Cell Differentiation and Cancer Laboratory, ETI1, IQUIBICEN-UBA-CONICET, CABA, Argentina. [2]Normal and Neoplastic Development of the Mammary Gland Laboratory, IFIBYNE-UBA-CONICET, CABA, Argentina. [3]Department of Stem Cell and Regenerative Biology, Harvard University, Boston, USA.

In 2020, breast cancer ranked first regarding cancer incidence and mortality worldwide. Basal-like tumors represent approximately 15-20% of all breast cancers and show aggressive behavior, with a high probability of recurrence and few specific therapies. Our aim was to study *in vitro* and *in ovo* the effects of knocking down R-spondin 3 (Rspo3), which (as demonstrated previously) enhances the canonical Wnt pathway in the SCg6 basal-like tumor cell line. Using flow cytometry assays with Propidium Iodide staining, we observed that SCg6 Rspo3 knockdown cells showed increased G1 phase compared to control cells, which suggests that reducing Rspo3 expression affects cell cycling. However, cell viability, analyzed by MTT and Trypan Blue assays was moderately or not significantly altered by reducing Rspo3 levels. For the optimization of the *in ovo* assay, tumors from SCg6 spheroids were successfully grown on the chick chorioallantoic membrane (CAM), which constitutes an alternative tool to validate the results observed *in vitro*. Moreover, canine mammary biopsies were correctly grafted onto de CAM, which better recapitulates physiological conditions and tumor heterogeneity. Finally, single-cell RNA sequencing datasets from oncologic patients were processed and analyzed in order to explore cellular heterogeneity in basal-like tumors. Additionally, through measurement of the expression of Rspo3 and target genes of the Wnt pathway, it was possible to evaluate the relevance of therapies that modulate these genes. These results highlight the impact of the Wnt pathway in the acquisition of tumorigenic characteristics of basal-type breast cancer cells. In addition, grafting of biopsies onto the CAM will allow for *in ovo* testing of Wnt pathway inhibiting drugs, already available at the laboratory, to evaluate the efficacy of this therapeutic strategy *in vivo*.

503. (296) **EFFECT OF RUNX2 INHIBITION *IN VITRO* AND *EX VIVO* IN LUMINAL BREAST CANCER MODELS**

María Sol Rodríguez[1], John Bushweller[2], Isabel Lüthy[1], Claudia Lanari[1], Cecilia Pérez Piñero[1].
1. CONICET-Instituto de Biología y Medicina Experimental, CABA, Argentina, 2. Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA

We have previously shown that RUNX2 overexpression in luminal breast cancer models leads to an increased FGFR2 expression and generates endocrine and PD173074 (PD, FGFR inhibitor) resistance. Many inhibitors against FGFR are used in breast cancer patients with endocrine resistance and FGF/FGFR alterations, but several are refractory to this therapy. The aim of this work was to evaluate the effect of a RUNX2 inhibitor (AI14-91, AI) in T47D and MCF7 breast cancer cells *in vitro* and *ex vivo*. We performed proliferation assays using control (C), RUNX2 overexpressing (RUNX2), and constitutively active FGFR2-transfected cells (R2CA). In all cell lines, AI diminished proliferation as compared with control cells (C, RUNX2, R2CA vs AI14 10uM $p<0.0001$). Next, we explored if AI could reverse the FGF2- induced stimulation of cell proliferation in a hormone-depleted culture setup. In MCF7 cell lines, FGF2 stimulation was reversed by AI (FGF2 + AI vs FGF2 $p<0.001$). T47D-R2CA showed a higher sensitivity to AI, while this inhibitor did not reverse FGF2 stimulation in T47D-RUNX2. T47D-C showed little FGF2 stimulation and no AI reversion. Then, we used an *ex vivo* model and treated tumor slices for 48h with control media, AI (10uM) or PD (0,1uM), and performed Ki67 immunohistochemistry to analyze the proliferation index in response to the treatments. T47D-C tumor slices showed a lower Ki67 index when treated with AI and PD as compared to untreated ones (T47D: C vs AI $p<0.0001$; vs PD 0,1uM $p<0.0001$). T47D-RUNX2 tumor slices were resistant to both treatments, showing no changes in the Ki67 index. In summary, high levels of RUNX2 expression may induce FGFR-inhibitor resistance suggesting that RUNX2 expression could be used to exclude patients who will receive FGFR target therapies. In addition, our data suggest that AI is effective in cells with endogenous levels of RUNX2 suggesting that RUNX2 inhibitors with a higher potency may be necessary to counteract the overexpression of RUNX2.

ONCOLOGY II *Thursday, November 17, 14-15:30 hr*
Chairs: M. Florencia Gottardo - Mauricio Menacho Márquez - Javier Cotignola

504. (59) **THYROID HORMONES (THS) ACTING THROUGH INTEGRIN AVB3 INDUCE ONCOGENIC SIGNALING PATHWAYS INVOLVED IN T CELL LYMPHOMA (TCL) PROGRESSION**

Debernardi MM[1*], Diaz Albuja JA [1#], Sterle HA[1*], Paulazo A[1], Díaz Flaqué MC[1*], Campos Haedo M[1*], Gonzalo G[1], Rosemblit C[1*], Cremaschi GA[1*], Cayrol F[1*].
1- Laboratorio de Neuroinmunomodulación y Oncología Molecular, Instituto de Investigaciones Biomédicas (BIOMED-UCA-CONICET), Buenos Aires, Argentina.
** - SAIC*
 # - SAFIS

Decoding the molecular mechanisms leading to TCL progression is a complex issue due to the diversity of these malignancies. In most TCL patients the JAK/STAT and NF-κB pathways are over-activated. To find new therapeutic targets, these oncogenic pathways, and the activating factors should be studied deeply. Our previous results show that THs, acting via integrin αVβ3, promotes cell proliferation and survival, and induces an angiogenic program in TCL cells. Here we study if THs are one of the factors involved in the activation of these oncogenic pathways. First, we analyzed TCL cell lines corresponding to immature (CUTLL1) and mature (OCI-Ly12, OCI-Ly13.2) human subtypes after 10, 15, and 30 minutes THs treatment. We found that physiological levels of THs significantly increase STAT1, 3, 5, NF-κB, and ERK phosphorylation in all TCL cells ($p<0.05$). As GATA3 overexpression is associated with a poor prognosis in TCL patients, we also analyzed it and found that, after 48 hours, THs significantly increase GATA3 protein levels ($p<0.05$). Similar results were found in EL-4 murine TCL cells ($p<0.05$). Interestingly, THs effects on STATs phosphorylation and GATA3 expression were blunted by the integrin αVβ3 inhibitor, cilengitide ($p<0.05$). Moreover, we found that cilengitide was able to decrease *in vivo* STAT1 and 5 phosphorylation ($p<0.01$) in EL4 tumors growing *in vivo* in a syngeneic murine model. Finally, we study how THs actions affect the sphingomyelinases (SMases) pathway. We found that the inhibitors of the neutral (imipramine), or acidic (GW4869) SMases significantly revert TH effects on cell proliferation ($p<0.05$), highlighting the importance of this signaling pathway in TCL cells.
Our results provide the rational basis to continue studying the molecular mechanisms of THs actions in malignant cells; this could lead to the identification of new therapeutic targets, like integrin αVβ3, to improve current treatments for TCL patients.

505. (185) **HIF-1α REGULATES TUMOR PROGRESSION IN A HUMAN EPITHELIAL OVARIAN CANCER MODEL**

España De Marco María José[1], Marinoni Rocio, Tesone Marta[1], Pérez Piñero Cecilia[2].
[1] Laboratorio de Fisiología y Biología Tumoral del Ovario – Instituto de Biología y Medicina Experimental (IBYME) – CABA, Argentina
[2] Laboratorio de Hormonas y cáncer – Instituto de Biología y Medicina Experimental (IBYME) – CABA, Argentina

Ovarian cancer is the 7th most common cancer in women and the 8th cause of cancer-related death. The development of specific therapies is needed, as the treatment remains the same after decades. Hypoxia is a characteristic of solid tumors usually associated with a more aggressive phenotype. The main transcriptional factor involved in this process is Hypoxia Inducible Factor 1 alpha (HIF1α). This work aimed to study the inhibition of HIF1α by Acriflavine (ACR) on SKOV3 and IGROV1 human ovarian cancer models, both in vitro and in vivo. We performed proliferation assays with ACR. The results showed a diminished proliferation starting from 0.5 uM ACR (SKOV3: 56.0±7.8 % $p<0.0001$; IGROV1: 64.2±7.2 % $p<0.0001$). To study the effect of ACR on cell migration, we performed wound healing assays for 18 hours. The migration was reduced between 20-40% with 0.5 uM of ACR (SKOV3: 63.4±1 % $p<0.0001$; IGROV1: 83.0±10.3 % $p=0.0123$). For the in vivo experiments, $5x10^6$ cells (IGROV1 or SKOV3) were s.c. injected into the flank of NSG mice. The treatment was ACR ip daily injections (8 or 12 mg/kg, 15 days). ACR-treated tumors were significantly smaller than control tumors in both models (8mg/kg: from day 8 SKOV3 $p= 0.0006$, from day 11 IGROV1 $p<0.0001$; 12mg/kg: from day 8 SKOV3 $p=0.0012$, from day 8 IGROV1 $p=0.0127$), showed a lower proliferation index (Ki67), a lower VEGF and GLUT1 expression through immunohistochemistry and Western Blot as compared with control tumor samples. c-MYC expression was also reduced in ACR-treated tumors. Lung metastases were number-reduced in SKOV3 ACR-treated animals. VEGF and GLUT1 expression are used to evaluate the transcriptional activity of HIF1α, as both proteins are HIF1α downstream targets. In summary, HIF1α plays an important role in the proliferation, migration, tumor growth and metastatic spreading of ovarian cancer models. We conclude that ACR could be a potential drug for the treatment of ovarian cancer, alone or in combination with other drugs.

506. (238) **ALL-TRANS RETINOIC ACID AND CISPLATIN COMBINED TREATMENT IMPAIR MOTILITY CELL PARAMETERS IN CHEMORESISTANT NON-SMALL CELL LUNG CANCER (NSCLC) CELL LINES. IMPLICATION OF CANCER STEM CELLS (CSC)**

Bechis A, Brítez Neira DJ, Ariza Bareño LA, Cañonero L, Schey AM, Urtreger AJ, Todaro LB.
Área de Investigación, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina.

Cisplatin is a non-small cell lung cancer (NSCLC) standard therapy, although there is a frequent resistance acquisition, where the presence of cancer stem cells (CSC) can be associated with. The Retinoic Acid System has been implicated in the maintenance and expansion of CSC, making it as a potential target therapy. The objective of this work is to evaluate the involvement of all-trans retinoic acid (ATRA) in cell growth modulation in a chemoresistance context, in NSCLC human cell lines. To evaluate that, we have developed two cisplatin-resistant variants from NSCLC cell lines: A549 cell line (A549cpr) and NCI-H125 cell line (H125cpr). Monolayers of parental and cisplatin-resistant cells were treated with ATRA (0.3 - 70μM) and/or Cisplatin for 72h. While the Cisplatin treatment induce growth inhibition, ATRA addition did not modify proliferative capacity. Although all cell lines express all nuclear Retinoic Acid Receptors, the cisplatin-resistant cells showed lower levels of the RARβ/RARγ ratio, indicating a less-differentiate phenotype ($p<0.05$, determined by RT-qPCR). According to this, we observed an increase in pluripotential genes expression (NANOG/OCT4 mRNAs) in the cisplatin-resistant cells, followed by an elevated oncosphere (CSC enriched culture) growth rate. Regarding cellular parameters, we observed that cisplatin-resistant cells have less migration and adhesion capacity that control cells. ATRA and cisplatin combined treatment increased adhesive capacity only in cisplatin-resistant cell lines. On the other hand, all treatments increased migration capacity in control cell lines, but decreased this parameter in cisplatin-resistant cell lines. Our results reinforce the hypothesis that cisplatin resistance may be mediated by an increase in CSC renewal. These findings lead us to propose different combination therapies for targeting CSC, as retinoic-acid treatment, to differentiate and sensitize this cell subpopulation.

507. (291) **ANALYSIS OF CARBONIC ANHYDRASE IX AND HYPOXIA DRIVEN METABOLIC ADAPTATION RELEVANCE FOR TUMOR CELL SURVIVAL AND ANTITUMOR IMMUNE RESPONSE IN LUNG CANCER**

Mariel Fusco[1], Marco Aurelio Díaz Gutiérrez[1], Flavia Piccioni[1], Paula Roselló[1], Manglio Rizzo[1,2], Mariana Malvicini[1].
[1]Laboratorio de Inmunobiología del Cáncer, Instituto de Investigaciones en Medicina Traslacional (Universidad Austral-CONICET), [2]Servicio de Oncología del Hospital Universitario Austral

Warburg glycolysis allows cancer cells to consume glucose under hypoxia and increase lactic acid, glutamine, and CO2 production, resulting in acidification of the tumor microenvironment. Tumor cells

regulates the pH through the modulation of extracellular carbonic anhydrases (CA) such as CAIX and exchange H+ from CAIX activity releasing lactate, which reduces antitumor T cells function. Hypoxia also induces tumor-associated macrophages infiltration and stimulates the immune checkpoint Programmed Death-Ligand 1 (PD-L1). We aim to evaluate the relevance of hypoxia-associated factors and immune markers in lung cancer patients. Since we have reported that coumarin 4-methylumbelliferone (4Mu) modifies tumor HIF1 levels, and are able to inhibit CAIX, we tested the effect of 4Mu in Lewis Lung Carcinoma cells (LLC). First, we analyzed TCGA data of patients with adenocarcinoma (LUAD) and squamous cancer (LUSC), subtypes of non-small cell lung carcinoma (NSCLC). We found that HIF1a, CAIX and GLUT1 are differentially expressed in both tumors ($p<0.001$). Patients with high expression of CAIX shows a decrease in overall survival (Kaplan-Meyer). We also found a strong positive correlation between the expression of HIF1a and PD-L1 ($r=0.48$, $p<0.001$) in LUAD, and PD-L1 with CD68 ($r=0.52$, $p<0.001$), HIF-1a vs CAIX $r=0.22$, GLUT1 vs CAIX $r=0.26$; GLUT1 vs PD-L1 $r=0.26$. PD-L1 and CD68 also correlates in LUSC patients, in which we also found a positive correlation between GLUT1 and CAIX ($r=0.36$), HIF1a and CAIX ($r=0.22$) and HIF1a with CD68 ($r=0.24$). Then, we analyzed viability, metabolic activity in hypoxia/normoxia and CAIX/HIF-1 by qPCR in LLC cells. Hypoxia induces an increase in CAIX levels compared to normoxia ($p<0.05$) and reduces extracellular pH ($p<0.05$). We also observed that CAIX returns to normal in presence of 4Mu. We suggests that HIF1a/CAIX markers are relevant for NSCLC patients' prognosis and maybe for immunotherapy success. Besides, in our hands, 4Mu could contributes to regulate CAIX.

508. (292) **HYALURONAN INHIBITION IMPROVES PACLITAXEL EFFICACY IN A LUNG CANCER MODEL *IN VIVO***

Flavia Piccioni[1], Mariel Fusco[1], Marco Aurelio Díaz Gutierrez[1], Pamela Ribatto[1], Paula Rosello[1], Juan Bayo[2], Manglio Rizzo[1,3], Mariana Malvicini[1].

[1]Laboratorio de Inmunobiología del Cáncer- Instituto de Investigaciones en Medicina Traslacional (IIMT-Universidad Austral-CONICET), [2] Laboratorio de Terapia Génica (IIMT-Universidad Austral-CONICET), [3] Servicio de Oncología del Hospital Universitario Austral

Patients with non-small cell lung cancer (NSCLC) progress after treatment with conventional chemotherapy. Cancer stem cells (CSC), a subpopulation in tumor microenvironment (TME), form residual cellular niches and have a key role in resistance and recurrence. CSC express CD133, CD44, ALDH1 and SOX2, among other markers and factors. Hyaluronan (HA), a TME glycosaminoglycan, promotes CSC' function. We showed that coumarin 4-Methylumbelliferone (4Mu), reduced HA expression in murine Lewis Lung Carcinoma (LLC) cells and increased sensibility to paclitaxel (Pa), reducing the clonogenic and tumor-forming ability of LLC CD133+ treated with Pa *in vitro*. We also observed that Pa treatment induced the expression of HA-synthases genes HAS2 and HAS3. We aim to investigate a possible link between Pa treatment and the increase in HA metabolism and to evaluate the effect of Pa + 4Mu *in vivo*. We performed a bioinformatic analysis to find other molecules involved in HA-metabolism and CSC in tumors using TCGA Lung Cancer database. The analysis revealed that ABCC5 is differentially expressed in tumors compared with normal adjacent tissue ($p<0.001$). ABCC5 is an HA transporter and a multidrug resistance protein involved in Pa efflux and resistance. We found that ABCC5 has a strong correlation with SOX2, ALDH1 and CD44 ($R=0.74$, $R=0.48$, $R=0.40$, respectively. $p<0.001$). We observed that LLC cells expressed higher levels of SOX2 and ABCC5 mRNA after treatment with Pa. On the other hand, mice inoculated with $2x10^6$ LLC cells were treated with 4Mu [200 mg/kg] in water and/or Pa [5 mg/kg]i.p. every 5 days. Pa+4Mu significantly reduced LLC tumor volume ($p<0.05$), confirming the effects previously found *in vitro*. We suggest that 4Mu improved Pa treatment reducing LLC tumors. HA-inhibition in CSC population, might be the responsible of the higher sensibility to Pa. *In silico* analysis indicates that resistance in LLC cells could be due, at least in part, by Pa efflux by ABCC5, which might be expressed by CSC.

509. (300) **TUMOR PERIRENAL ADIPOSE TISSUE STIMULATES ANGIOGENESIS**

Matías Ferrando1*, Nadia Bannoud2*, Fiorella Campo-Verde-Arbocco1, Rubén Walter Carón1, Flavia Alejandra Bruna1, Diego Omar Croci2, Virginia Pistone-Creydt1, 3. *Ferrando and Bannoud had equal contribution to the work.

1IMBECU – CONICET, CCT Mendoza, Argentina, 2IHEM – CONICET, CCT Mendoza, Argentina, 3Universidad Nacional de Cuyo, Facultad de Ciencias Médicas Mendoza, Argentina.

Tumor growth and metastasis require the interaction of tumor cells with the stromal environment, in wich adipose tissue is one of the most abundant. We show that the conditioned media (CMs) of human renal adipose tissue from patients with renal tumors (hRAT) increases the migration of tumor and non-tumor renal epithelial cells compared to CMs of normal adipose tissue (hRAN). Angiogenesis is a necessary process for tumor growth and metastasis. In this work, we evaluated: 1) HIF1α, HIF2α and VEGF mRNA expression in hRAN ($n=14$) and hRAT ($n=14$), by RT-PCR; 2) VEGF protein expression in hRAN ($n=11$) and hRAT ($n=18$), by ELISA; 3) tubule formation by HUVEC cells, incubated with hRAN- ($n=4$) and hRAT-CMs ($n=4$); and 4) migration of HUVEC cells incubated with hRAN- ($n=6$) and hRAT-CMs ($n=6$), by wound healing assay. We found a higher gene expression of HIF1α, HIF2α in hRAT *vs.* hRAN explants ($p<0.05$). Also, we observed that VEGF protein expression were significantly increased in hRAN *vs.* hRAT explants ($p<0.001$). In addition, an increased tubulogenesis of HUVEC cells incubated with hRAT-CMs *vs.* hRAN-CMs was observed ($p<0.05$). Finally, we found that hRAT-CMs significantly stimulated the migration of HUVEC cells compared to control- and hRAN-CMs after 6h ($p<0.05$). In conclusion, renal peritumoral adipose tissue secretes angiogenic factors that would stimulate neovascularization of the tumor, favoring its growth and metastasis.

510. (326) **4-METHYLUMBELLIFERONE INDUCES SENESCENCE AND SENSITIZES THE RESISTANT CML CELL LINE Ki562 TO THE EFFECT OF IMATINIB**

Martín Ledesma[1], Daniela Poodts[2], Sofía Amoia[2], Carlos Vay[3], Ariela Fundia[4], Élida Álvarez[2], Silvia Hajos[2], Matías Pibuel[2], Silvina Lompardia[2].

[1]Unidad de Conocimiento Traslacional, Hospital de Alta Complejidad del Bicentenario de Esteban Echeverría.

[2]Instituto de Estudios de la Inmunidad Humoral (IDEHU, UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

[3] Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires

[4] Instituto de Medicina Experimental, CONICET- Academia Nacional de Medicina.

CML is a myeloproliferative neoplasia, and the first-line therapy is constituted by BCR-ABL inhibitors such as Imatinib (IM). Although IM is effective, its prolonged use exerts pressure, leading to the selection of resistant leukemic cells. Previously, we demonstrated that 4-methylumbelliferone (4MU) has a synergistic effect with IM on the growth of K562 and Kv562 cells, inducing senescence. Subsequently, Ki562 cells were obtained by culturing K562 cells with increasing doses of IM from 0.1μM up to 1μM. Control cells derived from K562, Ko562 were cultured in parallel but without the selection pressure of IM. K562 and Ko562 are sensitive to IM, while Ki562 and Kv562 cells are resistant through overexpression of BCR-ABL and by Pgp and PI3K activation, respectively. This work aimed to evaluate the effect of 4MU on Ko562 and Ki562 cells and to characterize the protein profile of all CML cell lines described above, studying the effect of 4MU. Metabolic activity was evaluated by XTT, senescence induction by SA-β-gal (X-gal) and SAHF (DAPI), cell death by IP (FC), and protein profile by MALDI-TOF-MS. 4MU decreased the metabolic activity on Ko562 and Ki562 cell lines ($p<0.01$), increasing the percentage of cells SA-β-gal+ and with SAHF ($p<0.05$) without modifying the percentage of PI+ cells concerning untreated control. Moreover, the co-treatment with 4MU+IM inhibited metabolic activity more than each drug alone in both cell lines ($p<0.01$) without modi-

fying the percentage of PI+ cells. MALDI-TOF-MS patterns analysis allowed the differentiation of the four CML cell lines by their chemotherapy-resistant phenotype (PC1+PC2 > 70% of variation). 4MU modulated these protein profiles, which were also differentiated by the former technique. We conclude that 4MU shows a cytostatic effect mediated by the induction of senescence and enhances the IM effect, supporting the hypothesis of 4MU as a potential drug for CML treatment even against IM-resistant leukemia cells.

511. (411) **GALECTIN-1 AND ITS NOVEL LIGAND CD13 IN LIVER TUMOR-DERIVED SINUSOIDAL ENDOTHELIAL CELLS**

Sarrias L[1], Fernández MM[2], Sarti F[1], Espelt MV[1], Malchiodi EL[2], Rabinovich GA[3], Elola MT[1], Troncoso MF[1].
[1] Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) (UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA. [2] Instituto de Estudios de la Inmunidad Humoral (IDEHU) (UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA. [3] Instituto de Biología y Medicina Experimental (IByME-CONICET), Facultad de Ciencias Exactas y Naturales, UBA.

Galectin-1 (Gal1), a β-galactoside-binding protein, is upregulated in hepatocellular carcinoma. Previously we identified CD13 as a ligand for Gal1 in human SKHEP1 liver tumor-derived sinusoidal endothelial cells (LSEC) using a proteomic approach. CD13 is a glycosylated membrane exopeptidase upregulated in endothelial cells during tumor-related angiogenesis. Here we aimed to study direct, specific and glycan-dependent Gal1/CD13 interaction as well as the role of both proteins in SKHEP1 LSEC proliferation and migration. Gal1 interaction with CD13 was studied by Surface Plasmon Resonance (SPR) on a Biacore T100 system. CD13 was isolated from SKHEP1 LSEC membrane fraction using a Gal1-affinity column and coupled to a sensor chip CM5. Human recombinant Gal1 (0.075-20μM) was injected in each cycle. As a blank, a fraction of membrane proteins isolated from CD13 knockout (CD13 KO, CRISPR-Cas9-based) SKHEP1 LSEC was immobilized on another chip. SPR assays confirmed Gal1 and CD13 interaction (Dissociation constant, K_D: 3.1±0.2 x 10^{-6}M). Preincubation with 1mM lactose, a well-established galectin inhibitor, abrogated this interaction while sucrose (1mM), a disaccharide not recognized by galectins, had no effect. Cell proliferation rate (t_{72h} vs. t_0 fold-change, MTT assay) in Gal1-silenced (shGal1) SKHEP1 LSEC showed a trend towards a decrease, while CD13 KO cells showed a significant lower rate of cell proliferation (3.43±0.27) versus control cells (scrambled (*scr*): 4.79±0.14, p<0.05; wild type (*wt*): 5.03±0.27, p<0.01). Cell migration (24h, wound healing assay) was reduced in shGal1 cells (25.4±1.7%) (*scr:* 42.3±4.8%, p<0.05) and in CD13 KO cells (31.1±1.3%) (*wt:* 40.4±2.7%, p<0.05). Our findings demonstrate that Gal1 interacts with CD13 directly and in a glycan-dependent manner. Both Gal1 and its partner CD13 are involved in SKHEP1 LSEC proliferation and migration. These results suggest that Gal1 and CD13 might cooperate in liver tumor-associated angiogenesis.

512. (468) **CHARACTERIZATION OF ACYL-COA SYNTHETASE 4 IN OVARIAN CANCER**

Jesica G. Prada[1], Andrea Hernández[2], Maritza P. Garrido[2, 3], Ulises D. Orlando[1], María M. Bigi[1], Carmen Romero Osses[2, 3], Ernesto J. Podesta[1], Ana F. Castillo[1], Paula M. Maloberti[1]
[1]INBIOMED (UBA-CONICET), Departamento de Bioquímica Humana, Facultad de Medicina, UBA. [2]Laboratorio de Endocrinología y Biología de la Reproducción, Hospital Clínico Universidad de Chile, Santiago, Chile. [3] Departamento de Obstetricia y Ginecología, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Acyl-CoA synthetase 4 (ACSL4) is involved in arachidonic acid metabolism and steroidogenesis. In pathological scenarios, increased ACSL4 level is associated with promotion of highly aggressive phenotype in breast and prostate cancer. We developed and characterized the effect of PRGL493, a specific ACSL4 inhibitor, in triple negative breast cancer and castration-resistant prostate cancer cell lines, demonstrating that decreased aggressive phenotype based on inhibition of steroidogenesis, chemotherapeutic resistance and tumor growth. Ovarian cancer is the third most common gynecologic malignancy but first in mortality rate and there is evidence that sex-steroid hormones have a role in ovarian carcinogenesis. Therefore, the aim was to analyze the role of ACSL4 in epithelial ovarian cancer (EOC). Bioinformatic analysis based on the crossover between genetic signatures described in ovarian tumors from patients with EOC and genes regulated by ACSL4 related to processes of proliferation, invasion, migration and transduction signals was performed. The study showed a positive correlation for 32 of 48 genes (p<0.05), with a correlation coefficient of 0.8, and 0.46 (p<0.05) when analyzing genes associated with drug resistance. Immunohistochemistry of human tissue samples showed significant increase of ACSL4 in EOC samples compared with normal tissue (p< 0.05). Western blot analysis showed an increase in ACSL4 levels in A2780, OV-90 and SKOV-3 EOC lines compared with the non-tumoral HOSE cells. A2780, OV-90 and SKOV-3 cells were treated with PRGL493 and MTT or BrdU assays were performed. A significant decrease in cell proliferation was observed in EOC cells incubated with the inhibitor vs control. The IC50 value of PRGL493 was about 40 uM for EOC cell lines, showing similar results obtained for breast and prostate cancer cell lines. This work led to the characterization of ACSL4 in EOC and may allow future studies combining ACSL4 inhibition with clinically used chemotherapeutics.

513. (593) **EXPRESSION AND FUNCTION OF THE HV1 PROTON CHANNEL IN HUMAN T LYMPHOMAS CELL LINES**

Paulina Finochietto[1], Diego Issourribehere[1], Florencia Cayrol[2], Mercedes Debernarfi[2] Graciela Cremaschi[2], Veronica Milesi[1], Clara Ventura[1].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), UNLP, CONICET, asociado CIC PBA, Facultad de Ciencias Exactas, Departamento de Ciencias Biológicas, La Plata, Argentina. [2]Instituto de Investigaciones Biomédicas (BIOMED), UCA-CONICET. Ciudad Autónoma de Buenos Aires, Argentina.

The Hv1 channel is a membrane protein present in the immune cells which allows the passive extrusion of H^+. In human B type leukemic cells, it was reported that its short, more active, isoform is associated with an increased cell proliferation. Then, we showed that in Jurkat cells, derived from a human T-cell acute leukemia, Hv1 inhibition induces apoptosis associated with a diminution of intracellular pH (pHi). Objectives**:** to expand the knowledge about the role of Hv1 in malignant T cells, we have explored two cell lines derived from human T lymphomas: HuT78 and K299. Methods and Results: by patch clamp technique, we observed that only HuT78 cells evoked H^+ currents mediated by Hv1 (65.8 ± 15.1 pA, p<0.05 vs 1.09 ± 1.09 pA in K299 cells). Hence, Hv1 inhibition using ClGBI 10 μM, reduced pHi in HuT78 (0.18 ± 0.03 pH units; p<0.01 vs. Control), but not in K299 cells (0.04 ± 0.03 pH units vs. control). Likewise, the proliferation experiments showed that Hv1 inhibition increases cell doubling time (Td) in HuT78 cells at concentrations of 5, 7 and, 10 μM (1.4 ± 0.1; 1.6 ± 0.3 and 2.0 ± 0.2 fold change, p<0.05) while in K299 cells an increase in Td was observed using 10 μM ClGBI (1.4 ± 0.1 fold change, p<0.05). Finally, using specific antibodies we showed a higher expression of Hv1 (long + short isoforms) in HuT78 cells (1.4 ± 0.2 times vs K299) but similar levels of the long isoform expression. These results suggest that HuT78 cells present a higher expression of Hv1 short isoform. Conclusions: Hv1 channel is expressed in both assayed cell lines, but we only could demonstrate its canonical rol in HuT78 cells, in which it mediates an H^+ efflux and is involved in pHi regulation. However, our results in K299 cells suggest a potential new function of the channel, which could be not associated with a H^+ efflux across the plasmatic membrane. Further experiments are necessary to study this function. Analysis of the expression of the Hv1 channel at the mRNA level, using the cBioPorta database (RNA Seq RPKM) indicates that the Hut78 cell line expresses higher levels of mRNA corresponding to the channel (4,37), when compared to the cell line Karpas299 (0.81). In agreement with these, using the patch clamp technique, we recorded currents mediated by the Hv1 channel in Hut78 cells (65.8 ± 15.1 pA, p<0.05), which were not observed in Karpas299 cells (1.09 ±1.09pA). In the same sense, the pharmacological inhibition of the channel using

ClGBI 10 µM, reduced the pHi in Hut78 cells (0.18 ± 0.03 pH units; p<0.01), without changes in Karpas299 cells (0, 04 ± 0.03 pH units). Likewise, our results indicate that channel inhibition increases cell doubling time (Td) in Hut78 cells at concentrations of 5, 7 and 10 µM (1.4 ± 0.1; 1.6 ± 0.3 and 2.0 ± 0.2 times vs. Control, p<0.05). However, in Karpas299 cells, we only observed a significant increase in Td using 10 µM ClGBI (1.4 ± 0.1, p<0.05).

514. (599) **NITRIC OXIDE INHIBITION WITH L-NAME REDUCES BLADDER TUMOR GROWTH AND IMPROVES CYTOTOXIC IMMUNE CELLS INFILTRATION IN THE BLADDER**

Yanina Langle, Belén Amato, Denise Belgorosky, Ana María Eiján

Área Investigaciones, Instituto de Oncología Ángel H. Roffo, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Bladder cancer (BC) is a common malignancy of the urogenital tract. Tumors are classified according to their invasion degree into non-muscle invasive (NMI) and muscle invasive (MI). The expression of inducible nitric oxide synthase (iNOS), producer of high levels of nitric oxide (NO), is a poor prognosis marker in human BC, associated with invasion and early recurrence. Previously, using the NMI murine BC model MB49 (iNOS+), we demonstrated that NO inhibition with L-NAME increased the cytotoxicity and reduced the immunosuppression in tumor bearing mice (TBM). Objectives: 1) To evaluate the effect of NO inhibition using the orthotopic or subcutaneous (sc) MI BC model MB49-I (iNOS+++). **2)** To study the systemic and the local immune cell profile. Methods: Tumor growth was evaluated by diameter size or bladder weight, after MB49-I cells inoculation ($2x10^5$ in sc or $1.2x10^4$ MB49-I cells into the bladder, respectively). Bladder and spleen $CD8^+$, NK and Treg cells from MB49-I TBM treated or not with L-NAME (0.5 or 1 g/L in drinking water) were evaluated by flow cytometry. Results: In the sc model, only L-NAME 1 g/L reduced tumor growth (p<0.01), associated with an increase of $CD8^+$/Treg ratio in spleen (p<0.01). L-NAME treatment at doses of 0.5 and 1 g/L, significantly reduced orthotopic tumor growth (p<0.05). No significant differences in $CD8^+$, NK and Treg cells from spleen of orthotopic MB49-I TBM were found. Only 1 g/L of L-NAME increased $CD8^+$ and NK cells in the bladder of MB49-I TBM (p<0.05 and p<0.01, respectively). L-NAME treatment also increased $CD8^+$/Treg ratio in the bladder (p<0.01). Our results suggest that tumor growth inhibition induced by L-NAME is associated, at least in part, to the increase of cytotoxic immune cells infiltrating the tumor.

515. (716) **VOLTAGE-GATED PROTON CHANNEL OVEREXPRESSION IN HUMAN BIOPSY-DERIVED ACUTE MYELOID LEUKEMIA BLAST CELLS**

Issouribehere Diego [2 3*], Enrique Nicolás [1 2*], Ventura Clara [1], Martin Pedro [1 2], Moncada Melisa [1], Loudet Stella Maris [3], Bordone Javier [3], Scandizzo Emilia [3], Milesi Verónica [1 2]

[1]IIFP. CONICET CIC UNLP. La Plata, Argentina .[2]Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP. La Plata, Argentina. [3]Hospital de Alta Complejidad El Cruce SAMIC Dr. Néstor Carlos Kirchner. Florencio Varela, Argentina.

**Issouribehere Diego and Enrique Nicolas contributed equally*

Some tumor cells change their energy metabolism towards glycolytic pathways, which end up generating a higher concentration of acid species. The voltage-gated proton channel (Hv1) is a membrane protein capable of mediating H^+ efflux. There is a long and a truncated isoform, and the truncated isoform generates greater extrusion of H^+. Objectives: Our goal was to assess the expression of long isoform and total expression of the Hv1 channel in blast cells derived from acute myeloid leukemia (AML). Methodology: In collaboration with the Flow Cytometry Service of the El Cruce Hospital, we worked with bone marrow samples from patients for diagnosis of AML. We labeled the cells with two antibodies: Anti-Hv1L, which recognizes an epitope present only in the long isoform, and Anti-Hv1T, which recognizes an epitope present in both Hv1 isoforms. Flow cytometry was used to quantify the median fluorescence intensity of the antibodies and the values obtained in the pathological cells were compared with the values of the non-pathological cells of the same lineage. We had 3 bone marrow samples from patients with a negative diagnosis used as control, and 6 samples from patients diagnosed with different subtypes of AML. We evaluated monocytic and granulocytic differentiated cells. Results: In monocytes, the Anti-Hv1L intensity was 2.796 ± 598 in control cells vs 10.802 ± 2.377 in pathological cells (p<0.05) while the Anti-Hv1T intensity was 3.966 ± 1017 *vs* 28.269 ± 5.051 (p<0.05). Moreover, the ratio Anti-Hv1T/Anti-Hv1L was 1.39 ± 0.11 in control *vs* 2.69 ± 0.13 in pathological cells (p<0.05). For granulocytes we observed a trend of increased expression in the pathological samples, but it was not significant for both antibodies. Conclusions: The Hv1 channel has a higher expression in pathological monocyte cells and, this difference is due in part to an increase in the truncated isoform relative to the long isoform. The functional relevance will be studied in the near future.

516. (741) **SMYD2 AS A NEW THERAPEUTIC TARGET FOR HEPATOCELLULAR CARCINOMA**

Barbara Bueloni[1], Maria Jose Cantero[1], Luciana Dominguez[1], Catalina Atorrasagasti[1], Mariana Garcia[1], Esteban Fiore[1], Juan Bayo[1], Guillermo Mazzolini[1]

1 Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional, Universidad Austral-CONICET, Buenos Aires, Argentina

Introduction: Available therapies for hepatocellular carcinoma (HCC) have a modest impact on patient survival, making it necessary to develop new treatments. SMYD2 is a methyltransferase that acts as an oncogene in numerous types of cancers. The aim of our work is to assess the therapeutic potential of SMYD2 pharmacological inhibition in HCC. Methods: SMYD2 expression levels and correlated relevant pathways were explored using public HCC datasets. HCC cells survival, cell cycle and apoptosis following treatment with SMYD2 inhibitors (AZ505 and LLY507) were assessed by standard MTT assay and flow cytometry. Mechanistic study was performed with RNA-seq analysis of HuH7 cells treated with LLY507. *In vivo* therapeutic potential of SMYD2 inhibitors was evaluated on an orthotopic HCC model in C3H/HeN mice. Results: SMYD2 is significantly upregulated in tumoral tissue from patients with HCC. We further found a negative correlation between SMYD2 expression and immune-related genes and apoptotic processes that are downregulated in HCC. SMYD2 inhibition by LLY507 induces cell cycle arrest and apoptosis on HCC cells. RNA-seq of LLY507-treated HCC cells revealed that there is a downregulation of aggressive and cell cycle-related genes. Most importantly, LL507 and AZ505 strongly inhibits tumor growth *in vivo.* Conclusions: Public human HCC datasets bioinformatic analysis shows that SMYD2 can be considered a novel therapeutic target for HCC. Targeted inhibition of SMYD2 exerts a potent antitumoral effect both *in vitro* and *in vivo* and reverts oncogenic transcriptional programs.

517. (883) **ANTINEOPLASTIC EFFECT OF BACTERIAL SUPERANTIGENS IN A MODEL OF HUMAN ACUTE T-LYMPHOBLASTIC LEUKEMIA**

Alejandra Duarte, Daniela Montagna, Florencia Todero, Mercedes Pastorini, Raúl Ruggiero, Mercedes Alemán.

Instituto de Medicina Experimental – CONICET – Academia Nacional de Medicina (IMEX-CONICET-ANM).

Superantigens (Sags) are bacterial and viral proteins that bind to major histocompatibility complex (MHC) class II molecules as unprocessed proteins and interact with T cells expressing the particular T-cell receptor (TCR) Vβ chains. We have previously described that Sags could induce apoptosis of neoplastic T cells in mice and even increase the survival of mice bearing T-cell lymphomas. Here we investigated the effect of Sag SEE (enterotoxin E from the bacterium *Staphylococcus aureus*), which specifically interacts with cells expressing the Vβ8 chain, on the human neoplastic cell line Jurkat, that carries the Vβ8 chain of the TCR. Preliminary experiments showed that SEE SAg could induce apoptosis of Jurkat cells by significantly reducing the tumor size and blood infiltration of neoplastic cells in the NOD/Scid mice model. In the present work, we

confirm these results, and we evaluated whether Sags were capable of improving the survival of mice carrying human neoplastic T cells. Jurkat cells ($10x10^6$) were inoculated intravenously (i.v.), and after two days, the mice were treated with Sag or PBS, monitoring weight and behavior every seven days. Sags-treated mice did not lose weight compared to untreated mice (SEE vs. PBS *p<0.05), whose low weight predicted deterioration and a 40% decreased in survival (*p<0.05). At the endpoint, the presence of Vβ8+ cells in peripheral blood, lymph nodes, spleen, and bone marrow was evaluated, being present in those mice that were not treated with Sag SEE, with no evidence of tumor growth confirming that Sags are capable of significantly prolonging the survival of mice carrying human neoplastic T lymphocytes restricted to a specific Vβ chain. These results encourage us to propose a possible new low-cost specific antitumor therapy with a personalized design for each individual according to the Vβ chain related to neoplastic cells in leukemias and T-lymphomas.

518. (904) **DIFFERENT SUSCEPTIBILITY TO THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA INDUCED BY DIETHYLNITROSAMINE IN FEMALES AND MALES C3H MICE**

Daniela Romina Montagna, María Florencia Todero, Alan Bernal, Mercedes Aleman, Mónica Vermeulen, Alejandra Duarte, Raúl Ruggiero
IMEX-CONICET, Academia Nacional de Medicina

Hepatocellular carcinoma (HCC) is gender-dependent in humans and rodents with a high prevalence in males. However, development of HCC in males and females is not completely studied. Therefore, the aim of this work was to characterize the differences between females and males C3H mice regarding their sensitivity to diethylnitrosamine (DEN) in HCC development. DEN was injected (10 mg/kg, i.p.) at 2 weeks old. Mice were euthanized 210 and 300 days after DEN administration. Liver sections were analyzed by histopathology and immunohistochemistry, and cell suspensions by flow cytometry. Liver tumors arose in 100% of males and 62.5% of females. Number of tumor foci was significantly higher in males (p<0.01). It correlates with congestive and chronic inflammatory areas. Accordingly, we have observed an increase in alanine aminotransferase and aspartate aminotransferase in males compared to females (p<0.05). Senescence was detected by a higher expression of beta-galactosidase in males than females (p<0.05). In addition, different immune populations were evaluated. Neutrophil-to-lymphocyte ratio was significantly higher in males than in females in blood (p<0.01). Surprisingly, a significant increase in CD8 T cell count has been observed both in spleen and lymph nodes in males (p<0.05). The count of CD11b/GR1$^+$, and the expression of PD-L1 are increased in males in lymph node (p<0.01), spleen (p<0.05) and peritoneum (p<0.05). Percentage of B cells that express PD-L1 is higher in males (p<0.05). It remains to be determined if tissue senescence, immunological or hormonal components are necessary conditions for tumor growth. Although it has been suggested that it is highly recommended that males C3H mice are used exclusively because females develop a lower and more variable percentage of liver tumors, it is precisely this difference that is worth characterizing, in order to propose animal models that guide us in the study of the broad aspects that contribute to carcinogenesis.

519. (918) **MITOTANE INDUCES THE ACTIVATION OF PROTEINS ASSOCIATED WITH TUMORIGENESIS AND DRUG RESISTANCE IN AN ADRENOCORTICAL CARCINOMA CELL LINE**

Mayra Ríos Medrano 1,2; María Mercedes Bigi 1,2; Paloma Martínez Ponce 1,2; Ulises Daniel Orlando 1,2.
1 Universidad de Buenos Aires-CONICET. Instituto de Investigaciones Biomédicas (INBIOMED), Buenos Aires, Argentina.
2 Universidad de Buenos Aires. Facultad de Medicina, Departamento de Bioquímica Humana. Buenos Aires, Argentina.

Acyl CoA synthetase 4 (ACSL4) is an enzyme participating in the metabolism of arachidonic acid during steroidogenesis. ATP-binding cassette (ABC) transporters are transmembrane proteins that translocate low molecular weight molecules through ATP hydrolysis. Mitotane is a steroidogenesis inhibitor and cytostatic antineoplastic medication. We have previously shown that ACSL4 participates in the resistance to chemotherapeutic agents by regulating the expression of transporters in cancer; thus, the objective of this work was to study the effect of mitotane on ACSL4 and ABCG2. The experimental model consisted in challenging H295R adrenal cancer cell, a line characterized by low aggressive phenotypes and low expression of ACSL4 and ABCG2 proteins, with non-lethal doses of mitotane (0.5 and 1 μM) and steroidogenic stimuli (ANGII 100 nM and KCl 14 mM). We evaluated cell functionality using viability (MTT) and proliferation (BrdU) assays, and compound exclusion (efflux) using fluorescent doxorubicin and Hoechst 33342. ACSL4 and ABCG2 were evaluated by western blot (WB). Treatment with mitotane improved H295R cell viability (MTT-p<0.001) and increased the expression of ACSL4 (WB-p <0.001) and ABCG2 (WB-p <0.001). The treatment also increased fluorescent compound exclusion (efflux-p <0.001), an effect reversed by the action of ABGC2 inhibitor Ko143. Treatment with ACSL4 inhibitor reduced the proliferation of H295R cells (BrdU-p <0.001). Stimuli with ANGII and KCl did not alter cell viability but increased the expression of ABCG2 in a time-dependent manner (WB-p <0.05). These results are in line with previous work by our group showing that ANGII and KCl regulate ACSL4 during steroidogenesis but have no effect on tumor progression or drug resistance. Therefore, ACSL4 and ABCG2 may constitute therapeutic targets at the initial stages of mitotane treatment to prevent an increase in tumor aggressiveness and drug resistance.

ONCOLOGY III *Thursday, November 17, 14-15:30 hr*

Chairs: Cecilia Pérez Piñero - Paula Maloberti - Ana María Eiján

520. (10) **NEGATIVE MODULATION OF THE AUTOPHAGY PATHWAY BY SURVIVIN/BIRC5 IN THE RESISTANCE OF PANCREATIC ADENOCARCINOMA CELLS**

Maximiliano A. Diaz[1], Maria Noé Garcia[1], Daniela L. Papademetrio[1], Martín Fernandez-Zapico[2], Elida Alvarez[1], Daniel Grasso[1].
[1]Instituto de Estudios de Inmunidad Humoral (UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.
[2]Schulze Center for Novel Therapeutics, Division of Oncology Research, Mayo Clinic, Rochester, Minesota, Estados Unidos.

Gemcitabine, the standard chemotherapy for pancreatic ductal adenocarcinoma (PDAC), induces low levels of apoptosis partially due to autophagy induction during the treatment. Autophagy inhibition allows apoptosis triggered by inhibitors of MAPKs pathway. In this context, a relationship between Survivin/BIRC5, from the IAPs family, and autophagy is observed. The aim of this work was to evaluate the modulation autophagy mediated by Survivin in PDAC cells. The MAPKs inhibitor U0126 increases Survivin (p<0.01), by western blot, that is dependent on autophagy since the result is lessened by the autophagy inhibitor 3MA (p<0.05). Moreover, TUNEL assay shows that apoptosis triggered by U0126 is prevented by a specific shRNA mediated Survivin depletion (p<0.01). Additionally, this survivin-dependent cell death resistance is mediated by autophagy since it is reversed by the autophagy inhibitor 3MA. By immunofluorescence, Survivin partially colocalizes with the autophagy marker LC3 in the perinuclear zone and it is more evident when the autophagy flux is blocked with chloroquine. More specifically, we found that Survivin colocalize in the initial structures of autophagosome formation with DFCP1 and LC3. Interestingly, that behavior seems to be independent of the local production of PI3P since no differences are observed with spautin-1. Finally, the constitutive overexpression of Survivin decreases the autophagy flux measured by LC3. All in all, our results suggests that Survivin is intimately implicated in the molecular mechanism of the autophagy pathway being a negative modulator of the autophagy flux and protecting the pancreatic can-

cer cell integrity from chemotherapeutics.

521. (194) **THE AUTOPHAGY PATHWAY STATUS INFLUENCIATES THE EXTRACELLULAR VESICLES PROFILE RELEASED FROM PANCREATIC ADENOCARCINOMA CELLS**

Daniel Grasso, Daniela L. Papademetrio, Giuliana Narváez, Maximiliano A. Diaz, Elida Alvarez, Maria Noé Garcia.
Instituto de Estudios de Inmunidad Humoral (UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Pancreatic ductal adenocarcinoma (PDAC) is a tough challenge due to its mortality and its extremely short life expectancy. Most cells release extracellular vesicles (EVs) that can transmit messages to distant target cells including effector actions. Autophagy is a homeostatic degradative program with implication in several cellular processes including, as it was suggested, the EVs biogenesis. This work was focused on the possible influences of the autophagy pathway over the EVs from PDAC cells. Immunofluorescence in PDAC cell line Panc-1 showed that the starvation (STV)-mediated induction of autophagy provokes a significant redistribution of the EVs markers CD81 and CD9 towards the plasma membrane where they colocalize partially. Moreover, STV induces a slight increase of extracellular signal for CD81 and CD9 (p<0.05). The blocking of autophagy flux with chloroquine also increased the extracellular signal of CD9 and CD81 (p<0,05). Surprisingly, reduction of the autophagosomes biogenesis with spautin-1 (Sp1) combined with 1h STV resulted in the maximum mobilization of CD81 and CD9 signals to the extracellular space (p<0,01). By electron microscopy of purified EVs from Panc-1 supernatants, an increase of EVs (~400 nm) is observed upon Sp1 plus 1h STV. Curiously, for the same treatment, numerous EVs were adhered to the plate surface with a size of 100-240 nm. Finally, in cytometry analysis of small EVs isolated by magnetic beads mediated immunocapture we observed an increase in number and fluorescence intensity of CD63 signal either for CD9 and CD81 subpopulations upon STV induced autophagy (65,4% vs 95,9%; MFI p<0.01 for CD9 and 69,4% vs 96,0%; MFI p<0.01 for CD81). This observation was even more evident when STV is combined with Sp1 treatment (61,8% vs 95,3%; MFI p<0.01 for CD9 and 55,9% vs 92,7%; MFI p<0.01 for CD81). Here we demonstrate a relationship between the autophagy and the EVs that could be of relevance for the understanding of pancreatic carcinogenesis.

522. (245) **IFNβ SENSITIZES PANCREATIC TUMOR CELLS TO GEMCITABINE BY MODULATING AUTOPHAGY**

Santiago Behr, Daniela Poodts, Daniel Grasso, Maria Noé Garcia, Elida Alvarez, Daniela L. Papademetrio.
Instituto de Estudios de Inmunidad Humoral (UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Infiltrating pancreatic ductal adenocarcinoma (PDAC) accounts for over 95% of all exocrine pancreatic malignancies, with a median survival of only 4–6 months. Despite its poor efficacy, Gemcitabine is a first line agent for PDAC treatment. Previously, we identified that autophagy is involved in gemcitabine chemoresistance. Now, we propose to evaluate the effect of IFNβ on autophagic flux and its ability to sensitize MIAPaCa-2 and PANC-1 cell lines to the effects of gemcitabine. The treatment for 48h with IFNβ in a dose of 1000UI/ml showed a dose and time dependent inhibition of cellular proliferation, by IFNβ by ^{3}H-timidine incorporation (67.3±5.1% and 52.3±1.9% for MIAPaCa-2 and PANC-1 cells respectively (p<0.001)). To analyse the effect of IFNβ on autophagic flux we determined the levels of LC3-II obtained by IFNβ alone or in combination with chloroquine (CQ) by western blot and microscopy evaluating the colour LC3-II puncta in cells transfected with the plasmid pBABE-puro-mCherry-EGFP-LC3B. Both assays showed that IFNβ inhibited the autophagic flux in MIAPaCa-2 and PANC-1 cells. Then, TUNEL assays were performed to determine the induction of cell death by IFNβ alone and in combination with gemcitabine. For all tested doses, co-treatment with IFNβ and gemcitabine induced an increased % of TUNEL+ cells versus gemcitabine alone, reaching values of 65.1±6.5% and 46.5±3.5% for MIAPaCa-2 and PANC-1 respectively. Moreover, the pre-treatment of PDAC cell lines with IFNβ decreased the gemcitabine-induced cell migration, determined by wound healing assay (p<0.001). All in all, the inhibition of autophagy by IFNβ sensitizes MIAPaCa-2 and PANC-1 cells to gemcitabine, leading to an increase in cell death, and it also prevented the migration process induced by the chemotherapeutic agent, suggesting that the combination of IFNβ plus gemcitabine, could be a good therapeutic alternative to deal with infiltrating PDAC.

523. (387) **GENOTOXIC DAMAGE, LIPID PEROXIDATION AND CA 19.9 MODIFICATIONS DURING GEMCITABINE (GEM)+DICHLOROACETATE (DCA)+LOSARTAN (LOS) TREATMENT IN ADVANCED PANCREAS CANCER PATIENTS**

Cecilia Bianchi1; Sergio Ghersevich2; Alejandra Luquita3; Sandra Ostoich1; Alejandra Bártoli1; Herman Perroud4; Viviana Rozados4,5; O. Graciela Scharovsky4,5.
1Hospital Provincial del Centenario, Rosario, Argentina; 2Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario; 3Biofísica, Facultad de Ciencias Médicas, Universidad Nacional de Rosario; 4Instituto de Genética Experimental, Facultad de Ciencias Médicas, Universidad Nacional de Rosario; 5 CONICET.

A single-arm, non-randomized, open-label, phase II clinical trial was developed as a 1st-line treatment of patients with locally advanced, inoperable or metastatic pancreatic cancer with GEM+DCA+LOS. The objective of this work was to analyze the genotoxic damage, lipid peroxidation and CA19.9 biomarkers during patients' treatment. Eligibility criteria: patients 21-80 years old, locally advanced, inoperable or metastatic adenocarcinoma of the pancreas without previous treatment, at least one lesion according to RECIST criteria, ECOG 0-2 scale, adequate renal and hematological function, normal calcium, informed consent. Treatment: GEM 1000 mg/m² IV weekly for 7 weeks, then, every 28 days on days 1, 8, 15 + DCA 5 mg/kg for 21 days, then 7.5 mg/kg bod with adjuvant thiamine + LOS: 50 mg bod. Treatment was administered until toxicity or progression. The 4 patients included in the study presented neurological toxicity: 1 irreversible and 3 reversible, and DCA administration was discontinued. Blood samples were taken at the beginning and during the treatment. The initial level of CA19.9 (ECLIA) was very high (4800-6400 U/ml) in the 3 patients with bad evolution and normal (9.3) in the patient still in treatment, without significant variations during treatment. The genotoxic damage (GD) was determined by the comet assay. The % of change of GD respect to the initial value (100%) increased during treatment up to 150%, independently of patients' evolution. The increase in DCA dose was accompanied with a 20% decrease in TBARS (the lipid peroxidation index of the substances reactive to thiobarbituric acid). Conclusion: the basal CA19.9 level was predictive of tumor behavior; the treatment caused both, increase of the GD in patients with bad and good evolution and, on the contrary, decrease of TBARS and, hence, of reactive oxygen species and oxidative stress. However, the combined treatment with DCA showed toxicity, causing the premature close of the study.

524. (503) **DABRAFENIB RESISTANCE PROMOTES ANCHORAGE-INDEPENDENT CELL POPULATION GROWTH AND MESENCHYMAL MARKERS INCREASE IN BRAF V600E MELANOMA CELLS**

Arbe MF[1], Asparch YB[2], Glikin GC[1], Finocchiaro LME[1] y Villaverde MS[1].
1. Unidad de Transferencia Genética, Área Investigación. Instituto de Oncología "Ángel H. Roffo", Facultad de Medicina, Universidad de Buenos Aires, Argentina.
2. Instituto de Investigación e Ingeniería Ambiental, IIIA-CONICET, Universidad de San Martín, Argentina.
e-mail: marcelavillaverde@hotmail.com

Epithelial-mesenchymal transition (EMT) has a driving role in migration and invasion promoting the acquisition of metastatic potential by tumor cells. In this process, epithelial cells lose their cell contact, rearrange their cytoskeleton, acquire a migratory phenotype and

downregulate E-cadherin (epithelial marker) and increase vimentin and N-cadherin (mesenchymal markers) expression. BRAFV600E mutation has been suggested to be involved in the development of EMT. Our group previously reported a cell contact loss in 3D spheroids and an increase in anchorage-independent cell population in A375 Dabrafenib-resistant cells (A375-R). In this work, we investigated the EMT marker expression (E-cadherin, N-cadherin, and vimentin) in adherent and anchorage-independent or suspension A375-R cells using western blot, flow cytometry, and immunofluorescent techniques. Suspension A375-R cells showed a downregulation of E-cadherin and up-regulation of vimentin ($p<0.05$ of both makers by flow cytometry and western blot). On top of that, vimentin pattern expression changed in A375-R cells showing higher localization in the border region of the subconfluent layer. Related to the switch cadherin process, suspension A375-R cells downregulated their N-cadherin expression ($p<0.05$ by flow cytometry and western blot). The morphological changes and the increased mesenchymal phenotype in suspension A375-R cells suggest that the EMT process could be induced during Dabrafenib resistance.

525. (538) **MRP4 OVEREXPRESSION INDUCES AN ALTERED EPIGENETIC AND TRANSCRIPTIONAL PROGRAM THAT CONTRIBUTES TO TUMOR PROGRESSION IN PANCREATIC CANCER**

Samanta Gancedo[1], Ana Sahores[1], Natalia Gómez[1], Maximiliano De Sousa-Serro, Agustín Yaneff, Carina Shayo[2], Carlos Davio[1], and Betina González[1]

[1]Instituto de Investigaciones Farmacológicas, UBA-CONICET, Argentina.

[2]Instituto de biología y medicina experimental, CONICET, Argentina.

The multidrug resistance-associated protein 4 (MRP4/ABCC4) is highly expressed in pancreatic ductal adenocarcinoma (PDAC), and its overexpression is linked to increased tumor proliferation and metastatic capacity. The aim of this study was to determine how MRP4 overexpression collaborates in the establishment of malignant epigenetic and transcriptional programs that sustain tumor progression. For this, we performed RNAseq studies of BxPC3 cells stable transfected with a ABCC4-expressing (-MRP4) plasmid or an empty vector (-mock) in two different environmental growing contexts: cell culture or tumor xenografts obtained from subcutaneous inoculation of these cells in NSG mice. Transcriptome analysis (FDR<0.05; Log2FC>0.5) showed 2,043 differentially expressed genes (DEGs) between -MRP4 and -mock cells growing in culture, and 4,234 DEGs when comparing -MRP4 and -mock xenograft samples. Interestingly, we found common deregulated pathways shared by culture and xenograft samples related to a proliferative and metastatic signature in PDAC cells. In addition, xenograft samples showed specific pathways related to epigenetic changes, including histone acetylation and methylation terms (FDR<0.05). We confirmed by WB the findings obtained in RNAseq with respect to global histone epigenetic marks and some related enzymes. Our results evidenced a significant decrease of the heterochromatin mark H3K9me3 and a rise in the active promoter mark H3K4me3 ($p<0.05$). We also detected increased levels of G9a methyltransferase and LSD1 demethylase in -MRP4 compared to -mock xenografts, which are related to cancer proliferation and metastasis. These findings show that MRP4 contributes to the establishment of an aberrant epigenomic landscape and altered transcriptional program, that could lead PDAC cells to a more proliferative, undifferentiated, and metastatic phenotype.

526. (547) **ROR1 PROMOTES MELANOMA CELL SURVIVAL BY UPREGULATING STAT3-DEPENDENT REGULATION OF BCL2-FAMILY PROTEINS**

María Josefina Quezada [1,2], Paula Denise Mascolo [1,2], Jesús Barraza Sánchez [1], and Pablo Lopez-Bergami [1,2]

[1] Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina, 1405. [2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, 1425.

Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a tyrosine kinase receptor that play crucial roles in the development of various organs and tissues as well as in human disease. ROR1 is overexpressed in cancer and has been associated with cellular features that promote malignancy, namely cell proliferation, survival, migration/invasion. The role of ROR1 in melanoma has not been investigated to a great extent. The aim of this work is to study the role of ROR1 in melanoma cell survival. To this end, both gain- and loss-of-function approaches were employed by generating A375 cell lines with both overexpression and silencing of ROR1. Overexpression of ROR1 significantly increased the expression levels of several of the anti-apoptotic members of the Bcl2 family including Bcl2, Mcl1, Bcl-XL and Bcl2L10 ($P<0.01$, Student's t-test), as determined by densitometric analysis of Western blot membranes. In contrast, ROR1 silencing significantly reduced the expression of these proteins ($P<0.01$, Student's t-test). To evaluate the effects of ROR1 in cell survival cells stained with Annexin V/ propidium iodide were analyzed by flow cytometry. Following 48 hours of serum withdrawal, cells with ROR1 silencing showed a two-fold increase in apoptotic cells ($P<0.001$, Student's t-test). The role of ROR1 in cell survival was confirmed when using ROR1-overexpressing cells. Since we have previously demonstrated that Bcl2L10 transcription is regulated by STAT3 and that STAT3 is regulated by ROR1, we wanted to evaluate whether STAT3 mediates the effect of ROR1 on these proteins. Inhibition of STAT3 in ROR1-overexpressing cells significantly inhibited ROR1-dependent induction of Bcl2L10 as demonstrated in Western blots experiments ($P<0.01$, Student's t-test). These results demonstrate that the aberrant expression of ROR1 in melanoma contributes to cell survival. ROR1 exert this effect by upregulating STAT3 and anti-apoptotic members of the Bcl2 family.

527. (560) **THE ABERRANT EXPRESSION OF ROR2 IN MELANOMA PROMOTES THE EXPRESSION OF ENDOTHELIAL CELL MARKERS**

Paula Denise Mascolo[1,3], María Victoria Castro[2], María Josefina Quezada[1,3], Jesus Barraza Sanchez[1], and Pablo Lopez-Bergami[1,3]

[1]Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina, 1405. [2]Fundación Instituto Leloir. IIBBA-CONICET. Av. Patricias Argentinas 435, 1405 Buenos Aires, Argentina. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, 1425.

Receptor tyrosine kinase-like orphan receptor 2 (ROR2) is a membrane receptor of the Wnt5a ligand and a mayor component of the Wnt non-canonical pathway. ROR2 was shown to play a dual role in cancer by either suppressing or promoting tumor progression in different tumor types. Recent findings from our group have established that ROR2 can play this dual role simultaneously in melanoma cell lines and has been proposed to be a main regulator of melanoma cell plasticity.

The aim of this work is to further characterize the complex role of ROR2 in melanoma. To investigate the biological and biochemical mechanisms regulated by ROR2, both gain and loss-of-function approaches were employed by generating A375 cell lines with both overexpression and silencing of ROR2. Overexpression of ROR2 significantly increased the expression levels of the endothelial cell markers PECAM, PDGF-R, VCAM1 and Tie2 ($P<0.01$, Student´s T test), as determined by densitometric analysis of Western blot. In contrast, ROR2 silencing significantly reduced the expression of these proteins ($P<0.01$, Student´s T test). These results were confirmed in two additional melanoma cell lines. To assess the regulation of these proteins by ROR2 *in vivo*, we inoculated nude mice with either A375-ROR2 or A375-empty cells. The tumors were resected and immunohistochemical analysis was performed. Similar to the *in vitro* findings, tumors from mice injected with A375-ROR2 presented a very strong positive staining for VCAM1 and Tie2 whereas tumors from mice injected with A375-empty cells were negative. These results suggest that ROR2 might be implicated in the generation of tumor- vasculature patterns.

528. (645) **TRANSDIFFERENTIATION REDUCES THE AGGRESSIVENESS OF PANCREATIC DUCTAL TUMOR**

CELLS

M. Jimena Mosna[1], Lucía P. Petriz [1], Federico J. Garde[1], Marcelo G. Stinson[1], Daniela Di Bella[2], Abel L. Carcagno[1]
[1]Cell Differentiation and Cancer Laboratory, ETI1, IQUIBICEN-CONICET, FCEyN-UBA, CABA, Argentina. [2]Department of Stem Cell and Regenerative Biology, Harvard University, Boston, USA.

Pancreatic ductal adenocarcinoma (PDAC) represents a very aggressive type of pancreatic cancer (9% survival rate at 5 years). Ectopic expression of specific transcription factors can successfully transdifferentiate pancreatic tumor cells from the exocrine to the endocrine lineage. Specifically, PDX1 and Neurog3 promote transdifferentiation of PANC-1 cells from an exocrine to an endocrine "beta-like" phenotype, and their overexpression in PDAC patients increases survival rate. Therefore, our aim was to analyze the effect of the exocrine-endocrine transdifferentiation of PDAC cells on tumor aggressiveness. To induce transdifferentiation, PANC-1 cells were either treated with BRD7552 (a PDX1 inducer) for 9 days or transfected with a plasmid of expression of hNeurog3. No cytotoxic effect was observed by MTT nor Trypan Blue assays on treated cells. Wound healing assay showed a significant reduction in migration rate compared to control. Flow cytometry assay showed an increase in G1 phase in treated cells relative to control. Analysis of Ki67 immunostaining exhibited no significant difference in cell proliferation rates. Treated PANC-1 cells were implanted onto the chorioallantoic membrane of chick embryos and tumor growth was measured at different stages, observing decreased tumor size at day 7 post-implantation compared to control. Neurog3 transfection showed no morphological effect on PANC-1 cells. Through scRNA-seq and bulk RNA-seq analysis we identified genes associated with tumor aggressiveness in human ductal pancreatic cells and with unfavorable clinical parameters in PDAC patients. In conclusion, transdifferentiation through BRD7552 treatment affects cell cycle and inhibits migratory potential *in vitro* and tumor growth *in ovo* in pancreatic ductal tumor cells.

529. (698) **EFFECT OF A PENICILLIN DERIVATIVE ON THE EXPRESSION LEVELS OF B-CATENIN IN MELANOMA CELLS WITH DIFFERENT SENSITIVITY TO BRAF INHIBITORS**

Sofia V. Bajicoff[1], Elizabeth Barrionuevo[1], Patricia G. Cornier[2], Carina M.L. Delpiccolo[2], Ernesto G. Mata[2], Viviana C. Blank[1], Leonor P. Roguin[1]
1. Instituto de Química y Fisicoquímica Biológicas, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 2. Instituto de Química Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.

We have previously demonstrated that TAP7f, a synthetic derivative formed by penicillin linked to the dipeptide Leu-Phe through a triazole group, inhibited melanoma metastasis by downregulating β-catenin. Since it has been reported that melanoma cells resistant to BRAF inhibitors (BRAFi) express higher levels of β-catenin, blocking Wnt/β-catenin signaling could be considered a useful strategy to improve cell response. In this study we examined the mechanism of action of TAP7f in melanoma cells sensitive (A375, $BRAF^{V600E}$) and resistant (SB2, $NRAS^{Q61K}$) to BRAFi. The cytotoxic potency of TAP7f on these cell lines was first determined, being IC_{50} values of 10.0 ± 1 μM and 9.0 ± 3 μM for A375 and SB2 cells, respectively (72 h, n=3). In addition, IC_{50} values of 7.7 ± 0.5 nM and 2.1 ± 0.9 nM were obtained for dabrafenib (D, BRAFi) and Trametinib (T, MEKi) in A375 cells, whereas SB2 cells were more resistant to these inhibitors (IC_{50} D: > 30 μM; IC_{50} T: > 20 nM). Western Blot assays revealed that TAP7f (20 μM) reduced 67.5 ± 0.6 % and 59.1 ± 0.1 % the expression levels of β-catenin in A375 and SB2 cells, respectively, after 24 h of incubation. Under similar experimental conditions, TAP7f increased the phosphorylation of Ser 33/37 of β-catenin in both cell lines, a target region for β-catenin degradation. Furthermore, when A375 and SB2 melanoma cells were incubated with TAP7f in the presence of MG132, a proteasome inhibitor, a significant reversion of TAP7f inhibitory effect on β-catenin levels was observed. In summary, our results suggest that TAP7f downregulates β-catenin levels by promoting its degradation via proteasome in melanoma cell lines with different genetic profile, regardless of sensitivity to BRFA inhibitors. This behavior positions this penicillin derivative as a promising agent for the treatment of BRAFi-resistant cells.

530. (701) **CIRCULATING GALECTIN-1 DELINEATES RESPONSE TO BEVACIZUMAB IN MELANOMA PATIENTS AND REPROGRAMS ENDOTHELIAL CELL BIOLOGY**

Nadia Bannoud[1], Alejandro J. Cagnoni[2,3], Juan C. Stupirski[2], Pablo F. Hockl[2], Juan M. Pérez Saez[2], Pablo A. García[1], Yamil D. Mahmoud[2], Julián Gambarte Tudela[1], Marco Scheidegger[2], Karina V. Mariño[3], M. Romina Girotti[2], Diego O. Croci[1] and Gabriel A. Rabinovich[2,4]
[1]Laboratorio de Glicobiología y Biología Vascular. IHEM-CONICET-UNCUYO, Mendoza, Argentina. [2]Laboratorio de Glicomedicina. IBYME-CONICET, Buenos Aires, Argentina. [3]Laboratorio de Glicómica Funcional y Molecular. IBYME-CONICET, Buenos Aires, Argentina. [4]Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

Blockade of vascular endothelial growth factor (VEGF) signaling with bevacizumab, a humanized anti-VEGF monoclonal antibody (mAb), has improved progression-free survival and, in some indications, overall survival across several types of cancers by interrupting tumor angiogenesis. However, the clinical benefit conferred by this therapy is variable, and tumors from treated patients eventually reinitiate growth. Previously we demonstrated, in mouse tumor models, that galectin-1 (Gal1), an endogenous glycan-binding protein, preserves angiogenesis in anti-VEGF resistant tumors by co-opting VEGFR2 signaling. However, the relevance of these findings in clinical settings is uncertain. Here we explored in a cohort of melanoma patients from AVAST-M [a multicentre, open-label, randomized controlled phase 3 trial of adjuvant bevacizumab (N=94) versus standard surveillance (N=95)], the role of circulating Gal1 as part of a compensatory mechanism that orchestrates endothelial cell programs in bevacizumab-treated melanoma patients. We found that increasing Gal1 levels over time in patients on the bevacizumab, but not on the observation arm, significantly increased their risks of recurrence and death ($p<0.0001$). Remarkably, plasma Gal1 was functionally active as it was able to reprogram endothelial cell biology, promoting migration ($p<0.05$) and tubulogenesis ($p=0.029$) *in vitro*. Interestingly, blockade of Gal1, using a newly-developed fully human anti-Gal1 neutralizing mAb (mAb42), prevented these effects but only in patients under bevacizumab arm. Notably, exposure to mAb42 resulted in both anti-angiogenic and immunostimulatory effects, highlighting the dual benefits of Gal1 blockade. Thus, using samples from the larger-scale clinical trial from grade II-III melanoma patients, we validated the clinical relevance of Gal1, an endogenous lectin widely associated with angiogenesis and immunomodulation, as a potential resistance mechanism to bevacizumab treatment.

531. (733) **UNDERSTANDING THE ROLE OF Vav3 IN MELANOMA**

Nahuel Cesatti Laluce[1,2]; Macarena Mamberto[1,2]; Luciano Anselmino[1,2]; Florencia Malizia[1]; Mauricio Menacho Márquez[1,2,3]
[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER, UNR-CONICET); [2]CONICET; [3]CIPReB (FCM-UNR) Universidad Nacional de Rosario
cesattilaluce@idicer-conicet.gob.ar

At present, melanoma is the skin cancer type with the poorest prognosis. A deeper understanding at molecular level of this disease may hold the key to improving its treatment. Vav proteins are guanosine nucleotide exchange factors (GEFs) which activate -principally- Rho-Rac GTPases. This family is involved in several processes linked to tumoral and metastasis development. As GEFs, they normally are considered as protumorigenic proteins. Our team is focused on characterizing the role of these proteins in cancer.
Previously, we found an unexpectedly antagonistic role between Vav2 and Vav3 in the context of melanoma. Through a bioinformatic approach, we found that Vav3 has the highest expression levels among Vav proteins in normal skin and that expression decreas-

es after UV exposure, while the opposite is observed for Vav2. We previously shown that Vav3 modulates migration and proliferation in melanoma cells. In this report, we demonstrate by annexin V staining and flow cytometry that Vav3 expression triggers apoptosis *in vitro* in the absence of death stimuli (p=0.05). Considering the GEF role of Vav3 on Rho-Rac, we focused on actin cytoskeleton in mouse melanoma cells after modulating Vav3 expression by transfection techniques. We found that overall cellular architecture is deeply affected by the modulation of Vav3 expression. Indeed, reduced Vav3 levels promote typical migratory actin structures on the membrane. By microarray-based expression assays with these cells, we analyzed transcriptional changes associated to Vav3 expression, noting that this protein modulates the expression of a wide group of genes. An analysis of these differentially expressed genes shed light on biological processes underlying different phenotypes we previously found *in vitro* and *in vivo*, as the altered migratory ability of cells associated to Vav3 expression (FDR <0.001). Taking together, our *in silico* and *in vitro* data provide us evidences to consider Vav3 as a tumor suppressor protein in melanoma, in contraposition to what is reported for this protein in other types of cancer.

532. (736) **CHARACTERIZING THE ROLE OF GAMMA-SYNUCLEIN IN MELANOMA**

Lucía Zanotti[1,2], Florencia Malizia[1,2], Mauricio Menacho Márquez[1,2,3]

1. Instituto de Inmunología Clínica y Experimental de Rosario. Universidad Nacional de Rosario (IDICER, UNR-CONICET); 2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); 3. Centro de Investigación y Producción de Reactivos Biológicos (CIPReB, Facultad de Ciencias Médicas-UNR)

Gamma-synuclein (γS) elevated expression has been reported in various cancers such as ovarian, breast and colorectal cancer, but to date there are no rigorous studies to address the role this protein could have in progression of melanoma. Our goal was to analyze if γS was related to melanoma development. For that, we worked with mouse (B16F0, B16F10) and human (SKMEL28, A375) melanoma cells. By Western Blot (WB), real-time PCR (qPCR) and immunocytochemistry (ICC) we observed the expression of γS in these cells. Then, we modulated (by shRNA and expression vectors) γS levels in murine melanoma cells (confirmed by qPCR (P<0.01) and WB). Growth studies (MTT) indicated that reduced expression of γS leads to proliferative (P<0.05) and migratory defects (P<0.05) were observed by wound healing assays. We confirmed these observations *in vivo* by injecting subcutaneously B16F0 (control and decreased levels of γS) in the right flank in 8-week-old female C57BL/6 mice ($2x10^5$ cells; n=5/group). We analyzed melanoma growth measuring tumor volume periodically. Growth kinetics indicated that low levels of γS in melanoma cells significantly reduced tumor growth (P<0.05). On the other hand, to analyze the generation of metastases, we injected B16F10 cells (control and decreased levels of γS) in female C57BL/6 mice. We analyzed melanoma growth measuring tumor volume periodically. When tumors reached a certain value of tumor volume (fixed in our research group) the mice were anesthetized and the tumors were surgically removed. At 4 weeks, they were sacrificed and lungs and lymph nodes were collected for histological analysis. Preliminary results indicate the development of a lower number of metastases in the mice that were injected with cells with decreased expression of γS (P<0.05). Interestingly, melanoma cells were able to uptake exogenous γS. This treatment increased proliferation, clonogenic capacity and migration of melanoma cells (P<0.05). Altogether, our results indicate that γS have a role in melanoma growth and development. Further studies should be addressed to confirm and complement our observations.

533. (766) **THYROID HORMONES INFLUENCE METASTATIC AND NON-METASTATIC MELANOMA PROGRESSION**

Cayrol F[1], Sterle HA[1], Debernardi MM[1], Gonzalo G[1], Diaz Albuja JA[1], Campos Haedo M[1] , Cremaschi GA[1].

[1] Instituto de Investigaciones Biomédicas (BIOMED) - UCA - CONICET.

Thyroid hormones (THs) can affect tumor behavior by direct actions on cancer cells through molecular pathways involved in tumorigenesis, cancer metabolism, tumor proliferation/survival, invasiveness, and angiogenesis. To evaluate if THs can influence melanoma (ME) cell growth we first evaluated nuclear (TR) and membrane (integrin αVβ3) THs receptor levels in human (A375 and WM35) and mouse (B16F10 and B16F1) ME cells. We found transcriptional and protein levels of these receptors in all the ME cells analyzed. We also evaluated THs effect on ME cell proliferation and found that physiological and supraphysiological levels of these hormones induce 15 to 40% ME cell proliferation (p<0.05); and that this effect is reduced by the pharmacological inhibition of integrin αVβ3 (p<0.05). Importantly, we found expression of αV and β3 integrins in ME patient samples from the TCGA-SKCM project, suggesting that the membrane receptor for THs can be a possible target to improve ME therapy. Increasing evidence indicates that THs can also affect the composition of the tumor microenvironment and the immune responses, thus influencing tumor progression. Therefore, we analyzed the effect of thyroid status on B16F10 and B16F1 *in vivo* syngeneic mouse models. In both models, hyperthyroid mice showed an increased tumor growth rate (p<0.05), but no significant effect of hypothyroidism on tumor growth has been observed. We also analyzed the distribution of immune cell subsets in spleens and tumor-draining lymph nodes from these animals and we observed that thyroid status affects the distribution of cytotoxic $CD8^+$ T cells, B lymphocytes, and myeloid-derived suppressor cells. Further experiments should be performed to determine the effect of THs on the functionality of these cells. Our results indicate that THs can induce ME cell proliferation through αvβ3 integrin and are also involved in the systemic anti-ME immune response.

534. (864) **CYTOSINE DEAMINASE::URACIL PHOSPHORIBOSYL TRANSFERASE/5-FC SUICIDE GENE SYSTEM DECREASES CLONOGENIC CAPACITY IN SURVIVING HUMAN MELANOMA CELLS**

Bernabei-Cornejo SG[1], Villaverde MS[1], Glikin GC[1], Finocchiaro LME[1]

1. Unidad de Transferencia Genética, Área Investigación, Instituto de Oncología "Ángel H. Roffo", Universidad de Buenos Aires.

The yeast cytosine deaminase::uracil phosphoribosyl transferase fusion protein associated to its prodrug 5-fluorocytosine (CDU/5FC), has been proposed as a suicide gene (SG) system for cancer therapy. Once expressed in the cell, CDU converts the 5FC prodrug in 5-fluorouracil (5-FU), which alters DNA and RNA processing and synthesis, leading to cell death. Previously, we have reported the cytotoxic effects of this system on eight human melanoma derived cell lines (A375, hM1, hM2, hM4, hM9, hM10, SB2 and M8). Five of these cell lines where derived from surgically excised human melanoma tumors, established and characterized in our laboratory (hM1, hM2, hM4, hM9 and hM10). We have shown that lipofection with CDU/5FC elicited a significant 5FC concentration-dependent cytotoxic response in these cell lines, compared to control lipofected cells. Even at the highest prodrug concentrations tested (1 mM 5FC), there were some remaining viable cells. In a clonogenic assay on A375, M8, hM4 and hM9 cell lines, we tested whether these remaining cells were still able to proliferate. We found that after 6 days of treatment of CDU- or control-lipofected cells, the clonogenic capacity of surviving cells was not altered at 1 μM, diminished at 10 μM and abolished at 100 μM 5FC. In parallel, dual acridine orange/ethidium bromide (AO/EB) staining was performed at 24 h, 48 h and 96 h after treatment. Control cells mostly appeared as green-stained healthy cells. In CD::UPRT/5FC suicide gene treated cells, the number of late apoptotic/necrotic cells was significantly increased at 96 h, as evidenced by the increased rise of red-stained nuclei. We conclude that the CDU/5FC suicide system can significantly decrease human melanoma cells' survival and that remaining cells are unable to proliferate.

ONCOLOGY IV *Friday, November 18, 9-10:30 hr*
Chairs: Marianela Candolfi - Alejandra Duarte -

Daniela Montagna

535. (320) **POTENTIAL ROLE OF ENDOTHELIAL CELLS AND BMP7 IN VASCULOGENIC MIMICRY FORMATION IN CERVICAL CANCER**

Cintia Birkenstok[1], Luana Homann[2], Pedro Carriere[2], María Belén Novoa Díaz[2], Claudia Gentili[2], Natalia Calvo[2]
1 Departamento de Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina, 2 Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)- INBIOSUR (CONICET-UNS), Bahía Blanca, Argentina.

Vasculogenic mimicry (VM) is an alternative microvascular system in which highly invasive tumor cells mimic endothelial cells by forming blood vessel-like structures. This process is correlated with poor prognosis, metastasis, and resistance to anti-angiogenic therapy in various cancers, including cervical cancer (CC). We previously observed that after treatment with conditioned media from CC-derived HeLa cells (TCMs) for 24 hours, HMEC-1 cells acquire characteristics related to endothelial cells found in the tumor microenvironment niche. In addition, we found that the signaling pathway of bone morphogenetic protein 7 (BMP7) could be involved. The objective of this work was to investigate whether endothelial cells with characteristics related to those found in the tumor microenvironment are involved in the formation of VM in CC and the potential role of BMP7 in this process. Initially, we evaluated whether the factors released by endothelial cells with characteristics related to those found in the tumor niche promote VM formation in CC. The HMEC-1 cells were treated for 24 hours with TCM from HeLa cells, followed by a medium renewal to obtain a new conditioned medium (ECM-T). Using qRT-PCR, we observed that exposure of HeLa cells with these ECM-T increase the mRNA levels of markers associated with VM, VE-cadherin, EphA2 and vimentin. The protein-protein interaction network by the STRING database identified interactions between BMP7 and 12 genes involved in MV formation. Furthermore, incubating HeLa cells with the ECM-T also promotes tube-like structure formation. These effects were partially reversed by pre-incubating TCM from HeLa cells with an antibody against BMP7. These results suggest a potential indirect role of BMP7 in the VM in CC by acting on endothelial cells. Expanding the knowledge of the molecular mechanisms associated with VM in CC will help identify potential biomarkers that predict the prognosis and resistance to anti-angiogenic therapy.

536. (327) **ADVANCES IN THE STUDY OF NORCANTHARIDIN AS AGENT FOR TRIPLE NEGATIVE BREAST CANCER TREATMENT**

Lizeth Aixa Ariza Bareño, Aldana M. Schey, Luciana Cañonero, Andrés Bechis, Diego Britez Neira, Laura B. Todaro, Alejandro J. Urtreger.
Research area. Oncology Institute Ángel H. Roffo, University of Buenos Aires, Argentina.

Triple negative breast cancer (TNBC) has a very aggressive clinical course lacking specific therapies since they do not express estrogen or progesterone receptors, neither overexpress HER2. Therefore, new strategies for the treatment of this pathology are required.
The Norcantharidin (NCTD) is a demethylated form of cantharidin an active constituent of Mylabris beetle, which has less systemic cytotoxicity. Although it can inhibit lung and liver tumor progression, its effect on TNBC has not been studied yet. In the present work we have evaluated the effect of NCTD on human (HS578T) and murine (4T1) TNBC progression. Previously we could determine that NCTD exhibits a significant antiproliferative effect, with an inhibitory concentration 50 of 56 and 35 μM for HS578T and 4T1 cells respectively, obtained by MTS assay. This effect was consequence of a sustained induction of the apoptotic process determined by fluorescence microscopy with acridine orange/BrEt staining, flow cytometry using Annexin V/IP ($p<0.05$ ANOVA) and the modulation of cleaved caspase 3 and Parp levels (Western Blot assays). In vivo assays showed that the systemic NCTD administration (2.5 mg/Kg) in BALB/c mice, significantly reduces tumor size after 17 and 21 days ($p<0.01$; $p<0.001$ respectively, ANOVA). This probably indicates a direct effect on both tumor mass and tumor stem cells, since NCTD affected in vitro oncospheres formation ability. Finally, the signaling pathways modulated by NCTD were studied. Surprisingly, Western blot assays showed that ERK/MAPK, AKT and NFkb pathways, associated to cell survival and proliferation processes, were activated after NCTD treatment. Although some results are encouraging, we must continue searching the molecular bases that support our biological results. Probably the combination with negative modulators of different signaling pathways may to enhance NCTD effect, encouraging its use in clinical settings.

537. (350) **THE RNAI-MEDIATED DOWNREGULATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS EFFECTIVELY INHIBITS PROLIFERATION, METALLOPROTEINASES ACTIVITY AND SPHEROIDS GROWTH IN HUMAN MCF-7 BREAST CANCER CELLS**

Dileyvic Giambalvo, Lourdes Materazzi, Macarena Acebedo, Eugenia Diez, Paula Ferraiolo, María Gabriela Lombardi.
Laboratorio de Oncoinmunología Molecular. Centro de Estudios Farmacológicos y Botánicos (CEFyBO)-CONICET, Segunda Cátedra de Farmacología, Facultad de Medicina, Universidad de Buenos Aires.

RNAi-mediated gene silencing (RNAi) has shown potential for cancer therapy. It has been reported that activation of muscarinic receptors (MR), subtypes 3 and 4, with the agonist carbachol (Carb), promotes tumoral progression of human breast cancer MCF-7 cells. We demonstrated that downregulation of MR has an inhibitory effect on colony formation and angiogenesis. We reported that intratumoral delivery of RNAi against MR affects the growth kinetics of MCF-7 *in vivo*. In this work, we silenced M_3 or/and M_4 subtypes of MR by RNAi (siM_3, siM_4 and siM_3M_4) in MCF-7 cells. Then, the different groups were treated or not with Carb (10^{-8}M) and we analysed the effect on different parameters of tumoral progression. By MTT assay, we observed that silencing of the MR_3 subtype affects the ability of MCF-7 cells to proliferate in response to cholinergic activation (siM_3+Carb:105±7; siM_3M_4+Carb: 105±12 vs MCF-7+Carb:151±11 $p<0,0001$; n=11; control: considered as 100%). In comparison to control, MR activation increased the activity of two isoforms (pro and active, detected by zymography) of metalloproteinases (MMP) 2 and 9. We observed that the inhibition of the Carb´s effect was most significantly in siM_3M_4 group (actMMP-2:158±6; proMMP-9:110±3; actMMP-9:139±4; vs MCF-7+Carb:334±15; 157±7; 210±8; $p<0,001$; control: considered as 100%).The activity of proMMP-2 was only detected in Carb treated groups and silencing also decreased the activity of this MMP isoform.Tumor spheroids were formed using the hanging droplet method. We observed that siM_3M_4+Carb spheroids showed a significant decrease in their diameter from day 4 of growth, compared to non-silenced spheroids (day40: siM_3M_4+Carb:1911±103µm vs MCF-7+Carb:2135±69µm; $p<0,0001$). In the siM_3+Carb and siM_4+Carb groups this decrease was only evident after day 32. Our results confirm the important role of muscarinic activation in tumor growth and support the possible therapeutic potential of RNAi gene silencing of MRs in breast cancer.

538. (356) **COMBINED TREATMENT OF RETINOIC ACID WITH A HER2-INHIBITOR PREVENTS CELL GROWTH AND INDUCES APOPTOSIS IN A TRIPLE NEGATIVE HUMAN BREAST CANCER MODEL**

Britez Neira DJ[1], Bechis A[1], Ariza Bareño LA[1], Cañonero L[1], Schey A[1], Urtreger AJ[1], Todaro LB[1].
[1]Instituto de Oncología "Ángel H. Roffo", Buenos Aires, Argentina

Cancer stem cells (CSC) are resistant to both chemotherapy and radiotherapy and are considered the seed of metastasis. Previously, we observed that CSCs derived from HER2-negative breast cancer lines over-express this receptor. With the aim of validating a new therapeutic strategy against this cellular component, we study the effect of combined treatment of All-Trans Retinoic Acid (ATRA) with Lapatinib (a HER receptor inhibitor) in triple negative human breast tumor cell line HS578T cultures. Presence of Lapatinib (5μM) on cell cultures induced two morphological changes: increasing of spin-

dle cells and reduced contact between them. In addition, Lapatinib treatment combined with ATRA showed a decrease in proliferation respect to untreated control at 72 hours and 96 hours ($P<0.05$ and $P<0.01$, respectively). We observed by flow cytometry with propidium iodide staining, that only combined treatment induced cell arrest in G0/G1 and a decrease in G2, respect to control ($p<0.5$). In addition, an increase in apoptotic and necrotic cells was evidenced in combined treatment, when measured Annexin V FITC by cytometry We have explored by qPCR some genes of the retinoid system. We observed a positive modulation of the retinoic receptor RARα by Lapatinib treatment. Regarding the expression of RARβ and RARγ: they both showed a significant increase respect to control ($p<0.05$) when treated with ATRA or Lapatinib/ATRA combined. Finally, a notable increase in retinol binding protein CRBP, was observed in the combined treatment. In the present work, cytostatic effects and a retinoid profile compatible with cell differentiation have been evidenced as a consequence of the presence of ATRA as treatment. Which is added a cytotoxic effect of the inhibitor Lapatinib that leads to cell death. These results would provide in vitro evidence of the potential use of this combined treatment in triple negative breast cancer.

539. (365) **MICRORNA EXPRESSION PROFILE OF TRIPLE NEGATIVE BREAST CANCER IN HUMAN TISSUE AND MURINE MODELS AND THEIR REGULATION BY METABOLIC SYNDROME**

Leandro Vera Sanchez[1;5], Paula Lucía Farré[1;5], Rocío Belén Duca[1], Karen Daniela Graña[1], Georgina Daniela Scalise[1], Guillermo Nicolás Dalton[1], Cintia Massillo[1], Juana Moro[1], Ezequiel Lacunza[2], Flavia Piccioni[3], Fiorella Campo Verde Arbocco[4], Adriana De Siervi[1], Paola De Luca[1].

1. Instituto de Biología y Medicina Experimental (IBYME-CONICET).

2. Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA), Facultad de Ciencias Médicas, Universidad Nacional de La Plata.

3. Laboratorio de Inmunobiología del cáncer - Instituto de Investigaciones en Medicina Traslacional (IIMT) - Universidad Austral - CONICET.

4. Laboratorio de Hormonas y Biología del Cáncer - Laboratorio de Endocrinología de la Reproducción y Lactancia, IMBECU CONICET. Universidad de Mendoza, Facultad de Ciencias Médicas.

5. Los dos son primer autor.

Triple negative breast cancer (TNBC) is the breast cancer (BC) subtype with worst prognosis and fewest therapeutic options. Metabolic syndrome (MS) is a risk factor for BC and its prevalence is higher in TNBC. Previously, we identified several miRNAs that could explain the link between MS and BC. We found that let-7b-5p, miR-28-3p and -877-5p were altered in the plasma from women with alterations associated to MS (AAMS), as well as in BC tissue. The aim of this work was to identify new key miRNAs in the development of TNBC associated with MS in human samples and to validate their expression in murine models of TNBC and MS. Our hypothesis is that miRNAs whose expression is exclusively altered in TNBC could explain the high aggressiveness of this molecular subtype and its association to MS. We found 222 miRNAs exclusively altered in TNBC tumors compared to normal adjacent tissue by bioinformatic analysis of GDC TCGA Breast Cancer patient database. We selected mir-19b-2, -135b, -29c and -138-1 for further analysis since they have MS-related functions, according to a bibliographic search. We evaluated their impact on patient survival from TCGA BRCA dataset using UCSC Xena tool. Interestingly, miR-877-5p expression correlates with lower BC patient survival while miR29c-5p and -3p increase survival and progression-free interval of TNBC patients. We validated these results in two murine models of TNBC and MS by determining the expression of these miRNAs in normal mammary tissue (MT) and tumors derived from 4T1 and MDA-MB-231 cells generated in mice with AAMS or control. Expression of miR-19b-3p was increased in 4T1 tumors compared to MT while miR-29c-5p and let-7b-5p were diminished. On the other hand, miR-877-5p and -28-3p were increased in MDA-MB-231 xenografts from AAMS mice compared to control. Our results suggest that miR-19b-3p, -29c-5p and let-7b-5p could be specific therapeutic targets for TNBC while miR-877-5p emerges as a molecular target for TNBC associated to MS.

540. (376) **NICOTINE NEGATIVELY MODULATES THE INHIBITORY EFFECT OF PACLITAXEL ON CELL VIABILITY IN TRIPLE NEGATIVE BREAST CANCER CELLS BY MODIFYING THE SIGNALING PATHWAYS INVOLVED**

Abigail Vásquez[1], Yamila Sanchez[1], Paloma Dejtiar Itzkov[2], Tobías Kohan Casa[2], David Krawiec[2], Alejandro Español[1]

1 Laboratorio de Inmunofarmacología Tumoral, CEFYBO-CONICET-UBA

2 Área de Química y Biotecnología, Escuela ORT

Breast cancer is classified into different subtypes depending on the expression of certain molecular biomarkers. Triple negative (TN) is the subtype with the worst prognosis due to the lack of a consensus about the treatment. Paclitaxel (PX) is one of the chemotherapeutic drugs frequently used. In smoker patients, nicotine (NIC) stimulates lung cancer progression, so we aimed to analyze its effect in breast cancer. We studied if it increases the cell viability of TN breast cancer cells MDA-MB231, the effect of its chronic administration (216 h) on the efficacy of 48 h PX treatment and the signaling pathways involved. We determined by Western blot assays that these cells express a7 and a9 nicotinic receptors (NR). By MTT assays, we demonstrated that they are functional since nicotine concentrations 10^{-9}M to 10^{-7}M induced an increment in cell viability ($p<0,001$). We pretreated the cells with nicotinic antagonists and kinases inhibitors and determined that the smokers' plasma concentration of NIC (10^{-7}M) increased cell viability (147.79±13.61% $p<0.001$ vs basal) through NRa7 (81.28±7.52%) and NRa9 (93.28±10.06%) and the participation of PKC (68.92±9.98%), ERK1/2 (71.4±11.18%) and NF-kB (102.12±12.08%) (all $p<0.001$). We confirmed that these cells are sensitive to PX in a concentration-dependent manner (CI_{50}PX: $4.52x10^{-7}$M) and determined that PX in a therapeutic concentration (10^{-7}M) exerts its effect (71.93±6.87%) by modulating Ras (107.48±4.7% $p<0.001$) and NF-κB (38.83±4.82% $p<0.001$). The pretreatment with NIC shifted the PX concentration-curve to the right, indicating a sensitivity reduction to this antitumor therapy (CI_{50}NIC+PX: $2.25x10^{-6}$M $p<0.05$). Moreover, NIC presence modifies the signaling pathway of the PX effect (125.82±10.82%), involving Ras (159.49±14.7% $p<0.001$) and MEK (152.32±1.79 $p<0.001$). We conclude that in TN breast cancer cells MDA-MB231 NIC negatively modulates the inhibitory effect of PX on cell viability by modifying the signaling pathway involved.

541. (409) **WHEN LEFT DOES NOT SEEM RIGHT: EPIGENETIC AND BIOELECTRIC DIFFERENCES BETWEEN LEFT AND RIGHT-SIDED BREAST CANCER**

Sofía Masuelli[1,2], Sebastián Real[1,2], Emanuel Campoy[1,2], María Teresita Branham[1,3], Diego Matías Marzese[4], Matthew Salomon[5], Gerardo De Blas[1,2], Michael Levin[6] and María Roqué[1,7]

[1]National Council of Scientific and Technological Research (IHEM-CONICET), Mendoza, Argentina.

[2]Medical School, National University of Cuyo,Mendoza, Argentina.

[3]Medical School, Mendoza University, Mendoza, Argentina.

[4]Cancer Epigenetics Laboratory, Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain.

[5]Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles,USA.

[6]Allen Discovery Center at Tufts University, Medford, USA.

[7]Exact Science Faculty, National University of Cuyo, Mendoza, Argentina.

Background: During embryogenesis lateral symmetry is broken, giving rise to Left/Right (L/R) breast tissues with distinct identity. L/R-sided breast tumors exhibit consistently-biased incidence, gene expression, and DNA methylation. We postulate that a differential L/R tumor-microenvironment crosstalk generates different tumorigenesis mechanisms. Methods: We performed in-silico analyses on

human breast tumors of public datasets, developed xenografted L/R tumors in mice, and conditioned MDA-MB-231 cultured cells with L/R human mammary extracts. Results: We found L/R differential DNA methylation involved in embryogenic and neuron-like functions (Gene Enrichment Analyses, adjusted p values < 0.05). Focusing on ion-channels, we discovered significant L/R epigenetic differences (53.8% vs 66.6%, OR 0.5, 95%CI 0.11–2.13 in the in-vivo experiment; 49.01% vs 50%, OR 0.96, 95%CI 0.35–2.59 in the in-silico experiment) and bioelectric differences. Specifically, L-sided cells presented increased methylation of hyperpolarizing ion channel genes and increased Ca^{2+} concentration (unpaired T-test with Welch's correction, $p < 0.003$; Kruskal–Wallis test, $p < 0.0001$) and depolarized membrane potential (L/R-fluorescence ratio, One-sample T-test with hypothetical R value = 1, $p = 0.04$), compared to R-ones. Functional laterality consequences were associated with increased proliferation in left tumors, assessed by KI67 expression (Unpaired T-test, $p = 0.002$) and mitotic count (Unpaired T-test, $p > 0.5$). Conclusions: Our findings reveal considerable L/R asymmetry in cancer processes, and suggest specific L/R epigenetic and bioelectric differences as future targets for cancer therapeutic approaches in the breast and many other paired organs.

542. (433) **ANALYSIS OF THYROID HORMONE EFFECTS ON THE RELEASE OF EXTRACELLULAR VESICLES AND THEIR ROLE IN CHEMOTHERAPY RESPONSE IN BREAST CANCER**

Johanna Díaz Albuja1 °, Pablo Fagúndez2, Juan Pablo Tosar2, Mateo Campos Haedo1*, María Mercedes Debernardi1*, Gonzalo Gonzalez1, Florencia Menay1#, Graciela Cremaschi1*, María Celeste Díaz Flaqué1*.

1. Instituto de Investigaciones Biomédicas BIOMED-UCA-CONICET.

2. Unidad de Bioquímica Analítica, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República, 11400, Montevideo, Uruguay.

** Sociedad Argentina de Investigación Clínica (SAIC).*

° Sociedad Argentina de Fisiología (SAFIS).

#Sociedad Argentina de Inmunología (SAI)

Chemoresistance is a major cause of cancer treatment failure. Extracellular vesicles (EVs) from resistant cells have been associated with the transfer of drug resistance to sensitive cells. Many breast cancer cells acquire multidrug resistance (MDR) by upregulating the level or activity of membrane proteins such as MDR1, which enables the exclusion of cytotoxic substances from the intracellular environment. Previously we have demonstrated that thyroid hormones (THs) modulate Doxorubicin response in T lymphoma cells. However, related to the chemoresistance of breast cancer cells, little is known about TH- induced mechanisms that influence tumor chemotherapy response. To this aim we first generate and characterized MDA-MB-231 Doxorubicin-resistant cells (MDA-DR). In these cells, we found that THs induce the expression of proteins involved in drug response such as MDR1, BCRP, and CYP3A4 ($p < 0,05$). In addition, we found that THs induce the release of EVs of 80-400 nm in size, as could be seen by nanoparticle analysis and transmission electron microscopy. In these EVs, we found protein expression of MDR1 and BCRP, the major proteins involved in the efflux of cytotoxic agents in breast cancer. Also, CD44 protein, associated with MDR1 transfer from vesicles to cells, was also found by western blot. Interestingly, the transfer of these EVs to doxorubicin-sensitive MDA-MB- 231 cells modulates the tolerance of sensitive cells to this drug. In conclusion, THs regulate the release of EVs and their protein cargo, containing MDR-transporters that could transfer drug tolerance to sensitive breast cancer cells.

543. (469) **MOUSE TRIPLE NEGATIVE BREAST CANCER 4T1 INTRADUCTAL MODEL CHARACTERIZATION FOR FUTURE STUDIES OF T2 ACTION**

Sólimo Aldana1-2, Filkiensztein Liliana3, Eiján, Ana María1,2, Lodillinsky Catalina1-2, Callero Mariana1-2.

1. Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigaciones, Departamento de Inmunobiología

2. Consejo Nacional de Investigación Científicas y Técnicas, CONICET

3. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Farmacología, Cátedra de Química Medicinal

Breast cancer is a neoplastic disorder of the mammary gland that remains one of the leading causes for cancer-induced death among women worldwide. Breast tumorigenesis is classically studied in mice by inoculating tumor cells in the adipose compartment of the mammary gland. Alternatively, the mammary ducts, which constitute the luminal mammary gland compartment, also provide a suitable inoculation site to induce breast cancer in murine models. Previously, our group has described an *N*4-aryl thiosemicarbazone (T2) action on fat pad 4T1 triple negative breast cancer model and found that this compound acts as an anti-invasive and anti- metastatic agent. However, since breast microenvironments influence tumor cell progression, we search for a more suitable model to clarify this action. In order to characterize intraductal 4T1 model, injection of several numbers and growth time of 4T1 cells have been tested. After whole mount staining of the mammary glands, we could observe positive mammary ducts (tumor cells inside the duct) with 15,000 cells 7 days after injection (a.i.). Later on, the presence of infiltrating tumor foci in the surrounding stroma was observed consistent with the presence of discontinuous SMA-alpha cell layers. The incidence rate (glands with tumor/ inoculated glands) was 100%. The percentages of positive and infiltrated ducts after 7 or 14 days a.i were 30.84 ± 4% and 4.2 ± 2.1% or 14.6 ± 2.2% and 14.3 ± 6.6, respectively. In order to characterize the immune infiltration in this model, we evaluated the tumor associated CD4/CD8 cells rate and we observed that it decreased from 3.87 ± 1.6 (7 days a.i.) to 1.48 ± 0.51 (7 days a.i.) at the expense of an increase in the number of CD8 cells. Although further tumor progression markers are being studied, we conclude that intraductal 4T1 model could be used for characterization of T2 action on tumor progression and inflammatory infiltrate as preclinical mouse models that mimic the complex human disease process from primary tumor growth to metastasis.

544. (479) **SEARCH OF PI3K AND CDK4-6 PATHWAY EFFECTORS AND ASSOCIATED MICRORNAS AS BIOMAKERS OF BREAST CANCER RESISTANCE**

Natalí Salgueiro[1], Karen Graña[1], María Cecilia Perrone[1], María Jimena Rodriguez[1], Marina Riggio[1], Andrea Werbach[1], Alexis Ostinelli[2], Sergio Rivero[2], Federico Waisberg[2], Adriana De Siervi[1] and Virginia Novaro[1].

Affiliations: 1. Instituto de Biología y Medicina Experimental (IBYME-CONICET) 2. Instituto Alexander Fleming (IAF) Buenos Aires, Argentina

salgueironatali@gmail.com

Dysregulation in the PI3K/S6 and CDK4-6/Rb pathways is associated with breast cancer progression and endocrine resistance. Therefore, the use of selective inhibitors is well established in clinical practice. In previous works we showed that high S6 and Rb protein levels are associated with poor prognosis in luminal tumors. In this study, we focus in the search of PI3K and CDK4-6 effectors and signs of pathway activation that could be used to predict which patients will respond to specific inhibitors. We determined the presence of S6 and Rb by immunohistochemistry in 55 primary carcinomas, and found that levels tend to increase in patients that relapse earlier. Furthermore, tumor cells that metastasize lymph node, liver and bone express high S6 and Rb levels. Consistently, in T47D and MCF-7 tamoxifen- and palbociclib-resistant cell variants, acquired resistance is associated with higher levels of S6 and CDK4-6 ($p<0.01$). We next performed stem loop RT-qPCR to evaluate expression of S6 and CDK4-6-associated miRNAs and found that miR-21, -126 and -200a are altered in resistant T47D variants (miR-21 is reduced, whereas -126 and -200a are increased, in comparison to wild type cells, $p<0.05$). Likewise, miR-21, -126 and -200a tend to be reduced in tumors that relapse earlier. Furthermore, in a prospective study we found these miRNAs in the plasma of patients that have relapsed to endocrine therapy. The levels remain higher in comparison to plasma from healthy women ($p<0.001$). We are

evaluating these and other miRNAs in patients that follow a second line therapy with palbociclib for at least 2 years or till a new relapse. In summary, we postulate that high levels of S6 and Rb could anticipate which patients will respond to selective kinase inhibitors and could constitute relevant predictive markers for clinical application. Moreover, circulating miRNAs that are associated with S6 and CDK4-6 are good candidates to follow patients under therapy to anticipate tumor relapse.

545. (525) **THE BLOCKAGE OF THE IL6-STAT3 PATHWAY RESTORES TRASTUZUMAB RESPONSE OF HER2+ RESISTANT TUMORS**

Sebastián Real[1,2] and María Roqué[1, 3]
[1]National Council of Scientific and Technological Research (IHEM-CONICET), Mendoza, Argentina
[2]Medical School, National University of Cuyo,Mendoza, Argentina.
[3]Exact Science Faculty, National University of Cuyo, Mendoza, Argentina.

Introduction: Resistance to the Mob Trastuzumab (TZM) is the main cause of dead in HER2+ breast cancer patients and it occurs in around 30% of primary and up to 70% of metastatic tumors. Several pathways are involved in this resistance, and new drugs are used as second and thirst line of treatment. Previous in vitro studies have suggested that the IL6-STAT3 pathway could be involved in TZM resistance, probably by altering the stem cell proportion in the tumor. In the present work we demonstrate in a HER2+ PDX model generated in our lab, that the inhibition of IL6 receptor (IL6R) restores the TZM response in resistant tumors. M&M: A human HER2+ breast tumor was implanted in an immunosuppressed mice, and then transplanted to generate a HER2+ PDX line. ER, PR and KI67 status was confirmed by IHC. PDX mice were afterwards treated with increasing doses of TZM to obtain a resistant tumor. Mob Tocilizumab was used to inhibit IL6R. Results: The TZM resistant tumors were able to grow and resist almost doubled doses of TZM (*p=0,045*). To evaluate the effect of the blockage of the IL6-STAT3 pathway, we applied Tocilizumab (Tocili) that is used in clinic to treat advanced Covid19 patients and rheumatoid disease. The use of Tocili alone generated a small reduction in the tumor growth of TZM resistant tumor (*p=0,009*), suggesting a basal effect of the drug. And the combination of Tocili + TZM generated a stronger reduction in the kinetic of growth when compared to TZM alone (*p=0,004*), similar to the non-resistant tumor. Conclusion and perspectives: the blockage of IL6-STAT3 pathway can restore the sensitivity to TZM in a human HER2+ resistant tumor, suggesting the possible repurposition of Tocilizumab as a treatment for TZM resistant patient. Actually, we are expanding the analysis to more HER2+ PDX tumors.

546. (543) **ESTROGEN INDUCES ID4 SILENCING THROUGH PROMOTER METHYLATION IN ER+ BREAST TUMORS**

Daniela Nasif[1], Sebastian Real [1,2], Sergio Laurito[1,3], María Roqué[1,3], Branham María Teresita [1,4]
[1]National Council of Scientific and Technological Research (IHEM-CONICET), Mendoza, Argentina.
[2]Medical School, National University of Cuyo, Mendoza, Argentina.
[3]Exact Science Faculty, National University of Cuyo, Mendoza, Argentina.
[4]Medical School, University of Mendoza, Mendoza, Argentina.

Background: Inhibitor of differentiation protein 4 (ID4) is a dominant negative regulator of the basic helix-loop-helix (bHLH) family of transcription factors. Our group has previously shown that, in breast cancer, ID4 behaves as a tumor suppressor only in estrogen receptor positive (ER+) tumors and that ID4 expression is downregulated through methylation. Taking these observations into consideration, we decided to explore into the study of the molecular mechanisms that lead to the silencing of ID4 through methylation. Given that ID4 is methylated only in ER+ tumors, we hypothesize here that estrogen via estrogen receptor α induces ID4 methylation. Methods: In vitro experiments involved cell culture of MCF7 and T47D ER+ breast cancer cell lines, cells were treated with estradiol, 5-Azacitidine or Tamoxifen. Gene and protein expression were analyzed by RT-qPCR and western blot and methylation by ddMSP. In-silico analyses involved the evaluation of ID4 expression and methylation status on human breast tumors of public datasets according to breast cancer molecular classification and estrogen receptor status. Results: Estradiol treatment induced a reduction in ID4 expression through increased methylation on ID4 promoter ($p<0.05$). 5-Azacitidine induced a reduction in ID4 methylation which was reverted by estradiol treatment in line with a methylating role of estrogens ($p<0.05$). To confirm that the effects induced by estradiol were through the estrogen receptor α, cell lines were treated with Tamoxifen (an antagonist of ER in breast). Tamoxifen treatment decreased ID4 methylation and increased its expression ($p<0.01$). Conclusions: Our findings reveal that estrogens through estrogen receptor α induce the silencing of the tumor suppressor gene ID4 through methylation of its promoter in ER+ breast tumors.

547. (546) **E2F1 AND RB ARE COMMON MEDIATORS OF THE INHIBITORY EFFECTS PROMPTED BY THE COMBINATION OF MIFEPRISTONE AND PALBOCICLIB ON BREAST CANCER CELLS EXPRESSING PROGESTERONE RECEPTOR ISOFORM A**

Gabriela Pataccini[1], Claudia Lanari[1], Sebastián Giulianelli[2]
[1]Instituto de investigaciones Biomédicas y Medicina Experimental (IByME) [2]Instituto de Biología de Organismos Marinos, IBIOMAR-CCT CENPAT-CONICET, Argentina

Palbociclib (PALBO), a CDK 4/6 inhibitor, is currently used in combination with endocrine therapy targeting estrogen receptors to treat advanced luminal breast cancer. However, with time tumors become resistant to these treatments highlighting the need to develop other therapeutic strategies. Our laboratory focuses on the use of therapies targeting progesterone receptors (PR). We have previously shown that PALBO inhibits luminal breast cancer cell proliferation regardless of the prevailing PR isoform expressed and that mifepristone (MFP), an antiprogestin, potentiates this effect only in cells expressing PR isoform A (PRA). The aim of this study was to evaluate the role of two key cell cycle proteins, RB and E2F1 as mediators of this effect. T47D-YA or T47D-YB cells, expressing respectively PRA or PRB were treated with MFP, PALBO, or MFP+PALBO. The expression of E2F1 and pRB was evaluated by western blots. In agreement with data obtained in cell proliferation studies, a significant decrease of both protein levels ($p<0.05$) was observed only in T47D-YA cells treated with MFP+PALBO, whereas slight decreases were noted with single treatments. Contrarily, in T47D-YB cells, the effects of combined drugs were similar to those induced by PALBO. The *in vivo* growth of T47D cells expressing equimolar levels of PRA and PRB was also inhibited by the combined therapies and the strongest inhibition of pRB was registered by immunohistochemistry in tumors treated with both agents. Our results suggest that E2F1 and RB are key players mediating the inhibition of cell proliferation induced by PALBO and MFP combination exclusively in PRA-expressing cells. Mechanistic studies are underway to explore the direct involvement of PRA on the E2F1 promoter.

548. (551) **REGULATION OF HORMONE-RELATED PROTEINS IN TUMOR-ADJACENT BREAST TISSUE BY MIFEPRISTONE TREATMENT**

Leo Saldain[1], Silvia I Vanzulli, Paula Martínez Vazquez[2], Javier Burruchaga[2], Eunice Spengler[2], Marcos Liguori[2], Claudia Lanari[1]*, Paola Rojas[1]*
**: equal contribution*
1. Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina.
2. Hospital de Agudos "Magdalena V de Martínez", General Pacheco, Argentina.

Preclinical data suggests that antiprogestins inhibit the growth of luminal breast carcinomas expressing higher levels of progesterone receptor (PR) isoform A (PRA) than isoform B (PRB), named PRA-H. Therefore, we designed a window-of-opportunity trial (MIPRA; NCT02651844) in order to study the benefits of mifepristone

(MFP) in breast carcinomas from postmenopausal patients selected by their PR+ expression (>%50) and PR isoform ratio (PRA/PRB≥1.5), resulting in a decrease of Ki67 expression after treatment (median=49.62%). Data regarding the effect of MFP in non-neoplastic human mammary glands (nnMG) is limited and points, in premenopausal women, to a decrease in the Ki67 index after treatment. Thus, we decided to evaluate the expression of biomarkers that were regulated by MFP in tumors from the MIPRA trial, in the nnMG adjacent to the tumor tissue of PRA-H postmenopausal patients, who were treated (n=6) or not (n=6) with MFP. Whereas Ki67, PR, estrogen receptor alpha (ER) and pSer118ER were down-regulated by immunohistochemistry in most MIPRA tumors studied after MFP treatment, Ki67 index was slightly increased in MFP-treated nnMG compared with those from untreated patients (p=0.03) and no difference of PR, ER, and pSer118ER expression was observed. Contrarily, whereas pSer167ER expression was either upregulated or remained without changes in the PRA-H tumors after MFP treatment, a significant decrease of pSer167ER expression was observed (p=0.004) in the MFP-treated nnMG, suggesting a tissue-dependent modulation in the AKT-mediated activation of ER. In conclusion, a selective modulatory effect of MFP in PRA-H tumors, but not necessarily in the adjacent breast tissue is suggested, probably due to the fact that adjacent nnMG have presumably equimolar levels of PRA and PRB. The basal quiescent status of nnMG in postmenopausal women may explain the slight stimulatory effect observed in Ki67 expression.

549. (553) **ANDROGENIC REGULATION OF ACYL-COA SYNTHETASE 4 IN BREAST CANCER CELLS**

Melina A. Dattilo, Paula F. Lopez, Evelyn M. Adler, Yanina Benzo, M. Mercedes Bigi, Estefanía Clavero, Cristina Paz, Ernesto J. Podestá, Paula M. Maloberti.
INBIOMED (UBA-CONICET), Dpto. de Bioquímica Humana, Facultad de Medicina, UBA

It is known that ACSL4 overexpression is involved in the development of a very aggressive phenotype in breast and prostate cancer. Androgen receptor (AR) expression or androgenic stimulation decrease mRNA levels of ACSL4 in prostate cancer cells. However, the effects of androgenic regulation in breast cancer cells have not yet been described. The aim of this work is to study whether AR and androgens regulate ACSL4 transcription in breast cancer cells. We observed that AR mRNA is detected in the slightly aggressive breast cancer cell line MCF-7. In contrast, this expression is not detectable in the very aggressive cell line MDA-MB-231. Bioinformatic analysis performed with the Genomatix tool and contrasted with the Alggen Promo software, showed one AR element (ARE) in a 1.8 kb fragment of the human ACSL4 promoter with a score close to 1. Transient transfection of AR in MDA-MB-231 cell line reduced ACSL4 1,8 kb promoter activity (p<0.001) as measured by the Nano-Glo® Luciferase Assay System (Promega) and this decrease was translated into decreased ACSL4 mRNA levels (p<0.001), quantified by real-time qPCR. Cells treated with 10 nM dihydrotestosterone (DHT) in steroid-free medium, showed a decrease of transcriptional activity of the promoter and of ACSL4 mRNA levels, both in MCF-7 cells (p<0.001) and in MDA-MB-231 with ectopic expression of human AR (p<0.001). This effect is not observed in MDA-MB-231 cells when they are not transiently transfected with the human AR. These results demonstrate the role of androgens in the expression of ACSL4 in breast cancer cells and suggest that androgen treatment could be a tool to reduce the expression of ACSL4 in breast cancer and thus lay the groundwork for the study of androgen-containing hormone therapy for breast cancer.

550. (600) **AN INCREASE OF GRANULOCYTES AND LEUCOCYTES EXPRESSING S100A9 IN PATIENTS WITH CERVICAL CANCER IS ASSOCIATED WITH A WORSE PREDICTIVE RESPONSE**

Yanina Verónica Langle*[1], Pablo Damián Cresta Morgado*[2], Denise Belgorosky[1], Florencia Guerra[2], Pablo Marenco[3], Pablo Menéndez[3], Valeria Cáceres[4], Andrea Aguilar[4], Marcela Ostojich[5], Laura Lay[5], Ana María Eiján[1], Eduardo Omar Sandes[1].

**Share first authorship.*
[1]Área Investigaciones. [2]Unidad de Investigación de Transferencia. [3]Área de Terapia Radiante y Medicina Nuclear. [4]Área Médica, Departamento de Tumores Femeninos. [5]Área Quirúrgica, Departamento de Ginecología. Instituto de Oncología Ángel H. Roffo, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Cervical cancer (CCa) is the second most common type of cancer affecting women. Leukocytosis is related with treatment failure and with the induction and accumulation of myeloid derived suppressor cells (MDSC) in peripheral blood. S100A9 is a useful marker to identify MDSC in whole blood. In this study, we analysed in blood of patients with locally advanced CCa (Pts) and in healthy donors (CRLs), the number of total leukocytes, monocytes (CD14+) and granulocytes (CD15+) that express S100A9. Peripheral blood from CRLs and Ptes with CCa (stage IB3-IVA) treated between 2018-2021 with concurrent chemoradiotherapy and brachytherapy were collected. Leukocytes were isolated and stained for S100A9, CD14 and CD15 and then analysed by flow cytometry. The predictive value of S100A9+ cells between Pts and CRLs was evaluated and associated with therapeutic response. Results: An increase in S100A9+ leukocytes and granulocytes were observed in peripheral blood from Pts before treatment compared to CRLs (p=0.0003 and p=0.0005, respectively). Tumor response was dichotomized as complete or insufficient response (persistence + progression), associated to S100A9+ cells. Using a logistic regression model, we observed that the percentage of leukocytes and granulocytes S100A9+ exhibited a trend close to significance of being associated with tumor response [Leukocytes odds ratio (OR): 0.908, 95% confidence interval (CI): 0.819-1.006, p=0.06; Granulocytes OR: 0.907, 95% CI: 0.821-1.002, p=0.057]. In this way, it represents that 1% increase in S100A9+ leukocytes or granulocytes are associated with a 9% reduction of having a complete response. Conclusions: Pts with locally advanced CCa presented an increase in total leukocytes and granulocytes that express S100A9. The association between S100A9+ leukocytes and granulocytes with tumor response might suggest that Pts with greater levels of these cells could define a population with higher risk of therapeutic failure.

551. (828) **SOLUBLE GUANYLYL CYCLASE ALFA1 AND BETA1 SUBUNITS PLAY ANTAGONIC ROLES IN A HUMAN CERVICAL CANCER MODEL**

Acosta Lucas Hernán[1,2], Rocca María Victoria[1], Pino María Teresa[1,2], Duvilanski Beatriz Haydée[1,2], Cabilla Jimena Paula[1,2]
[1]Centro de Altos estudios en Ciencias Humanas y de la Salud, Universidad Abierta Interamericana, [2]Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET)

Soluble guanylyl cyclase is the main nitric oxide receptor and is comprised by alpha1 (α1) and beta1 (β1) subunits. We have shown that α1 is upregulated after estrogen treatment in several tissues and cell lines, while β1 is downregulated or not affected. α1 is involved in cell growth, migration, and invasion in ER- and ER+ cancer cells but β1 effects remains almost unknown. The aim of this study was to explore the role of each subunit on cell proliferation, protein expression and their interrelation in HeLa cells. β1 and α1 were overexpressed using adenoviral vectors (sGCβ1-GFP or sGCα1-myc, MOI 1-100) or empty virus (control). Cells were incubated for 48 h. Cell viability (MTT and flow cytometry), protein expression (western blot), migration (scratch motility assay) and metalloproteinases activity (gelatin zymography) were determined. β1 overexpression (MOI 1-10) decreased cell viability (14% p<0.05), increased the percentage of subGo/G1 DNA content (1.6-fold) and decreased PCNA expression (66% p<0.01). Besides, α1 and Akt protein levels were reduced (54% p<0.01 and 60% p<0.05, respectively). ERα66 was not detected but ER-truncated variant ERα46 increased (64% p<0.01). β1 decreased cell migration (20% p<0.05) and MMP-2 activity (20% p<0.05). Unexpectedly, β1 overexpression (MOI 50-100) increased α1 correlated with PCNA and Akt protein levels (p<0.05). α1 overexpression augmented cell viability in basal conditions (28% p<0.05) and after serum deprivation (40% p<0.05) at all MOI assayed. Be-

sides, α1 increased MMP-2 activity (70% p<0.001), VEGF expression (20% p<0.05), and downregulated ERα46 levels (34% p<0.05). Our results suggest that α1 promotes cell proliferation and survival while β1 effects seems to be biphasic and depending on its expression grade in HeLa cells. It has been reported that β1 can stabilize α1 protein levels thus explaining α1 increase. Both subunits differentially regulated ERα46 which is associated with better prognosis in some cancers.

ONCOLOGY V *Friday, November 18, 14-15:30 hr*
Chairs: Victoria Fabris - Patricia Pennisi - Paola De Luca

552. (284) **THE NON-NEURONAL CHOLINERGIC SYSTEM INDUCES THE INHIBITION IN THE VEGF PRODUCTION IN A GLIOBLASTOMA MULTIFORME U251 CELL LINE**

Alejandra Infante[1], Paula Saibene[1], David Rosso[1], Micaela Rossato[1], Marianela Candolfi[2], Juan Iturrizaga[1,4], Martín Abelleyro[1], Carolina Jancic[1], Mónica Vermeulen[1], Marcela Villaverde[3] and Gabriela Salamone[1].

1. Instituto de Medicina Experimental (IMEX)-CONICET Academia Nacional de Medicina.
2. INBIOMED (UBA-CONICET)
3.Unidad de Transferencia Genética. Instituto de Oncología Ángel H. Roffo
4.División Neurocirugía del Instituto de Investigaciones Médicas A Lanari. Universidad de Buenos Aires. Universidad de Buenos Aires.

Glioblastoma multiforme (GBM) is the most common and deadly cancer of the central nervous system. This is at least in part due to the complex interaction of GBM cells with the brain microenvironment and their tendency to infiltrate normal brain tissue. This tumor is characterized by the increase in vascularity due to hypoxic regions of tumor driven by VEGF that stimulate the angiogenic activity and tumorigenesis. Acetylcholine (ACh) is a neurotransmitter, which can also modulate cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells and different tumors. The aim of this work was to evaluate the influence of ACh in the growing of tumor. We examined the expression and function of ACh receptors in GBM datasets using RNA seq from Illumina HiSeq 2000 sequencing platform, by analysing GBM samples from The Cancer Genome Atlas (TCGA) using the TIMER2.0 web server. There is a decrease in the survival in the GBM patients who had increased expression of M3 cholinergic muscarinic receptors (CHRM3); we confirmed in human biopsies of tumors of patients with GBM the expression of CHRM3, by Immunohistochemistry. On the other hand, we evaluated the proliferation of U251 cell line in a 3D culture in presence of ACh for 9 days using the acid phosphatase assay (APH) and we did not find significative difference respect to the control. In addition, we evaluated the VEGF production in the supernatant of 2D (18 h) and 3D (9 d) of U251 cell cultures incubated in presence or absence of ACh by ELISA assay. Interestingly, we observed a decreased in the VEGF production in the cells cultivated with cholinergic agonist (p<0.05). In conclusion: our findings suggest that the cholinergic system inhibit the production of VEGF in 2D and 3D culture suggesting a possible relevance in the comprehension in the resistance of the GBM to different treatment.

553. (307) **ACTION OF THYROID HORMONES IN SORAFENIB TREATMENT OF THYROID CANCER**

Campos Haedo MN[1], Diaz Flaqué MC[1], Sterle HA[1], Díaz Albuja JA[1], Perona M[2,3], Juvenal GJ[2,3], Cremaschi GA[1], Rosemblit C[1].

1 BIOMED UCA-CONICET, Buenos Aires, Argentina.
2 Departamento de Radiobiología (CAC), Comisión Nacional de Energía Atómica (CNEA), Buenos Aires, Argentina.
3 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Background. Thyroid carcinoma (TC) is the most common endocrine neoplasia. Its incidence has increased in the last 40 years worldwide. Sorafenib (Sor), a tyrosine kinase inhibitor, was approved for the treatment of TC, being hypothyroidism the most frequent consequence of Sor-induced endocrine dysfunction. We have described that thyroid hormones (TH) increase cell proliferation in TC lines. Thus, hormone replacement therapy to treat Sor-induced hypothyroidism could negatively affect the antitumor action. We have also shown that the selective inhibition of the TH membrane receptor, αVβ3 integrin, diminishes proliferation. Objective. To study integrin αVβ3 inhibition to enhance Sor antineoplastic activity in TC and the molecular mechanisms involved. Results. We first studied the role of αvβ3 integrin in Sor inhibition of TH-induced proliferation in TC cells. The treatment of the human anaplastic 8505C TC cells with the αvβ3 integrin antagonist Cilengitide (Cile) inhibits TH-induced proliferation by MTS assay (p<0.0001), confirming the participation of the integrin. As expected, Sor treatment decreases proliferation (p<0.05) besides the addition of Cile did not change the level of inhibition. Also, we found that Sor and Cile treatment diminished PCNA and Cyclin D1 expression by western blot. Finally, cells were preincubated with Sor and Cile and then treated or not with TH for 48h to analyze apoptosis by annexin V and propidium iodide staining, followed by flow cytometry. Sor treatment increases the number of apoptotic cells relative to untreated control (p<0.0001) and the presence of TH reduces the rate of apoptosis (p<0.01). The combination of drugs enhances apoptosis independently of TH presence. Conclusion. Our results establish that the effective dual Sor and Cile treatment can significantly drive tumor proliferation inhibition and apoptosis, and it could provide alternatives to the treatments currently used for this disease.

554. (447) **EFFECTS OF iNOS AND METABOLIC INHIBITION IN HUMAN PRIMARY GLIOMA CULTURES**

Elsa Lourdes Hincapié Arias[1,4], Denise Belgorosky[1], Martín Merenzon[2], Eduardo Roberto Seoane[2], Ezequiel Yasuda[3], Rodolfo Recalde[3], Mariano Socolovsky[3], Roberto Zaninovich[3], Marcela Villaverde[1,4*] and Ana María Eiján[1,4*]

*[1] Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, [2] Hospital General de Agudos Dr. José María Ramos Mejía, [3] Hospital de Clínicas José de San Martín, Universidad de Buenos Aires, [4] CONICET, * sharing direction*

Introduction: Gliomas (GM) are a group with varied cellular origins, from low to high grade (I-IV, OMS). Despite treatment with surgery/radiotherapy/chemotherapy, malignant and some benign gliomas in eloquent areas or of vital importance are inoperable and/or have a dismal prognosis. It has been suggested that inducible nitric oxide synthase isoform enzyme (iNOS) is related to glioma growth, progression and cancer stem cell (CSC) maintenance; and that the selective inhibition of glycolytic pathways is related to a decreased proliferation of glioma cells. Objective: Evaluate the effect of iNOS specific inhibitor, S-methylisothiourea (SMT); and the effect of glycolytic inhibitor, 2-Deoxy-D-glucose (2DG), alone or in combination with TMZ, on 2D (monolayer) and on the glioma stem cell (GSC) niche. Methodology: Endorsement was obtained from the ethics committees of 3 hospitals (Ángel H. Roffo, Clínicas, Ramos-Mejía); and signed informed consents. Samples were obtained between 2018 and 2022 from the operating room and immediately processed by mechanical disintegration with DMEM-F12 and 10% fetal bovine serum; seeded in 2D and under sphere conditions (low adhesion and high dilution). Cell viability was determined in 2D by MTS. The number of CSC was established by sphere forming efficiency (SFE) in relation to the number of seeded cells and their diameters were measured with imageJ. Results: 11 human GM samples were processed. 6 cultures prospered with at least 1 cell passage. In 2D, 2DG decreased cell viability (50 - 70%); SMT has no significant effect. In the GSC niche, SMT alone decreased SFE (40 - 60%, p<0,001 vs. control) and sphere diameters (30 - 50%, p<0,001 vs. control); 2DG showed only a tendency of decreased SFE. Conclusion: Primary cell cultures of human gliomas were obtained, 54% of them prospered. iNOS inhibition reduces the GSC niche. 2DG decreases the viability of more differentiated cells. Further studies are needed to validate these results.

555. (509) **EFFECT OF SUPERPARAMAGNETIC IRON OXIDE**

NANOPARTICLES (SPIONS) ON THYROID CELLS

Marina Perona[1,2], Cecilia Grissi[3,4], Susana I Nievas[2], Lisa Thomasz[1,2], Romina Oglio[1], Carla Rodriguez[1], Cinthia Rosemblit[5], Mateo Campos Haedo[5], María A Dagrosa[1,2], Cremaschi G[5], Hebe A Durán[3,4], Guillermo Juvenal[1,2], Irene L Ibañez[3,4]

[1]Departamento de Radiobiología, Comisión Nacional de Energía Atómica (CNEA), [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), [3]Gerencia de Investigación y Aplicaciones, CNEA, [4]Instituto de Nanociencia y Nanotecnología (INN), CNEA-CONICET, [5]Biomed UCA-CONICET, [6]Escuela de Ciencias y Tecnología, Universidad Nacional de San Martín (UNSAM), Buenos Aires, Argentina.

Introduction: Thyroid carcinoma is the most frequent malignancy of the endocrine system. Well-diFFerentiated thyroid carcinoma, especially papillary (PTC) and follicular (FTC) variants, account for about 94% of the cases and the prognosis is favorable. However, there remains a subset of patients with advanced or recurrent disease with a poorer response to conventional therapy. External beam radiotherapy (EBRT) is a treatment option especially for papillary thyroid carcinoma. Superparamagnetic iron oxide nanoparticles (SPIONs) have been used in cancer diagnosis and therapy. SPIONs or mPEG-SPIONs increase sub-toxically ROS levels. This characteristic could be combined with radiotherapy to optimize the clinical outcome. Objective: The aim was to study the effect of SPIONs in different thyroid cell lines. Methods: SPIONs were synthesized and stabilized by methyl-poly(ethylene glycol). Papillary thyroid cancer cells (TPC-1) were incubated with different concentrations of coated (mPEG) and non-coated SPIONs. Cell viability was measured 24 and 48 hours later by MTT method. Intracellular SPION content by measuring the Fe concentration *per* cell was performed by ICP-AES at 2, 4 and 24 hours. Intracelullar ROS levels were measured using the fluorescent dye 2', 7'-dichlorofluorescein-diacetate (DCFH-DA). Results: Increasing doses of SPIONs did not affect cell viability (0-250 μg/ml). Intracellular iron content *per* cell was significantly increased at 2, 4 and 24 hours in cells incubated with SPIONs ($p<0.001$ for 25 and 50 μg/ml *vs.* 100 μg/ml). Intracellular ROS levels were higher in cells incubated with SPIONs ($p<0.05$ control vs. uncoated 50 μg/ml SPIONs). Conclusions: SPIONs increased intracellular iron levels and ROS levels in a dose-dependent manner without affecting cell viability. Considering the above results, the radiosensitizing effect of SPIONs will be evaluated in thyroid cell lines.

556. (528) **ROLE OF GLYCOSYLATION IN THE INTERACTION BETWEEN INTEGRIN αV AND UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR IN HUMAN GLIOBLASTOMA**

Nogueira Aylén Camila[1], Ferreira Gretel Magalí[1,2], Rojo Selene[1], Gulino Cynthia Antonella[1], Castillo Jeremías Omar[1,2], Segatori Valeria Inés[1,2] and Gabri Mariano Rolando[1,2]

1Centro de Oncología Molecular y Trasalcional, Universidad Nacional de Quilmes,

2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

It is well described that aberrant glycosylation has an important role not only in tumor transformation but also in tumor progression. Glioblastoma (GBM) has a median survival of 15 months and a long-term survival of less than 5% in human patients. This poor patient survival points out the importance of new therapeutics targets to be discovered in this type of tumors. Integrin alpha V (IαV) and urokinase-type plasminogen activator receptor (uPAR) are glycosylated plasma membrane proteins described as key players in GBM malignancy. Even though IαV has been reported to act as a co-receptor of uPAR, there is no information regarding how these two proteins interact with each other in GBM. Taking this information into account, the aim of our work was to evaluate if the interaction between them in GBM is mediated by glycans and to identify which type of glycosylation is involved. We first evaluated the expression of IαV and uPAR on a set of human GBM cell lines by flow cytometry, and found that the 4 cell lines showed expression of both proteins with mean fluorescence values of 9,56 ± 3,82 and 1,36 ± 0,2, respectively. Since A172 cells showed the higher expression of uPAR, we decided to continue the experiments using this cell line as our model. In order to increase uPAR expression, at the same time we transfected A172. We then evaluated the interaction between IαV and uPAR in A172 wild type cells by co-immunoprecipitation, and found that both protein interact since the pull down of IαV allows us to observe the presence of uPAR by western blot. Interestingly, swainsonine treatment disrupts this interaction. These results suggest that N-glycans may have a role in the interaction between IαV and uPAR in GBM, laying the foundation for further characterize and evaluate how N-glycosylation modulates downstream signaling and tumor behavior.

557. (529) **BIOLOGICAL EFFECTS OF IONIZING RADIATION ON TUMOR MICROENVIRONMENT OF THYROID CANCER CELL**

Rodriguez Carla[1,], Perona Marina[1,2], Oglio Romina[1,], Gambetta Adriana[1], Juvenal Guillermo[1,2], Dagrosa Alejandra[1,2], Thomasz Lisa[1,2].

[1] Department of Radiobiology (CAC), National Commission of Atomic Energy (CNEA), [2] National Council of Scientific and Technical Research (CONICET).

Thyroid Cancer (TC) is the most prevalent malignant endocrine system disease. Patients with differentiated thyroid carcinomas have a good prognosis, while some may progress to more aggressive phenotypes such as undifferentiated or anaplastic thyroid carcinoma, which has a very poor prognosis. Ionizing radiation is frequently applied to tumors in patients suffering cancer, with curative or palliative purposes. Nevertheless, tumor resistance and metastasis remain as a clinical concern after radiotherapy. The tumor microenvironment plays a key role in cancer development and progression and ionizing radiation also affects non-tumoral cells in the surrounding tissue. The irradiated cells are believed to communicate signals by affecting the function of non-irradiated cells. We propose to study the effect of soluble factors derived from irradiated thyroid cancer cells on cell proliferation and migration on undifferentiated non-irradiated thyroid cancer cells. Methods: Undifferentiated thyroid carcinoma cells were irradiated with 0, 2, 5 and 8 Gy. After 1, 24, 72 and 96 hours, the supernatants of the irradiated cells were collected and used as conditioned medium for cell proliferation (MTT) and migration (wound) assays. Results: Conditioned medium from irradiated cells after 1 and 24 h have no effect on cell proliferation and migration of non-irradiated cell. After 72 h conditioned medium stimulated significantly cell proliferation of non-irradiated cells by 1.3 fold at 2 Gy, 1.4 fold at 5 Gy and 1.4 fold at 8Gy ($p<0.01$) and after 96 hours by 1.3 fold at 2 Gy, 1.4 fold at 5 Gy and 1.5 fold at 8 Gy ($p<0.01$). Migration studies show that conditioned medium from irradiated cells with 5 Gy and 8 Gy after 76 hours stimulated migration of non-irradiated cells by 1.8 and 2 fold ($p<0.01$). Conclusion: Irradiated cells secreted factors to the medium with pro tumorigenic activity.

558. (561) **EFFECT OF METRONOMIC CHEMOTHERAPY (MCT) WITH LOSARTAN (LOS) AND CYCLOPHOSPHAMIDE (CY) ON THE ANTI-TUMOR RESPONSE OF MACROPHAGES IN THE M-406 TRIPLE-NEGATIVE MAMMARY ADENOCARCINOMA MODEL IN FEMALE MICE WITH METABOLIC SYNDROME (MetS)**

Barranco MM[2;3], Hillotte G[1], Scharovsky OG[1;3], Rico MJ[1;3], Rozados VR[1;3], García F[2;3], Mainetti LE[1;3]

[1]Instituto de Genética Experimental Facultad de Ciencias Médicas, [2]Laboratorio de Fisiología Metabólica. Facultad Ciencias Médicas, [3]CONICET

Breast cancer frequently coexists with Metabolic Syndrome (MetS), a pathology that is associated with cancer progression and a worse prognosis. Our aim was to evaluate the effect of MCT with CY+LOS on the anti-tumor response of macrophages, in mice with MetS bearing a mammary adenocarcinoma. CBi female mice (5 weeks) were fed with standard diet (SD) or diet with 40% calories of fat (HFD) throughout the experiment. At 20 weeks, the development of MetS was confirmed on the HFD group by biochemical and morphological parameters. Once the MetS features were settled, all mice

were challenged orthotopically with M-406 tumor (day 0); when the tumor was palpable, mice of SD and HFD diets were distributed into 2 groups, GI: Control, with no treatment; GII: Cy 25mg/kg/day+Los 150mg/kg/day in the drinking water. Mice were weighted and tumor volume measured 3 times/week. When tumors were exponentially growing, mice were euthanized, tumors excised, fixed and paraffin included. *Peritumoral* and *intratumoral* macrophages [Total-Mt-(F4/80$^+$), M1 (iNOS$^+$, antitumoral) and M2 (MRC1$^+$, protumoral)] were analyzed by immunofluorescence. GI-HFD mice showed a significantly higher number of Mt and M2 *peritumoral* macrophages compared to GI-SD mice ($P<0.05$). MCT with CY+LOS produced a significant increase in Mt and M1 peritumoral macrophages in both dietary groups when compared to the respective controls ($P<0.05$). The *intratumoral* macrophages of HFD mice showed a significantly higher number of Mt and M1 than that observed in GI-SD mice ($P<0.05$). Interestingly, MCT with CY+LOS produced an increase of Mt and M1 macrophages, only in the SD group. Conclusion: HFD increase protumoral macrophages in the invasive front of the tumor and MCT with CY+LOS reverts such an effect, increasing M1 macrophages. HFD has a deleterious effect on the M1 polarization of the MCT with CY+LOS, which would explain its lower antitumor effectiveness, as shown previously. Hence, the comorbidity impairs the treatment effect.

559. (575) **TARGETING OF MITOCHONDRIAL PEPTIDE HUMANIN TO IMPROVE CHEMOSENSITIVITY IN GLIOBLASTOMACELLS**

Jorge Armando Peña Agudelo[1], Matías Pidre[4], Antonela S. Asad[1], Matías García Fallit[1,2], Melanie Pérez Küper[1], Alejandro J. Nicola Candia[1], Leilane Glienke[1], Sofía Sagripanti[1], Abril Marchesini[4], Leslie C. Amorós[4], Mariana Belén Vera[5], Nazareno González[1], Guillermo Videla Richardson[5], Adriana Seilicovich[1,3], Marianela Candolfi[1]*

[1]Instituto de Investigaciones Biomédicas (INBIOMED, CONICET-UBA), Facultad de Medicina, Universidad de Buenos Aires, Argentina. [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. [3]Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad de Buenos Aires, Argentina. [4]Instituto de Biotecnología y Biología Molecular, Universidad Nacional de la Plata, Argentina. [5]Fundación para la lucha contra las enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina.

**Correspondence: maru.candolfi@gmail.com*

Humanin (HN) is a mitochondrial peptide with a robust cytoprotective many cell types. HN can interact with proteins of the bcl-2 family or be released and bind with two membrane receptors: a trimetric receptor, and the FPR-2 receptor. HN protects normal tissues from chemotherapy, and the administration of HN analogs has been proposed as a therapeutic approach for degenerative diseases. However, its role on the pathogenesis of cancer is poorly understood. Here we aimed to evaluate whether HN affects chemo-resistance of glioblastoma (GBM) cells. We first assessed the effect of chemotherapy on HN expression in murine (GL26) and human (U251) GBM cell lines, as well as in primary cultures from GBM biopsies. By immunofluorescence we observed that cisplatin upregulates HN in all the cells evaluated. To analyze the effect of HN on chemotherapeutic cytotoxicity, we used a HN analog peptide (HNG). In human GBM cells we observed that HNG abolished the cytotoxic and antiproliferative effect of cisplatin, restoring viability and clonogenic capacity (Two-way ANOVA $p<0.05$). Blockade HN interaction with the FPR-2 receptor, using a specific antagonist (WRW4), limited the cytoprotective function of both endogenous and exogenous HN in human GBM cells exposed to cisplatin, as assessed by MTT assay and BrdU incorporation (Two-way ANOVA $p<0.05$). To explore the effect of endogenous HN on GBM cell chemosensitivity, we developed a baculoviral vector encoding a HN-specific shRNA for the transcriptional silencing of its expression. These vectors showed excellent transduction efficiency in these cells. We observed that the inhibition of endogenous HN exerts an inhibitory effect on the viability of GBM cells and increases their sensitivity to cisplatin. Our study suggests that HN favors chemoresistance in GBM cells and that it could hold value as a therapeutic target to improve their response to conventional treatment.

560. (579) **METRONOMIC PHOTODYNAMIC THERAPY *IN VITRO* EVALUATION FOR MALIGNANT GLIOMAS**

Paula Martina Oliveda[1], Matías Daniel Caverzán[2], Lucía Beaugé[2], Rodrigo Emiliano Palacios[2], Carlos Alberto Chesta[2], Luis Exequiel Ibarra[1]

[1]Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Rio Cuarto y CONICET, Río Cuarto, Argentina, [2]Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Rio Cuarto y CONICET, Río Cuarto, Argentina

Glioblastoma (GBM) is the most aggressive brain tumor. New therapies are proposed such as Photodynamic Therapy (PDT) that combines light, oxygen and photosensitizers (PTs) to overcome conventional treatment issues. An important disadvantage of PDT using high light flux rates (PDTc) is the abrupt oxygen consumption, which leads to resistance to the treatment. PDT metronomic regimens (PDTm) administering light at low irradiation intensity could be an alternative to validate PDT for GBM. The main objective of the present work was to compare PDT effectiveness with conjugated polymer nanoparticles (CPN) in two modalities: PDTc or PDTm based on cell viability, impact in tumor microenvironment modulation and HIF-1α activation as indicator of oxygen consumption. To accomplish this, GBM cell lines U87MG y T98G and THP-1 macrophages were used. PDT efficacy was assayed in GBM mono-culture and co-culture with macrophages using 10 and 40 J/cm^2 with 84 mW/cm^2 and 17 mW/cm^2 for PDTc and PDTm respectively. In order to determine the activation of HIF-1α, GBM MO59K cells genetically-modified to overexpress a GFP-associated with the hypoxia response element, where HIF binds, was used. GBM were incubated with CPN (3 and 6 ug/mL), then irradiated until reaching 10 J/cm^2 in both modalities, and GFP expression was measured by flow cytometry. At 6 and 24 h after PDT, PDTc increased HIF-1α activation regarding PDTm. Cell viability was also dissimilar between PDTc and PDTm at the same CPN concentration and light doses (10 and 40 J/cm^2). T98G cells were more resistant in both modalities and cell viability decreased significant using PDTm (16,1;13,8;15,6; and 31,40 % with 47,5, 23,75, 11,875 y 5,93 ug/mL CPN) compared to PDTc (19,4, 35,8, 36,9% y 72,3% corresponding to CPN concentrations of 47,5, 23,75, 11,875 y 5,93 ug/mL) in U87MG. PDTm resulted in a more pronounce cell death with less HIF-1 activation as a main resistant molecular mechanism triggered proposing this modality as most suitable for GBM treatment.

561. (580) **ANALYSIS OF PLAGL1 EXPRESSION IN THYROID CARCINOMAS**

Victoria Peyret[1], Demian Slodki[1], Romina Celeste Geysels[2], Bladimiro Lenis[1], Marcelo Monteros Alvi[1,3], Javier Jaldín-Fincati[1], Juan Pablo Nicola[2], Marta Alicia Toscano[1].

[1]Unidad de Conocimiento Traslacional Hospitalaria del Hospital Dr. Arturo Oñativia. Salta, Argentina; [2]Centro de Investigaciones en Bioquímica Clínica e Inmunología, Consejo Nacional de Investigaciones Científicas y Técnicas. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba, Argentina; [3] Programa de Anatomía Patológica y Genética. Hospital Dr. Arturo Oñativia. Salta, Argentina.

The PLAGL1 gene encodes for a zinc finger transcription factor proposed to act as a tumor suppressor involved in cell cycle arrest and apoptosis. However, PLAGL1 is overexpressed in some neoplasms suggesting an oncogenic function. Our aim was to study PLAGL1 expression in Thyroid Carcinomas (TC) and the association between high PLAGL1 levels and poor prognosis. PLAGL1 expression was evaluated by IHC in human thyroid tissue samples and by RT-PCR and Western blot on a large panel of human thyroid cell lines. For transcriptomic analysis we downloaded datasets from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) data from papillary thyroid carcinomas (PTC). DNA methylation analysis and clinical characteristics were obtained from the

PTC-TCGA dataset. We detected the expression of the large and short isoforms of PLAGL1 in different TC cell lines. Furthermore, in TC samples, we identified cytoplasmic and nuclear expression of PLAGL1. Transcriptomic analysis indicated a significant (P<0.0001) downregulation of PLAGL1 in differentiated and poorly differentiated carcinomas compared with nonneoplastic thyroid tissue, while PLAGL1 overexpression (P=0.003) was observed in anaplastic carcinomas. We have found no association between PLAGL1 expression levels and the methylation status on their promoter (P=0.09). In PTC-TCGA database, we found two subtypes of tumors that displayed distinct PLAGL1 levels (P<0.0001). PTC with high PLAGL1 expression tend to have a higher degree of malignancy regarding shorter disease-free survival (P=0.016), association with BRAF mutations (P<0.0001), increased dedifferentiation (P=0.046), advanced T stage (P=0.03), a greater number of lymph node metastases (P=0.03), and a strong correlation with the number of M2 macrophages (P<0.0001).Here we describe an association between high PLAGL1 expression and an aggressive phenotype of thyroid tumors suggesting that this transcription factor might serve as a cancer risk predictor.

562. (590) **GENETIC TESTING OF FINE-NEEDLE ASPIRATIONS FOR DIAGNOSIS OF THYROID CANCER**

Sofía Savy[1], Victoria Peyret[1], Romina Celeste Geysels[1], Francisco Andrés Montes[1], Eduardo Rafael Cuvertino[2], Juan Pablo Nicola[1]

[1] Departamento de Bioquímica Clínica (CIBICI-CONICET). Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.

[2] Cuvertino estudios ecográficos

Introduction

The frequency of palpable thyroid nodules is ~5% in the population, but only a small fraction of them are malignant (thyroid cancer). Fine-needle aspiration (FNA) with cytological evaluation is the most reliable tool for cancer diagnosis in thyroid nodules. However, ~20% of nodules are diagnosed as indeterminate by cytology, making it difficult to optimally manage these patients. The introduction of multigene molecular panels has improved the diagnostic accuracy among indeterminate thyroid nodules. Objectives: We sought to develop a multigene molecular panel to conduct genetic testing to improve the FNA diagnosis of thyroid nodules. Methods: We developed a PCR-based multigene molecular panel consisted of BRAF codons 600 and 601, H/N/KRAS codon 12, 13 and 61 point mutations (Sanger sequencing), and several RET, NTRK, ALK, BRAF and PPARγ rearrangements (multiplex PCR). Results: Genetic testing was conducted in a pilot retrospective study including 10 FNA samples with cytology suggestive of malignancy (Bethesda V). We purify genomic DNA and total RNA from residual material from FNA samples. The point mutations p.V600E BRAF (n=6) and p.G12V HRAS (n=1), and the RET/PTC1 (n=2) and ETV6-NTRK3 (n=1) rearrangements were detected. The presence of mutation was a strong indicator of cancer because anatomical-pathological analysis after surgery indicated thyroid cancer in all mutation-positive nodules. Conclusion: A combination of cytology and clinically applicable genetic testing showed significant advance in the diagnostic accuracy of malignancy in the nodules, improving presurgical malignancy risk assessment in FNA in order to avoid unnecessary diagnostic surgeries. Moreover, genetic testing allow to design personalized therapy specific to the needs of individual patients with thyroid cancer.

563. (591) **ROLE OF ASCL1 IN NEUROBLASTOMA TUMORIGENICITY**

Federico J. Garde[1], M. Jimena Mosna[1], Lucía P. Petriz Otaño[1], Marcelo G. Stinson[1], Daniela Di Bella[2], Abel L. Carcagno[1]

[1]Cell Differentiation and Cancer Laboratory, ETI1, IQUIBICEN-CONICET, FCEyN-UBA, CABA, Argentina. [2]Department of Stem Cell and Regenerative Biology, Harvard University, Boston, USA.

Neuroblastoma (NB) is the most common solid extracranial pediatric cancer derived from the sympathoadrenal lineage. Ascl1 is a proneural transcription factor whose transient expression is essential for the development of this lineage. Ascl1 remains overexpressed in NB cells and its high expression is associated with poor clinical prognosis. The aim of this work was to evaluate the role of Ascl1 in the tumorigenicity of NB cells. We evaluated the phenotype of Ascl1 knockdown (KD) SK-N-SH NB cells relative to control and analyzed scRNA-seq data from patients' tumors to understand NB tumor cell heterogeneity. By performing a Trypan Blue assay, we found that Ascl1 KD does not affect cell viability. Analysis of proliferation by clonogenic assay and immunohistochemistry against Ki67 showed that the KD of Ascl1 significantly reduces the proliferating capacity of NB cells. Moreover, Ascl1 KD cells exhibited changes in their morphology relative to control, showing a neuron-like shape. For scRNA-seq analysis we obtained transcriptomic data of human NBs from public repositories. We integrated the data into a single dataset, performed dimensional reduction and clustering, and analyzed the origin of the cells. We characterized the resulting clusters through analysis of functional enrichment and analyzed expression of Ascl1 and co-expressed factors using non-negative matrix factorization. We found that NB cells share a single origin: sympathoblasts of the adrenal medulla, that cellular heterogeneity can be explained by the phase of the cell cycle, and that Ascl1 is expressed across all cell types in association with neurogenesis-related genes. The obtained results show that: a) Ascl1 blocks terminal neuronal differentiation in NBs, supporting tumor progression, b) a therapy against Ascl1 could target several tumoral subpopulations and, together with the observed effects of the Ascl1 KD *in vitro* sets the basis for a therapeutic strategy based on the modulation of Ascl1.

564. (601) **sTn EXPRESSION IN EXTRACELLULAR VESICLES DERIVED FROM BREAST CANCER PATIENTS**

Manuela Oviedo[1], Giovanna Munive Shong[1], Betiana Ermili[1], Iván Álvarez[1], Marina Isla Larrain[1], María Virginia Croce[1], Martín Enrique Rabassa[1].

1Centro de Investigaciones Inmunológicas Básicas y Aplicadas, Facultad de Ciencias Médicas, UNLP, Argentina

Breast tumors may secrete extracellular vesicles (EVs) of varying sizes, which may present on their surface O-glycans related to immune evasion or the spread of tumors. The sialyl antigen Tn (sTn) is the result of the early addition of sialic acid to the core of Ser/Thr-GalNAc, which disrupts the elongation of the O-glycan chain and in turn associates it with greater aggressiveness in various adenocarcinomas. To analyze the expression of sTn in breast cancer EVs, 27 patients with stage I and II tumors were analyzed by immunohistochemistry and EVs were isolated from the serum of these patients and four controls. Differential centrifugation followed by size exclusion chromatography (SEC) in CL2B agarose columns was used to isolate EVs. The morphology and size of the EVs were verified by dynamic light diffraction (DLS) and electron microscopy (ME); the protein content was determined by Qubit assay and by calculating the absorbance at 330 and 260/280 nm for each fraction. The presence of the markers CD9 and CD63, as well as sTn in the fractions was analyzed by dot blot; positive fractions were concentrated and analyzed by Western blot (WB). The SEC fractions showed that 11 of 27 patients were positive for CD63 (40.7%) and 13/27 (48.1%) for CD9 and 7/27 (25.9%) for sTn. Only 1 in 4 patients positive for sTn in the primary tumor was also positive in the fractions from serum EVs. All sTn positive fractions were positive for CD9 and CD63 and showed small-sized EVs using ME and DLS (97 nm mean). WB analysis showed low and high molecular weight bands in the range of 60 to 120 kD. No relationship was found between the expression of sTn and the pathological variables of the patients. In conclusion, it was possible to detect the presence of sTn in EVs derived from few patients with breast cancer.

565. (618) **MICROBIOME RELEVANCE IN TUMOR TISSUE OF EARLY BREAST CANCER PATIENTS**

Leonardo Dandeu[1*], Pablo Aguilera[2*], Sofía Sidlik[1], Alberto Penas-Steinhardt[2], Pablo Marenco[3], Martín Vázquez[4], María del Pilar Carballo[3], María Fernanda Alsina[3], Norma A Chasseing[1], Vivian Labovsky[1].

[1]Instituto de Biología y Medicina Experimental (IBYME)-CO-

NICET, CABA, Argentina, [2]Universidad Nacional de Luján, Luján, Argentina, [3]Instituto de Oncología Ángel H. Roffo, CABA, Argentina, [4]Instituto Héritas, Rosario, Argentina. ˚First authorship.

Some microbes are known to be damaging to human health. Breast microbiome composition are poorly understood. In order to study the prognostic relevance of the microbiome in the evolution of early breast cancer, a study was carried out including samples of frozen breast tumor tissues from women with invasive ductal breast carcinoma, clinicopathological stages I/II with a 5-year follow-up minimum (*Hospital Roffo*, n=22). Samples were processed and DNA was extracted using the QIAamp-DNA-Mini-Kit. The bacterial profile was identified studying the16S rRNA gene using the Illumina Miseq platform. OTUs classification and Alpha and Beta diversity analysis were performed to study their possible associations with the classic parameters of breast cancer and tumoral progression. In this way, taxonomic analysis of these samples indicates that *Proteobacteria* (44.20%) and *Firmicutes* (23.22%) are the most representative phylum followed by *Actinobacteriota* (11.00%) and *Bacteroidota* (9.44%). Difference in microbial abundances (evenness index) has been found between patients with positive sentinel node and negative sentinel node (p=0.05) and patients with positive progesterone receptor (PR+) and (PR-) p=0.02. Moreover, diversity and richness metabolic pathways were found between patients PR(+) and PR(-) (Shannon index p=0.03) and Ki67 marker (Chao1 index=0.02). To conclude, our results provided insight into the possible relationship between difference abundance of certain bacteria, as well as difference richness and diversity of metabolic pathways with the classical prognostic parameters: sentinel node, PR and ki67. These results open up new paths to study the relevance of the host microbiome with the progression of early breast cancer.

566. (709) **GALECTIN-1 AND GALECTIN-3 PARTICIPATE IN GLIOMA STEM CELL SURVIVAL AND CONTROL THE EXPRESSION OF THE ANTI-APOPTOTIC FACTOR BCL-XL**

Mariana Belén Vera[1], Olivia Morris-Hanon[1], Luisina Belén Ripari[1], Gustavo Emilio Sevlever[1], María Élida Scassa[1], Gabriel Adrián Rabinovich[2], Guillermo Agustín Videla-Richardson[1].
[1]Laboratorio de Investigación Aplicada a Neurociencias (LIAN) - Instituto de Neurociencias Fleni-CONICET (INEU), Fleni, Buenos Aires, Argentina; [2]Laboratorio de Glicomedicina - Instituto de Biología y Medicina Experimental (IByME-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

Glioblastoma (GBM) is the most prevalent and lethal brain tumor. It is characterized by high radio- and chemoresistance and diffuse infiltration throughout the brain. GBM arises from the so-called glioma stem cells (GSC), which correspond to a subpopulation of cells defined by their tumorigenicity and chemo-resistance. In our laboratory we have successfully established several patient-derived GSC lines. Galectins, glycan binding proteins, participate in multiple cellular processes including apoptosis. Apoptosis in GBM cells is highly dependent on Bcl-2 family members, especially on Bcl-xL and Mcl-1. Previously, we found that GSCs express high levels of galectin-1 (Gal-1) and galectin-3 (Gal-3) and that Gal-1, but not Gal-3, downregulation increases cell death. This work aims to elucidate if Gal-1 and Gal-3 regulate cell death by controlling the expression of anti-apoptotic factors of the Bcl-2 family. Initially, by a fluorimetric assay, we found that silencing of Gal-1, but not Gal-3, effectively induced a 2- to 5-fold increment on Caspase-3 activity. However, when both galectins are silenced, the effect of Gal-1 downregulation is no longer observed. Moreover, by RT-qPCR and Western Blot (WB) we found that Gal-1 silencing decreases Bcl-xL expression up to 52% ($p<0.05$, n=3). In contrast, Gal-3 downregulation upregulates this anti-apoptotic factor up to 203% ($p<0.05$, n=3). Conversely, disruption of Gal-1 and Gal-3 expression did not affect Mcl-1 levels. Additionally, by RT-qPCR and WB, we observed that Gal-1 knockdown decreased up to 74% of STAT3 abundance (n=1), which may be associated with the reduced levels of its transcriptional target, Bcl-xL. In contrast, Gal-3 silencing up-regulated STAT3 at the protein level. Thus, Gal-1 and Gal-3 regulate GSC survival, presumably by controlling the expression of Bcl-xL. Involvement of these lectins in regulating GSC survival further highlights their relevance as potential targets of therapeutic strategies in GBM.

567. (770) **CANCER STEM-LIKE CELL MARKERS EXPRESSION IN HUMAN GLIOBLASTOMA NEUROSPHERES**

Castillo Jeremias Omar[1], Ferreira Gretel Magalí[1,2], Rojo Selene[1,2], Nogueira Aylén Camila[1], Gulino Cynthia Antonella[1], Segatori Valeria Inés[1,2] and Gabri Mariano Rolando[1,2]
[1]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes,[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults and remains incurable, with a median survival of 15 months and a long-term survival of less than 5%. In the last two decades, therapeutic advances for this disease have been minimal. Even though our understanding of GBM biology has significantly increased, better approaches are required to translate preclinical information into therapeutic benefits for patients. GBM cancer stem-like cells (CSC) in the tumor microenvironment are described as the main barrier to therapeutic success. The growth of GBM cells as neurospheres (NF) seems to be the best approach to enrich CSC *in vitro*. The aim of our work was to evaluate the presence of CSC in the U87MG cell line when they were grown as NF, by analyzing the expression of specific surface markers. U87MG cells were grown in a 1:1 mixture of DMEM and Ham's F-12 medium, supplemented with penicillin-streptomycin, L-glutamine, B27, b-FGF and EGF for two weeks. Culture medium was changed every three days and cells disaggregated after one week in culture. During the first week, cells were able to grow as NF, so called first generation NF (FGNF). After disaggregation of FGNF, cells were allowed to form the second generation NF (SGNF). The expression of CD44, CD133 and CD15 CSC markers was evaluated by flow cytometry on different days of FGNF and SGNF. Expression was significantly increased in SGNF, being CD133 the marker that showed the highest values with an eight-fold increase when compared to cells growing as monolayers in normoxia. CD44 and CD15 showed an increase of more than six and four-times, respectively. Interestingly, when cells were cultured as monolayers in a hypoxic atmosphere, no increment in surface markers was observed. Our results indicate that the CSC population is highly expressed in NF of U87MG cells, suggesting that this culture condition resembles the described phenotype of *in vivo* tumor growth.

568. (781) **ROLE OF GALECTIN-1 IN PATIENT-DERIVED GLIOMA STEM CELLS WITH ENDOTHELIAL FEATURES**

Luisina Belén Ripari[1], Mariana Belén Vera[1], Nicolás Ignacio Torres[2], Olivia Morris-Hanon[1], Gustavo Emilio Sevlever[1], María Élida Scassa[1], Gabriel Adrián Rabinovich[2], Guillermo Agustín Videla-Richardson[1]
[1]Laboratorio de Investigación Aplicada a Neurociencias (LIAN) - Instituto de Neurociencias FLENI-CONICET (INEU), FLENI, Buenos Aires, Argentina.
[2]Laboratorio de Glicomedicina - Instituto de Biología y Medicina Experimental (IByME-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

Glioblastoma is the most aggressive type of primary central nervous system (CNS) tumor. These tumors are characterized by high inter-tumoral heterogeneity, which is manifested in the different phenotypes exhibited by patient-derived glioma stem cells (GSC). Notably, it has been reported that GSCs can differentiate even into endothelial cells. Galectin-1 (Gal-1), a β-galactoside-binding protein, has become a potential therapeutic target in different neoplasms since it modulates a myriad of cellular processes such as proliferation, differentiation, migration, and survival. Importantly, Gal-1 triggers immune escape mechanisms in several tumors and promotes angiogenesis in tumors resistant to VEGF-targeted therapies. In our laboratory, we determined that different GSC lines exhibit a high expression of Gal-1 and that its silencing increases cell death and reduces cell proliferation. However, in one of these cell lines (G02), Gal-1 expression does not affect cell viability. RNA-seq showed that

this cell line displays a differential expression of several endothelial markers (KDR, ITGA3, ICAM1, EDN1, among others) and exhibits higher amounts of α2-6 sialic acid on its surface, which inhibits Gal-1 binding. We also observed that under hypoxic conditions, many of these endothelial markers increase their expression. Interestingly, an increased expression of Gal-1 and Neuraminidase-1, an enzyme that removes α2-6 sialic acid from cell surfaces, was also observed. Notably, in contrast to normoxic conditions, Gal-1 silencing under hypoxia led to increased cell death in the G02 cell line from 13% to 22% ($p<0.05$, n=3), suggesting that a reduction in α2-6 sialic acid on the cell surface allows the exposure of Gal-1 binding sites and that the binding of this lectin to membrane receptors can promote GSC survival. Therefore, the relevance of Gal-1 in patient-derived GSCs could depend on cell surface α2-6-sialylation and this should be considered when designing tailor-made therapies that target Gal-1.

ONCOLOGY VI *Friday, November 18, 14-15:30 hr*
Chairs: Julia Ferronato - Fernanda Castillo - Alejandro Urtreger - Virginia Novaro

569. (22) **HIGH FAT DIET ATTENUATES TUMOR SUPPRESSOR miRNAs EXPRESSION IN ANDROGEN-SENSITIVE PROSTATE CANCER TUMORS**

Duca Rocío Belén[1], Karen Daniela Graña[1], Juana Moro[1], Ezequiel Lacunza[2] & Adriana De Siervi[2]
[1]IBYME-CONICET-Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, Buenos Aires, Argentina.
[2]CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Buenos Aires, Argentina.

Prostate cancer (PCa) is the most common type of cancer and the third cause of death by cancer in Argentinian men. The incidence and mortality of PCa worldwide correlate with age and bad dietary habits. Recent evidence has revealed that high-fat diet (HFD) plays a role in PCa development and progression. MiRNAs are small non-coding RNA molecules that regulate gene expression. Our aim was to investigate the effect of HFD on the expression of PCa-related miRNAs and their relevance in PCa patients. C57BL/6J male mice chronically fed with HFD or control diet (CD) were s.c. inoculated with murine TRAMP-C1 PCa cell line. After tumor growth, mice were sacrificed, tumors were collected and miRNAs isolated and analyzed by GeneChip® miRNA 4.0 Array (Affymetrix) microarrays. We identified 6 up- and 18 down-regulated miRNAs (FDR<0.05) in prostate tumors under HFD conditions. Three down-regulated miRNAs: mmu-miR-133a-3p, -1a-3p and -29c-3p were validated in TRAMP-C1 mice prostate tumor by stem-loop RT-qPCR. Hsa-miR-133a-3p/1-3p expression levels were significantly decreased in PCa tumors compared to normal adjacent tissue (NAT) while hsa-miR-133a-3p was found to be decreased in metastatic prostate cancer tumors compared to non-metastatic PCa. Based on this, miR-133a-3p and miR-133b emerged as potential tumor suppressor miRNAs (tsmiRs). GOLPH3 and JUP, two hsa-miR-133a-3p and miR-1a-3p predicted target genes, were up-regulated in PCa. Also, ROC analysis showed that the combination of hsa-miR-133a-3p, miR-1a-3p, GOLPH3 and JUP is a promising biomarker panel to distinguish between PCa and NAT. In summary, we propose that HFD attenuates tsmiRs expression, which leads to an increase in the oncogenes expression, impacting PCa aggressiveness. Furthermore, our work proposes a novel biomarker panel for the diagnosis of PCa.

570. (127) **PERFORMANCE COMPARISON OF TWO QPCR PLATFORMS FOR THE DETECTION OF BRAF V600E MUTATION**

Nazarena Cardoso[1,2], Sandra Colli[2], Franco Mangone[1], Denisse Rozas[1], De Matteo Elena [1,2], María Victoria Preciado[1], Mario Lorenzetti[1]
[1] División Patología, Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires C1425EFD, Argentina.
[2] Laboratorio de Biología Molecular, División Patología, Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP), CONICET-GCBA, Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires C1425EFD, Argentina.

V600 mutation in BRAF modulates Ras-MEK signaling pathway in a ligand-dependent fashion and was described as a driver mutation in multiple tumor types, including central nervous system (CNS). A main drawback for gold-standard Sanger sequencing detection of V600 mutation in small, formalin-fixed paraffin-embedded (FFPE) CNS tumor biopsies is low DNA yield and integrity. Here, qPCR is a suitable alternative to detect the mutation in these cases. Our aim was to compare two qPCR commercial kits for the detection of BRAF V600E mutation in FFPE CNS tumor biopsies. The performance of the BRAF V600 Mutations Detection kit (AmoyDX) and LightMix BRAF V600E/K kit (Roche) was assessed and compared. DNA extracted from 18 FFPE Glioma biopsies, previously characterized by Sanger sequencing, 11 with V600E variant, were re-tested. AmoyDX kit correctly identified all mutated (11/11) and WT (7/7) samples. Roche kit failed to detect 3 mutated samples, while correctly identifying WT cases, thus rendering 3 false-negative results. Both kits showed 100% specificity; however, AmoyDX kit had a significantly higher sensitivity over Roche, 100% vs 73%, respectively (χ^2 test, $p=0.031$). Both kits had positive likelihood ratios (LR+) above 10 (15.3 for AmoyDX and 11.3 for Roche), but their LR+ difference was not significant. Similarly, and although not statistically significant, the LR- was lower for the AmoyDx kit (0.04 for AmoyDX vs 0.31 for Roche). The positive predicted value (PPV) was 100% for both kits, while the negative predictive value (NPV) was 1 for AmoyDX vs 0.7 for Roche. The kit from AmoyDX represents a better diagnostic tool for the detection of BRAF V600 variant since it presented higher sensitivity and a moderately higher LR+ and NPV; being an open-platform kit represents an additional advantage. Since qPCR detects small DNA targets, assessing BRAF V600 by qPCR is of paramount importance when dealing with scarce FFPE CNS biopsies, where DNA is usually fragmented during fixation.

571. (178) **TARGETING THE PRENYLATION PATHWAY IN CANCER: IDENTIFICATION AND CHARACTERIZATION OF NOVEL SALIRASIB DERIVATIVES**

Evelyn E. Arel Zalazar[1], Carla M. Borini Etichetti[2], Maria Sol Ballari[3], Agustina Cerri[1], Nabila Cocordano[1], Guillermo R. Labadie[3] & Javier E. Girardini[1].
[1]Instituto de Inmunología Clínica y Experimental de Rosario (UNR-IDICER-CONICET). [2]Instituto de Fisiología Experimental de Rosario (UNR-IFISE-CONICET). [3]Instituto de Química Rosario (UNR-IQUIR-CONICET).
E-mail: arelzalazar@idicer-conicet.gob.ar

ICMT (Isoprenylcysteine Carboxymethyl Transferase) is involved in the posttranslational modification process known as prenylation, which starts with the addition of an isoprenoid to a cysteine near the C-terminus, followed by the cleavage of terminal amino acids. Then, ICMT catalyzes the methylation of the newly generated C-terminus on the cysteine. This modification, regulates critical functional aspects of substrate proteins. ICMT has emerged as an interesting target for novel anti-cancer therapies. We have previously shown that ICMT enhances aggressive tumor phenotypes and that its expression is repressed by the p53 tumor suppressor. In order to identify novel ICMT inhibitors we generated derivatives of Farnesylthiosalisilic acid (FTS), commercially known as Salirasib. This molecule was reported to inhibit ICMT and recently has reached clinical trials for the treatment of Non Small Cel Lung Carcinoma (NSCLC). We analyzed the effects of a collection of 27 compounds on cell viability using H1299 (NSCLC) and MDA-MB-231 (Triple Negative Breast Cancer) cells. Through bioreduction-based assays we identified four compounds showing significant activity ($p < 0,02$; n=3). Among compounds which did not affect cell viability we analyzed the ability to reduce metastasis associated phenotypes such as migration and invasion. We performed wound healing assays on the H1299 cell line. Our results showed that four compounds significantly inhibited cell migration ($p= 0.0009$; 8: $p= 0.0017$; 9: $p= 0.0003$; 13: $p= 0.0202$; n=3). To further characterize these compounds, we tested their effect on invasion by transwell invasion assays using Matrigel-coated filters. We found that two of them significantly reduced invasion in vitro ($p= 0.0009$; $p= 0.0158$ n=3). In summary, we identified novel Salirasib derivatives that reduced cancer-associated phenotypes in

vitro and are interesting candidates for leading molecules in cancer therapy.

572. (181) **SELECTIVE DEGRADATION OF ONCOGENIC p53 MUTANTS THROUGH A DRUG REPURPOSING STRATEGY**

Nabila Cocordano[1], Carla M. Borini Etichetti[2], M. Candelaria Llorens de los ríos[3], Evelyn E. Arel Zalazar[1], Gastón Soria[3] & Javier E. Girardini[1].

[1]Laboratorio de Inmuno-Oncología, Instituto de Inmunología Clínica y Experimental de Rosario, UNR-IDICER-CONICET, Rosario, Argentina. [2]Instituto de Fisiología Experimental de Rosario, UNR-IFISE-CONICET, Rosario, Argentina. [3]Centro de Investigaciones en Bioquímica Clínica e Inmunología, UNC-CIBICI-CONICET, Córdoba, Argentina.

E-mail: cocordano@idicer-conicet.gob.ar

The presence of missense mutations in the p53 gene is among the most frequent alterations in human cancer. These mutations lead to the expression of mutant p53 proteins, which can actively collaborate with oncogenic processes. Mutant p53 forms have attracted great interest as therapeutic targets because their elimination could reduce the development of aggressive and metastatic tumors. Targeting mutant p53 would also provide highly selective therapies, since mutations are found exclusively in tumor cells, reducing the possibility of adverse effects. Also, the high mutation frequency of p53 would make this strategy useful in different cancer types. In tumor cells, p53 point mutants show a remarkable increase in stability compared with the wt protein. In order to identify drugs able to induce mutant p53 degradation, we performed a high-throughput screening, using libraries of drugs approved for clinical use in humans against various pathologies. Using In Cell Western Blot, we analyzed the effect of 1760 drugs on the MDA-MB-231 cell line, derived from Triple-Negative breast adenocarcinoma, which endogenously expresses p53R280K mutant. In this way, compounds capable of significantly reducing mutant p53 levels were identified. We further characterized the effect of a selected candidate. We demonstrated that the compound reduced the levels of other p53 point mutants in different cell lines ($p < 0,001$; n=3). Time course analysis using western blot showed that the drug decreased the half-life of mutant p53 ($p < 0,01$; n=3), associated with an increase in polyubiquitination. In contrast, wt p53 levels were not affected, suggesting that the effect is selective for cells that express mutant p53. Using wound healing assays we showed that the drug reduced the migration of cancer cells ($p < 0,01$; n=3), a characteristic trait of metastatic cells promoted by mutant p53. In summary, we identified a compound potentially useful in antitumor strategies based on mutant p53 degradation.

573. (254) **EXPLORING PROSTATE CANCER DERIVED EXOSOMES CONTRIBUTION TO METASTATIC BONE DISEASE**

Florencia Cascardo[1,2], Sabrina Ledesma-Bazan[1,2], Juan Bizzotto[1,2], Elba Vázquez[1,2], Geraldine Gueron[1,2], Javier Cotignola[1,2].

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina.

[2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

Metastatic prostate tumor cells have mainly tropism to the bone, triggering the disruption of bone formation/resorption balance. Although several studies have focused on soluble factors as the paracrine signaling between prostate tumor and bone cells, there is increasing evidence on the involvement of extracellular vesicles during the metastatic process. Prostate cancer derived exosomes, which carry diverse biomolecules and are found in body fluids, have been shown to favor tumor progression and may have the potential to emerge as biomarkers in liquid biopsies. Hence, to evaluate whether prostate tumor exosomes prepare the bone metastatic niche by trafficking molecules that modulate bone cell physiology, we co-cultured CFSE-stained PC3 cells with unlabeled preosteoblastic MC3T3 or preosteoclastic Raw264.7 cells. After 48h incubation, CFSE staining was observed in the originally unlabeled bone cells, suggesting an extracellular vesicle trafficking from prostate to bone cells. Next, exosomes were isolated by ultracentrifugation and further characterized by transmission electron microscopy, flow cytometry (anti-CD63 magnetic beads and anti-CD81-PE) and Western Blot (anti-CD81, anti-TSG101 and anti-flotillin-1). The presence of 60-100 nm vesicles containing exosomal markers was confirmed. In addition, we mined RNA-seq and microarray data (GSE109356, GSE117744 and GSE35813) and assessed the exosomal RNA cargos. For further analyses, we selected 16 miRNAs that overlapped in all 3 datasets and were among the 100 most abundant of each study. Gene ontology analysis revealed these 16 miRNAs together with their target mRNAs to be associated with inflammation, bone regeneration, epithelial-mesenchymal transition, cellular adhesion and cancer pathways. Altogether, we showed prostate tumor exosomes trafficking between tumor cell and bone progenitors, and shed light into relevant miRNAs as part of the vesicle cargo that might promote the tumor seeding in the bone.

574. (275) **TNFA BLOCKADE INCREASES SURVIVAL OF MICE DISPLAYING PREMALIGNANT LESIONS INDUCED BY TTP ABLATION AND K-RAS ACTIVATION IN THE TONGUE**

Ferri D[1], Ayre M[1], Ariza Bareño L A[3], Sánchez V.[2], Urtreger A[3]. Paparella M.L[2]. Kordon E[1]. Raimondi A. R[1].

1. Institute of Physiology, Molecular Biology and Neuroscience, School of Sciences, UBA-CONICET, Argentina.

2. Oral Pathology Department, School of Dentistry, University of Buenos Aires, Argentina.

3. Oncology Institute Angel H. Roffo, University of Buenos Aires, Argentina.

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer worldwide, being tongue SCC the most common malignancy found in the oral cavity. Tristetraprolin (TTP) is a RNA binding protein that binds to target mRNA and destabilizes it, reducing protein expression. TTP has the ability to regulate proinflammatory mediators such as TNFα and IL6 that promote tumorigenesis. Previously, we have characterized a mouse model where TTP is ablated in the oral cavity (TTP KO: K14-CreER^{TAM}/TTP$^{flox/flox}$). These mice developed mild dysplastic lesions in the tongue over time along with inflammatory infiltrate in the connective tissue (mast cells and CD11b cells). Besides, we analyzed the status of the NFkB pathway and we found increased levels of p65 and p-p65. Furthermore, we generated K14-CreER^{tam}/TTP$^{-/-}$/K-ras$^{G12D+/-}$ animals (compound mice) that exhibited a complete oral phenotype and presented a significant reduction in survival time. Here, to asses the mechanism underlying the development of the mentioned lesions together with the inflammatory infiltrate we tested the role of TNFα in this model. We treated TTP KO and compound mice with a TNFα decoy receptor (Etanercept: 0.01mg/g of body weight, 3 times a week during 2 months and 2-3 weeks respectively). Etanercept treatment reduced 37% mast cells infiltration in TTP-KO mice vs untreated control group (TB+/mm^2, $p<0.05$) without epithelial changes. Moreover, the survival of the compound mice was significantly increased by Etanercept treatment (Kaplan-Meier $p< 0.05$). Due to the increased lifetime these mice developed more severe lesions (verrucous carcinoma) without changes in mast cell infiltration when compared to K-ras or TTP KO mice. Thus, we provide evidence that blocking TNFα activity in the tongue of TTP KO mice prevented the local increase of mast cell infiltration. Furthermore, TNFα blockade extended survival of the compound mice.

575. (293) **TELOMERES EXPRESS THEIR LNCRNAS TERRA AND RECRUIT FKBP51 IN RESPONSE TO INCREASED ROS DURING THE EPITHELIAL-TO-MESENCHYMAL TRANSITION**

Natalia M. Galigniana[1], Romina Uranga[2], Graciela Piwien-Pilipuk[1]

1) Laboratory of Nuclear Architecture, Instituto de Medicina y Biología Experimental (IBYME-CONICET), Buenos Aires,

Argentina
2) Instituto de Investigaciones Bioquímicas de Bahía Blanca (IIBBB-CONICET), Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina

We have previously shown that telomeric lncRNAs TERRA increase by oxidative stress to protect the genome upon DNA damage. The epithelial-to-mesenchymal transition (EMT) plays an important role in the progression of primary epithelial tumors, and it was shown that ROS favor EMT. Thus, we hypothesize that the inherent rise in ROS during EMT triggers TERRA induction. We found that TERRA levels increase when NMuMG epithelial cells undergo EMT with TGFbeta1 treatment for 4 days. These cells have over 4-fold induction of telomere DNA damage (TAFs) than in their epithelial state, and TAFs co-localize with stress protein FKBP51. When NMuMG are treated with TGFbeta1 + 100μM H_2O_2, EMT is accelerated, evident at day 1, and accompanied by earlier TERRA induction and higher number of TAFs with FKBP51 co-localization than in EMT triggered by TGF-beta1 alone, suggesting that earlier TERRA induction is required for protecting telomeres from damage. Accordingly, ChIP shows higher FKBP51 recruitment to subtelomeric regions when EMT is triggered by TGFbeta1 in the presence of H_2O_2 than in its absence, suggesting FKBP51 is relevant for TERRA induction. HP1gamma, RNA-POL II and lamin A/C display the same recruitment pattern. FKBP51 co-immunoprecipitates with lamin A/C. Since FKBP51 lacks a DNA-binding domain, lamins may act as scaffolds for FKBP51 recruitment to subtelomeres. In conclusion, EMT induces TERRAs upon the onset of TAF formation where FKBP51 is recruited, partly through its interaction with lamin A/C, events that are magnified in a milieu of higher ROS levels that accelerates EMT.

576. (388) **IMMUNOLOGICAL AND ANGIOGENESIS BIOMARKERS IN HIGH RISK PEDIATRIC PATIENTS WITH SOLID TUMORS TREATED WITH METRONOMIC CHEMOTHERAPY (MCT)**

Cáceres JM[1], Adriana Rosé [2], Walter Cacciavillano [2], Marcelo Coirini [3], Andrea Schifino [3], Julia Jotomliansky [3], Graciela Scharovsky [1,4,5], Viviana Rozados [1,4]
[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, Rosario; [2]Hospital de Pediatría J.P. Garrahan, Buenos Aires; [3]Hospital de Niños Víctor J. Vilela, Rosario;[4]CONICET, Rosario; [5]CIC-U.N.R.

MCT consists in the chronic administration of low doses of chemotherapeutic drugs, without prolonged drug-free periods. It has demonstrated therapeutic efficacy with low to null toxicity. Among other effects, it can modulate the antitumor immune response and inhibit tumoral angiogenesis. Our aim was to identify biomarkers of angiogenesis and immune response as prognostic indicators of tumor relapse or progression. Twenty pediatric cancer patients (7-17 years old, median=13) (Ewings´ Sarcoma 8, Rhabdomyosarcoma 7, Osteosarcoma 3, Rhabdoid tumor 1, Renal anaplastic sarcoma 1) receiving maintenance MCT after achieving complete remission, were analyzed. Circulating (CEC) and Progenitor (CEP) Endothelial Cells, and TRegs lymphocytes were studied by Flow Cytometry; and sPD-1 (soluble programmed cell death protein 1) and sPD-L1 (soluble programmed cell death ligand 1) by ELISA. Patients were distributed in three groups: *Good evolution* (*GE*, n=10): those which ended MCT, without distant recurrence or event, *Metastatic Relapse (MR, n=5)*: patients with relapse after, at least, 6 months of successfully completing MCT and *Bad evolution (BE, n=5)*: MCT was discontinued due to relapse. Start (S) and End (E) of treatment values were determined. Medians (S-E) are shown: CEC [*GE*: 13.5-4 (p=0,027), *MR*: 34-4 (p=0,031), *BE*: 21-11]; CEP [*GE*: 67-63.5, *MR*: 41-8, *BE*: 91-29]; TRegs [*GE*: 270- 1165 (p=0,002), *MR*: 265-1460, *BE*: 122-76]; sPD-1 [*GE*: 85.4-129, *MR*: 41.7-80.7 (p=0,063), *BE*: 91-61.2]; sPD-L1 [*GE*: 27.4-12.6 (p=0.007), *MR*: 24.8-21.6, *BE*: 19.5-18.9]. Wilcoxon matched-pairs signed rank test. We conclude that: 1) Descent of CEC values may be associated with prolonged disease-free survival after completion of MCT; 2) Higher TRegs values along with low sPD-L1 values could be associated with prolonged disease-free survival and successful treatment. Confirmation of these putative biomarkers with a higher number of patients could give the basis for its further use in patients' treatment.

577. (389) **COMPARATIVE EVALUATION IN AN EXPERIMENTAL MODEL OF A NOVEL PROTOCOL OF BORON NEUTRON CAPTURE THERAPY (BNCT) VS THE STANDARD PROTOCOL EMPLOYED IN CLINICAL TRIALS**

Mónica A. Palmieri,[1] Debora N. Benitez Frydryk,[2] Yanina Langle,[3] Emiliano C.C. Pozzi,[2] Silvia I. Thorp,[2] Marcela A. Garabalino,[2] Andrea Monti Hughes,[2,4] Paula Curotto,[2] Paula S. Ramos,[2] María L. Paparella,[5] Lucas Polti,[5] Ana Eiján,[3,4] Amanda E. Schwint,[2,4] Verónica A. Trivillin[2,4]
[1]Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA), Argentina. [2]Comisión Nacional de Energía Atómica (CNEA), Argentina. [3]Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigación, Argentina. [4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [5]Facultad Odontología, UBA, Argentina.

Boron Neutron Capture Therapy (BNCT) combines selective tumor uptake of ^{10}B compounds and neutron irradiation. The aim of this study was to evaluate the therapeutic efficacy and radiotoxicity of (BPA/borophenylalanine+GB-10/Decahydrodecaborate)-BNCT (Comb-BNCT) alone or in combination with Oligo-Fucoidan (O-Fuco) or Glutamine (GLN), compared to the standard BPA-BNCT protocol. Also, the systemic immune response expressed in the spleen was evaluated for the Comb-BNCT group alone or in combination with O-Fuco or GLN. BDIX rats were injected subcutaneously in the right hind flank with DHD/K12/TRb syngeneic colon cancer cells. Three weeks later, the tumor-bearing legs were treated locally with BNCT at the RA-3 Nuclear Reactor. a- Comb-BNCT: BPA 31 mg ^{10}B /kg bw + GB-10 34 mg ^{10}B/kg bw, i.v. b- Comb-BNCT+O-Fuco: same as (a) + O-Fuco (200 mg/ml) once a week for 7 weeks, joint oral and topical admin. c- Comb-BNCT+GLN: same as (a) + GLN (40 mg/ml) once a week for 7 weeks, with wet compresses. d- BPA-BNCT: 46.5 mg ^{10}B /kg bw de BPA. e- Sham: same manipulation, no treatment. The post/pre-BNCT ratio of tumor volume at 7 weeks post treatment was significantly lower for all the groups treated with BNCT vs SHAM ($p < 0.05$). Using the end-point "incidence of tumors that underwent a reduction to ≤ 50% of initial tumor volume" to further assess therapeutic response, results were 62% for Comb-BNCT alone, 80% for Comb-BNCT+GLN, 73% for Comb-BNCT+O-Fuco and 30% for BPA-BNCT. The incidence of severe dermatitis at two weeks (when the peak occurs) was 100% for BPA-BNCT while for Comb-BNCT, Comb-BNCT+O-Fuco and Comb-BNCT+GLN it was below 70%, this difference being statistically significant ($p \leq 0.05$). At the systemic level, an increase in CD8 was observed for Comb-BNCT+GLN vs SHAM $p \leq 0.01$, and an increase in NK for Comb-BNCT vs SHAM $p \leq 0.05$. Comb-BNCT improves therapeutic efficacy and reduces radiotoxicity compared to standard BNCT (BPA-BNCT). A systemic immune response was activated after Comb-BNCT.

578. (489) **NSAID SCREENING FOR MRP4 INHIBITION: DRUG REPOSITIONING FOR PDAC TREATMENT**

Ramiro Héctor Cerviño[1], Natalia Gómez[1], Ana Sahores[1], Carina Shayo[2], Carlos Davio[1] and Agustín Yaneff[1]
[1]Laboratorio de Farmacología y Oncología Molecular, ININFA, FFyB, UBA-CONICET
[2]Laboratorio de Patología y Farmacología Molecular, IByME-CONICET

In a previous work, we validated the inhibition of the MRP4-dependant cAMP extrusion process as a promising therapeutic strategy for pancreatic ductal adenocarcinoma (PDAC). In view of the therapeutic challenge associated with this malignancy, we have identified non-steroidal anti-inflammatory drugs (NSAIDs) as an attractive pharmacological family for drug repositioning based on their ability to block MRP4 and on the fact that inflammation is critical for PDAC initiation and progression. In this work, we first selected several NSAIDs considering two aspects: the capacity to inhibit MRP4-cAMP transport and the potency to reduce cell proliferation

in experimental *in vitro* PDAC models. The initial screening was performed using HEK-T cells expressing the EPAC-S^{H187} sensor, which allows rapid and efficient monitoring of the rise in intracellular cAMP levels that occurs upon treatment with the inhibitors. We tested 18 NSAIDs and only 11 compounds successfully increased intracellular cAMP concentrations ($p<0.01$). We further evaluated the ability of these 11 compounds to inhibit cAMP transport in human pancreatic BxPC-3 cells using a Radio-Binding Protein assay (RBP) that allows us to measure extra- and intracellular cAMP levels. A total of 8 compounds significantly decreased the extracellular concentration of cAMP ($p<0.01$), proving to be potent MRP4 inhibitors and thus, we decided to analyze their effect upon BxPC-3 cell proliferation. Only 3 compounds (diclofenac, flurbiprofen, and indomethacin) were able to inhibit cell proliferation of BxPC-3 at concentrations below 25μM ($p<0.01$). Considering these results, we selected these NSAIDs as candidates for further *in vitro* studies in PDAC cell lines. Our results suggest diclofenac, flurbiprofen, and indomethacin are promising drugs to repurpose for adjuvant PDAC therapy, since they have a wide range of safety and modulate other pathways related to cancer development in other tumor models.

579. (513) **HEME OXYGENASE 1 (HO-1) MODULATES RELEVANT METASTASIS-ASSOCIATED GENES IN PROSTATE CANCER**

Sabater A[1;2;3], Toro A[1;2], Sanchis P[1;2], Pascual G[1;2], Seniuk R[1;2], Vazquez E[1;2], Cotignola J[1;2], Bizzotto J[1;2], Gueron G[1;2], Lage-Vickers S[1;2]

[1] Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica, Buenos Aires, Argentina.

[2] CONICET-Universidad de Buenos Aires. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

[3] Universidad Argentina de la Empresa (UADE), Instituto de Tecnología (INTEC), Buenos Aires C1073AAO, Argentina

Eighty percent of prostate cancers (PCa) metastasize to bone, showing a significant source of patient morbidity. PCa stem-like cells (PCSCs) are capable to outlast in a quiescent state remaining able to develop therapy-resistant tumors and metastatic lesions. We have previously reported that heme oxygenase 1 (HO-1, the rate limiting enzyme in heme degradation) overexpression leads to a less invasive PCa phenotype. However, its effect on metastasis-stemness (MS) remains unknown. In this work, we address the biological significance of HO-1 in association with MS genes relevant for PCa. Clonogenic assays were performed in PC3 and C4-2B cells subjected to hemin treatment (FDA approved drug and specific HO-1 inducer) to assess its effect on stem-like properties. Colony formation assays evidenced a reduction on stem-like properties of PCa cells treated with hemin ($p<0.05$). RNA-seq analysis was also performed and we identified 15 MS genes that are differentially expressed in PCa compared with normal gland ($n=1128$) whose expression profiles are reverted by HO-1 induction *in vitro*. Next, we used a custom-made bioinformatics tool (*Gene Hunter*) to analyze MS gene expression across multiple PCa datasets ($n=1287$) and defined a 5-gene signature (*ADAM15*, *BCL2L1*, *LTBR*, *MBNL2* and *SPINT1*) with consistent dysregulation in PCa compared with normal gland or adjacent tumor tissue. We provided a risk score that could predict disease progression ($p<0.05$). Low *MBNL2*, the main contributor of the signature, was associated with poor prognosis ($p<0.01$) and was significantly downregulated in PCa metastases. Interestingly, HO-1 reverts *MBNL2* expression as validated by RT-qPCR *in vitro*. We highlight *MBNL2* as a potential druggable target and point out to a potential mechanism associated with MS of PCa cells by which HO-1 modulation could halt progression, thus supporting HO-1 as a potential therapeutic target for disease intervention.

580. (548) **EXPLORING HO-1 NON-CANONICAL FUNCTIONS THROUGH ITS NUCLEAR INTERACTORS**

Rocio Seniuk[1,2], Juan Bizzotto[1,2], Sofia Lage-Vickers[1,2], Pablo Sanchis[1,2], Gastón Pascual[1,2], Agustina Sabater[1,2,3], Pia Valacco[1,2], Elba Vazquez[1 2], Javier Cotignola[1,2], Geraldine Gueron[1,2], Ayelén Toro[1,2]

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina; [2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina; [3]Universidad Argentina de la Empresa (UADE), Instituto de Tecnología (INTEC), Buenos Aires C1073AAO, Argentina.

Prostate tumor cells display abnormal expression of proteins resulting in an augmented capacity to resist chemotherapy and colonize distant organs. We have previously shown that heme-oxygenase 1 (HO-1), the rate limiting enzyme in heme degradation, has a strong anti-tumoral effect in prostate cancer (PCa). Several reports indicate that HO-1 translocates to the nucleus as a truncated isoform without enzymatic activity. Regarding its nuclear role, it has been suggested that HO-1 modulates the activity of different transcription factors; however, its non-canonical functions remain unclear. We hypothesize that HO-1 and its nuclear interactors reprogram prostate tumor cells, favoring a less aggressive phenotype. In this work, we carry out a mass spectrometry (MS)-based proteomics analysis to identify HO-1 molecular partners which might collaborate with its non-canonical functions in PCa. PC3 cells were treated with hemin (80 μM for 24 h), a specific pharmacological inducer of HO-1. After nuclear protein isolation, we immunoprecipitated HO-1 and its associated proteins and subjected them to LC-ESI MS/MS analysis. After filtering results with CRAPome we identified 33 proteins in the nuclear fraction of control cells and 34 proteins in the hemin-treated cells. Protein expression analysis reveals 11 differential proteins between control and hemin treated cells. STRING and Ingenuity pathway analysis suggested that the identified HO-1 interactors are implicated in several biological processes such as antioxidant activity, RNA splicing and hydrogen peroxide catabolic processes. Moreover, functions related with scaffold, stemness and gene expression regulation were also identified, pointing out a non-canonical function of nuclear HO-1 in PCa.

581. (573) ***HMOX1* POLYMORPHISMS AS PROGNOSTIC PREDICTORS FOR PROSTATE CANCER IN ARGENTINIAN POPULATION**

Gaston Pascual[1,2], Agustina Sabater[1,2,3], Rocío Seniuk[1,2], Ayelén Toro[1,2], Carlos Scorticati[4], Osvaldo Mazza[4], Elba Vázquez[1,2], Geraldine Gueron[1,2] and Javier Cotignola[1,2]

[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina;[2]CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina; [3]Universidad Argentina de la Empresa (UADE), Instituto de Tecnología (INTEC), Buenos Aires C1073AAO, Argentina; [4]Universidad de Buenos Aires, Hospital de Clínicas José de San Martín, Departamento de Urología, Buenos Aires, Argentina.

Heme Oxygenase 1 (HO-1), the rate-limiting enzyme in heme degradation and encoded by *HMOX1* gene, has a strong anti-tumoral effect in prostate cancer. *HMOX1* expression levels are modulated by the length of a dinucleotide $(GT)_n$ microsatellite polymorphism mapping in its promoter region. Prostate cancer is the second most incident cancer in men and the sixth leading cause of cancer-related deaths in men worldwide. Nowadays, one of the strongest predictors for newly diagnosed prostate cancer is the Gleason score, a histopathology-based classification system. However, Gleason score presents diverse limitations in capturing tumoral heterogeneity; thus, hindering accurate disease diagnosis. Consequently, the establishment of novel biomarkers is important to improve prostate cancer prognosis. In this work we genotyped the $(GT)_n$ promoter polymorphisms in the *HMOX1* gene and analyze its association with prostate cancer clinicopathological parameters. We studied 108 peripheral blood mononuclear cells (PBMC) samples from prostate cancer patients from Hospital de Clínicas “Jose de San Martín”. Genomic DNA was extracted and the $(GT)_n$ was genotyped by fluorescent PCR and capillary electrophoresis. Sanger sequencing was

performed to validate the results in random selected samples. The length of the $(GT)_n$ varied from 11 to 40 repeats. *HMOX1* genotypes were stratified as "Long allele (L)" (≥29 repeats), or "Short allele (S)" (<29 repeats) based on the median value of $(GT)_n$. We found an association between the $HMOX1_{SS}$ genotype and higher serum prostate specific antigen (sPSA) levels within patients with <10 ng/mL sPSA ($p<0.0022$). These patients also had higher Gleason scores ($p_{trend}<0.023$) and International Society of Urological Pathology grade (ISUP) ($p_{trend}<0.049$). Finally, $HMOX1_{SS}$ patients showcased increased risk of biochemical relapse (HR=4.03, $p<0.024$). In conclusion, *HMOX1* $(GT)_n$ polymorphism have the potential to predict the outcome of patients with prostate cancer.

582. (660) **IMPACT OF THE MICROBIOME ON PROSTATE TUMOR DEVELOPMENT ASSOCIATED WITH METABOLIC SYNDROME**

Karen Daniela Graña[1], Rocío Belén Duca[1], Juana Moro[1], Adriana De Siervi[1]

[1]IBYME-CONICET-Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, Buenos Aires, Argentina.

Prostate cancer (PCa) is the most common cancer type and the third cause of cancer death in Argentinean men. Metabolic syndrome (MS) is a risk factor for PCa and previous studies revealed that high-fat diet (HFD) is associated with PCa development and progression. The microbiome is made up of the environment of the microbiota that live in an organ of an individual. It impacts cancer development and varies according to different individuals metabolic states. MiRNAs are small non-coding RNA molecules that regulate gene expression. The interaction between the microbiome and the individual requires a regulatory network in which miRNAs could play a crucial role. Our aim was to characterize the bacteria and miRNA composition of the microbiome from mice with MS and PCa. Male C57BL/6J mice chronically fed with HFD or control diet (CD) were inoculated with the murine TRAMP-C1 cell line. After tumor growth, mice were sacrificed and tumor, intestine and fecal samples were collected. Fecal samples were collected every two weeks during the whole experiment. Using qPCR, we analyzed the five most dominant bacterial phyla of the human microbiota from the collected stool: Firmicutes (F), Bacteroidetes (B), Verrucomicrobia (V), Proteobacteria (P) and Actinobacteria (A). We found that the population of B and V were increased in CD, while F and P were increased in HFD. Additionally, we observed that the obesity index, established by the F/B ratio, was increased in HFD. We screened a panel of miRNAs selected from the literature to determine expression levels in stool samples from CD and HFD fed mice using stem loop RT-qPCR. We found that the presence of tumor significantly correlates with an increased expression of miR-21-5p while its absence with its expression reduction. These results suggest that the altered expression of miRNAs obtained from HFD fed mice may be related to changes in bacterial composition.

583. (717) **SIMPLE AND EFFICIENT NEW ADENO-ASSOCIATED VIRUS (AAV) QPCR TITRATION METHOD**

Camila Seoane Rocha[1], María Belén Palma[1, 2], Antonella Lombardi[2], Guadalupe Amin[2], Ailén Iribarne[1], Kevin Martinez[1], Ana María Inda[1], Santiago G. Miriuka [2, 3], Lucía N. Moro[2, 3], Marcela N. García[1]

1Catedra de Citología, Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Buenos Aires, Argentina 2Laboratorio de Investigación Aplicada a Neurociencias (LIAN), Instituto de Neurociencias (INEU), Fleni-CONICET 3Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Arg

Adeno-associated virus (AAV) are small nonenveloped viruses with a linear, single stranded DNA of approximately 4.7kb. AAV has gained growing interest as a human gene therapy vector for decades. This is due to its non-pathogenicity in humans, persists episomal, its high transduction efficacy, low inflammatory response, and broad host cell tropism. Currently, methods for purification of AAV can be expensive because they require equipment of ultrahigh speed gradient centrifugation and in the absence of this it is possible to obtain cell detritus together with the viral fraction. The strategy to titrate AAV particles is the quantification by qPCR using primers that target ITRs sequences of the viral genome, but these cell debris mentioned above could interfere with the titration. The aim is to propose a new AAV qPCR titration method including an additional step of transduction with the obtained viral fraction. We carried out qPCR titration of AAV2-GFPturbo produced from pAAV-CASI-Cas9C-P2A-turboGFP (#80942, AddGene), directly from the viral fraction or by the proposed new method. For this, HEK 293T cells are transduced with 1, 5 and 10uL of the viral fraction obtained, incubated for 6h and then the viral genome is extracted to quantify only the infective viral particles. Finally, qPCR was performed. The results show that we obtained a value 1,06x10ˆ9Vg/ml with the new titration method, compare with 2,19x10ˆ11Vg/ml obtained with the conventional titration method. Therefore, it can be said that the proposed method was efficient since we obtained a value similar to those published (1x10ˆ9Vg/ml). In summary, we have proposed a new, accurate, reliable AAV titration method. Furthermore, it´s a simple method which can be carried out in laboratories that do not have access to the necessary equipment to purify completely the viral fraction. In addition, as the additional step is not specific to the viral serotype, this method could be applied to the rest of the AAV serotypes.

584. (868) **DEVELOPMENT OF NOVEL MONO AND BIVALENT NANOBODIES AGAINST EGFR FOR TARGETED CANCER THERAPY**

Heitrich Mauro[1], Fernandez Marisa[2], Werbajh Santiago[1], Canziani Gabriela[1], Malchiodi Emilio[2], Podhajcer Osvaldo[1], Vinzón Sabrina[1]

[1] Laboratorio de Terapia Molecular y Celular, Fundación Instituto Leloir, IIBBA-CONICET, Avenida Patricias Argentinas 435, Buenos Aires C1405BWE, Argentina.

[2] Instituto de Estudios de la Inmunidad Humoral Prof. Ricardo A. Margini, (IDEHU-CONICET) - Cátedra de Inmunología, FFyB-UBA, Junín 956, Buenos Aires C1113, Argentina.

Introduction: The epidermal growth factor receptor (EGFR) has a central role in the tumorigenesis of many types of solid tumors, making it a target of interest for cancer therapy. Nanobodies (Nb) represent an attractive therapeutic approach, as they exhibit low immunogenicity, rapid clearance, and high targeting specificity. The aim of this work is to isolate, design and characterize Nbs directed against EGFR. Methods: The Nb library was constructed after llama immunization with cells overexpressing EGFR. After three rounds of panning the phage-display library (in solid phase and solution), single clones were screened for EGFR reactivity by ELISA against Virus Like Particles. Positive clones were sequenced, cloned into an expression vector and purified by IMAC. The Nbs were characterized by immunofluorescence (IF), flow cytometry (FACS) and surface plasmon resonance (SPR). The best candidates were used to design bivalent constructs which were further characterized by SPR and in a cell proliferation assay. Results: We successfully constructed an EGFR targeted Nb library which allowed the selection of several EGFR-specific Nbs after optimization of the panning strategy. Two of the candidates strongly bind native EGFR in cells and outperform the reference anti-EGFR Nb 7D12, showing enhanced binding parameters in SPR, with fast association and slow dissociation rates. The homo/heterodimeric bivalent constructs have improved affinity and are able to inhibit cancer cell proliferation. Conclusion: We were able to develop novel mono and bivalent nanobodies with enhanced binding properties which can be used for targeted cancer therapy.

585. (888) **NOTCH AND ANDROGEN RECEPTOR INVOLVEMENT IN PROSTATE CANCER DEVELOPMENT**

Agustina Chimento[1], Nadia Bonadeo[1], Sofía Perrone[1], María Lucia Romano[1], Ana Laura Fontanazza[2], Amilcar Osorio[3], Elena Casco[3], Kurt Villalba[4], Licina Tessone[5]; Fernanda Parenti[6], Carolina Cristina[1].

[1]CIBA – CITNOBA, UNNOBA – CONICET, J. Newbery 261, Junín, Provincia de Buenos Aires; [2]HIGA Abraham Félix Piñeyro, Junín, Provincia de Buenos Aires; [3]Centro Oncológico Pergamino (COP), Pergamino, Buenos Aires; [4]Clínica Cen-

tro, Junín, Provincia de Buenos Aires; [5]Laboratorio de Patología Dr Alberto Petraglia, Junín, Provincia de Buenos Aires; [6]Centro Medico Famyl, Junín, Provincia de Buenos Aires.

Prostate cancer (PCa) remains among the leading causes of cancer-related deaths in men. Standard therapies for castration resistant prostate cancer (CRPC) include second-generation anti-androgens, such as Enzalutamide (Enz), which prolong patient lifespan. There is strong evidence that involves Notch pathway in prostate development but its role in PCa generation and progression is poorly understood. In this work, we aimed to study the Notch system association with prostate tumor development and resistance to treatment with Enzalutamide. In prostate cancer PC3 cells, we demonstrated AR expression by RT-qPRC. Instead, PSA expression was absent when evaluated culture cell supernantants by chemiluminescence. We injected subcutaneously Nude mice with PC3 cells and we observed an increased mRNA levels of *Notch-1,* a target gene of the Notch pathway *Hes-1* and *TMPRSS,* an androgen dependent gene, in larger volume tumors compared to small tumors; mRNA levels of AR didn't show differences. Moreover, under Notch pathway inhibition with DAPT, the expression of *TMPRSS2*, showed lower levels after 24 h of treatment (10 and 30 μM) by RT-qPCR (p=0.02;n=2). In turn, Enz treatment (30 and 50 μM) reduced the levels of *HES1* determined by RT-qPCR (n=3). We observed significantly reduced viability, using MTS assay, of PC3 cells both with DAPT (p=0.0027;n=3) and Enz isolated treatments (p=0.0018;n=3), also with the combined treatment (p=0.0043;n=3). We observed reduced migratory abilities both with DAPT (p=0,0022;n=3) and Enz isolated treatment (p=ns; n=1.2), and also with the combined treatment (p=0,0526; n=3) using wound healing assay. Our study shows *in vitro* and *in vivo* activation of the Notch pathway in PC3 cells, which would be involved in cell proliferation and migration. Importantly, our results suggest an interconnection between Notch and AR pathways in PCa. A combined approach with inhibitors of both pathways could be more effective, especially in patients with aggressive PCa.

ONCOLOGY VII *Saturday, November 19, 9-10:30 hr*
Chairs: Juan Garona - María Sol Ruiz - Mara Giselle Peters

586. (659) **FACING THE ENEMY: MIRNAS 19B-3P AND 146A-5P AS POTENTIAL BIOMARKERS OF DOXORUBICIN RESISTANCE IN TRIPLE NEGATIVE BREAST CANCER**

Juana Moro[1], Rocío Belén Duca[1], Karen Daniela Graña[1] , Paola De Luca[1], Adriana De Siervi[1]
Instituto de Biología y Medicina Experimental (IBYME-CONICET).

Breast Cancer (BCa) is the most prevalent global malignancy and one of the leading causes of cancer deaths. Despite the novel therapies, resistance to chemotherapeutic drugs is a major challenge for effective therapy. Doxorubicin is a drug that is vastly used for BCa treatment which makes it an interesting target for the study of drug resistance. MiRNAs are short non-coding RNAs that act as post-transcriptional regulators of gene expression. Our aim was to identify a panel of miRNAs as possible biomarkers for doxorubicin resistance in triple negative breast cancer (TNBC) cells. Initially, we determined the IC50 to doxorubicin from several human and murine TNBC cell lines using MTS assays. Afterward, the expression levels of a panel of miRNAs were measured by RT-qPCR. This panel was selected based on previous results from our lab and bibliography. We have selected the most sensitive cell line, 4T1 (IC50= 0.33$\pm$ 0.08) to generate a doxorubicin-resistant variant cell line (4T1DR) by chronic exposition to sublethal increasing doses of doxorubicin for 8 months. We found that 4T1DR showed 4 times higher IC50 compared to control cells (4T1DR, IC50= 1.37 $\pm$ 0.11). We have determined miRNAs expression of these cells (4T1DR and 4T1 control) by RT-qPCR. We have found significantly up-regulation of miR-19b-3p and miR-146a-5p expression levels in 4T1DR in comparison to 4T1 control cells. These miRNAs are implicated in several KEGG pathways related to cancer and drug resistance such as p53 signaling, cell cycle, and apoptosis. In addition, miR-146a-5p is up-regulated in BCa tissue compared to normal adjacent tissue in one cohort of 16 paired samples (GSE97811). We have found miR-146-a-5p and miR-19b-3p expression levels increased in the plasma of BCa patients compared to healthy donors (GSE 73002, N=1280 per group). The results obtained so far are a promising landscape for finding an effective way to predict the failure of chemotherapy and eventually, inhibit drug resistance.

587. (667) **IDENTIFICATION OF A PUTATIVE REGULATORY REGION INVOLVED IN R-SPONDIN3 EXPRESSION MODULATION IN TRIPLE NEGATIVE BREAST CANCER CELLS**

Ana Laura Ortiz*[1], Carla María Felcher*[1], Pedro Javier Salaberry[1], Micaela Nadia Stedile[1], Camila Daiana Arcuschin[1], Ignacio Esteban Schor[1], Lucio Hernán Castilla[2], Edith Claudia Kordon[1,3]
1 Instituto de Fisiología, Biología Molecular y Neurociencias-CONICET-UBA Argentina, 2 University of Massachusetts-USA, 3 Departamento de Química Biológica-Universidad de Buenos Aires.
** Ana Laura Ortiz and Carla Maria Felcher contributed equally to this study.*

We have determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in basal breast cancer (BC). Besides, we have previously reported that blocking RUNX-CBFβ transcription factor (TF) activity inhibited RSPO3 expression in human triple-negative BC cells MDA-MB231. Interestingly, RUNX1-KO mice also showed reduced Rspo3 levels in their mammary glands. Then, the possible direct expression regulation exerted by RUNXs in human cells was assessed by an *in silico* analysis that revealed a putative DNA region containing three RUNX binding motifs (named R4) in the first intron of the human RSPO3 locus. The physical interaction between RUNX1 and R4 was confirmed by a ChIP-PCR assay in MDA-MB231 cells. Besides, we performed a combined bio-informatic analysis of publicly available data (including TF and modified histone CHIP-seq, ATAC-seq and TF binding motifs in the human genome), which revealed that this 363bp region may constitute a putative enhancer of the human RSPO3 in BC cells. Therefore, using Crispr-Cas9 technology, we proceeded to remove the putative regulatory region from these cells genome. Surprisingly, we determined that RSPO3 expression was increased at both mRNA and protein levels, by RT-qPCR and Western Blot analysis, in the edited ΔR4 cells. Therefore, we postulate that in the MDA-MB231 cells the R4 region may recruit a transcription repressor that might be absent in BC cells with higher RSPO3 expression. However, the role played by RUNX1 binding to the R4 in the RSPO3 locus remains to be elucidated. Finally, given the relevance of RSPO3 in basal-like mammary tumor cell behavior, we propose that the newly found regulatory region may be important for triple-negative BC progression.

588. (671) **EFFECTS OF DIETARY COMPOUNDS ON THE INTERCELLULAR COMMUNICATION MEDIATED BY EXTRACELLULAR VESICLES IN MAMMARY TUMORS**

Érica Daniela Solla[1,2], Franco Fabián Roldán Gallardo[1,2], Tamara Magalí Mazo[2,3], Victoria Ferrero[2,3], María Eugenia Pasqualini[2,3], Cristina Alicia Maldonado[1,2], Amado Alfredo Quintar[1,2]
[1] Universidad Nacional de Córdoba (UNC). Facultad de Ciencias Médicas. Centro de Microscopía Electrónica, [2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Investigaciones en Ciencias de la Salud (INICSA, CONICET-UNC), [3] Universidad Nacional de Córdoba (UNC). Facultad de Ciencias Médicas. Instituto de Biología Celular.

Carbohydrate- and fatty acid-rich dietary patterns (CAP and FAP), represented mainly by fructose (F) and palmitic acid (PA) respectively, are strongly associated with breast cancer, with the cellular mechanisms being far to be fully elucidated. We aimed to evaluate the dietary effects on the microenvironment interactions between

cancer-associated fibroblasts (CAFs) and tumoral cells mediated by extracellular vesicles (EVs). Balb/c mice fed on CAP, FAP or CAP+-FAP mixture for 2 months were s.c. implanted with LM3 mammary tumor cells for 20 days. When compared with chow diet, CAP+-FAP tumors grew faster and developed a larger volume ($p<0.05$); by electron microscopy, a higher frequency of CAFs, collagen and ultrastructural signs of increased secretion of EVs were seen. For *in vitro* studies, the mammary CAFs cell line F88 was cultured and stimulated with F40mM, AP250uM, F+PA combinations or their vehicles for 24h. Supernatants were collected and EVs isolated by sequential ultracentrifugation (2k, 10k, 100k), characterized by electron microscopy, and labeled with CD63 using immunogold technique. F+PA induced an increase in the frequency of 20-30 nm-sized EVs with respect to controls or F and PA alone ($p<0.05$). To evaluate the effect of EVs from CAFs on tumor cell proliferation, MCF-7 mammary tumor cells were stimulated for 24h with conditioned media or with EVs derived from F-, AP-, and F+PA-treated F88 cells. Conditioned media from F88 treated with F+PA increased cell proliferation of MCF7 cells, determined by incorporation of bromodeoxyuridine and cell count ($p<0.05$), with this effect being reproduced with EVs from F+PA-treated F88. This pro-tumoral action of EVs was inhibited by pre-incubating MCF7 with genistein, suggesting EVs uptake in a clathrin-independent manner. These results indicate a pathogenic effect of dietary patterns rich in F and PA on mammary tumor microenvironment, through the release of pro-proliferative EVs by CAFs.

589. (674) **TGFβ TYPE I RECEPTOR SIGNALING MEDIATES HISTAMINE-IONIZING RADIATION CROSSTALK IN EPITHELIAL MESENCHYMAL TRANSITION PROGRESS AND CANCER STEM-LIKE CELLS ENRICHMENT IN BREAST CANCER CELLS**

Tamara Galarza[1,2], Nora Mohamad[1], Graciela Cricco[1], Gabriela Martín[1,2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos. Buenos Aires, Argentina. [2]CONICET

Tumor radio-resistance favours recurrence and progression of the disease in patients due to cells that survive radiotherapy. As well, epithelial mesenchymal transition (EMT) is relevant for the acquisition and maintenance of cancer stem-like cells (CSC-like) properties in tumor cells. Likewise, activation of the TGFβ-1/TGFβ type I receptor (TβRI) pathway promotes radio-resistance by inducing EMT progression and CSC-like enrichment. We have previously demonstrated in breast tumor cells the dual role of histamine (Ha) on the radio-induced EMT process: favouring EMT at ≤ 1 μM and hindering it at ≥10 μM. In this study, we aimed to evaluate a link between Ha action on radio-induced EMT and the activation of TβRI signalling, in MCF-7 and MDA-MB-231 breast cancer cells. Cells were treated with 1 μM Ha (low Ha) or 20 μM (high Ha) and with the selective inhibitor of TGFβRI A83-01; 24 h later cells were 2Gy irradiated. After 5 days, indirect immunofluorescence assays were performed to evaluate positive nuclei for P-Smad2/3 (canonical TβRI effector), Slug and β-catenin proteins (EMT molecular markers). In addition, mammosphere formation (stem cells surrogate assay), cell migration using transwell chambers, morphology (EMT functional traits) and clonogenic capacity were evaluated. In both cell lines, A83-01 prevented the alteration in cell morphology and the increase in mammospheres, colonies and migrated cells number due to irradiation ($p<0.05$), low Ha treatment ($p<0.05$), or their combination ($p<0.01$). It also blocked the rise in Slug, β-catenin and P-Smad2/3 positive nuclei. High Ha showed no effect on mammosphere formation, EMT molecular markers and functional traits, but reduced irradiation-induced increments ($p<0.05$). In irradiated cells treated with high Ha+A83-01 the diminution was even deeper ($p<0.01$). Collectively, results suggest that TβRI signaling is involved in Ha-ionizing radiation crosstalk in EMT progress and CSC-like enrichment in breast cancer cell lines.

590. (679) **ANALYSIS OF TRISTETRASPOLIN (TTP) ROLE IN HUMAN BREAST CANCER BEHAVIOR**

Angela Lara Montero[1], Micaela Stedile[1], Karina Cicinelli[1], Noemi Jordanovski[2], Eliana Querol[2], Cecilia Temple[2], Florencia Costa[2], Carina Gon[2], Mary Carrasco[2], Daniela Maltagliatti[2], Verónica Sanchotena[2], Laura Leguina[2], Silvia Vornetti[2], Diego Flaks[2], Gabriela Acosta[2], Nicasio Cuneo[2], Eva Wertheimer[3], Claudia Arias[2], Edith Kordon[1]

[1]Institute of Physiology, Molecular Biology, and Neurosciences (IFIBYNE-CONICET), University of Buenos Aires, CABA, Argentina,[2]Marie Curie Oncology Hospital, CABA, Argentina,[3]Center of Pharmacological and Botanical Studies (CEFYBO-CONICET), University of Buenos Aires, CABA, Argentina.

Tristetraprolin (TTP) induces the degradation of multiple mRNAs of pro-inflammatory and oncogenic factors. We have previously reported TTP down-regulation in invasive breast cancer (BC) compared to normal tissue. However, we have also found that TTP expression is required for survival of mouse mammary progenitor cells. To better understand the role of this protein in BC development, we carried on an integrative analysis using data from The Cancer Genome Atlas (TCGA), the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC), the Clinical Proteomic Tumor Analysis Consortium (CPTAC), and studies performed on Hospital Curie´s BC patients. By bioinformatics, we determined a negative association between oncogenes and TTP expression in BC ($p < 0.001$) and found that all molecular subtypes, with the exception of the Claudin-low group, under-expressed this gene. Besides, TTP immunohistochemistry (IHC) analysis also showed a negative correlation of this protein with tumor size and proliferative marker Ki67. However, TTP high expression was associated with a worse prognosis in triple-negative breast cancer (TNBC) patients ($p<0.05$). We also aimed to determine whether mRNA levels of inflammatory cytokines that are TTP targets are negatively related to this protein in BC; and although we found it in relation to TNFα, a significantly positive correlation between TTP and IL-6 was determined not only in all BC molecular subtypes, but also in normal tissue. Besides, TTP expression was related to phosphorylated STAT3 (a transcription factor commonly activated by IL-6) levels in patient samples ($p<0.05$) and by CPTAC data. In summary, we have confirmed TTP putative tumor suppressor activities in most human mammary tumors. Nevertheless, our results may indicate that TTP can be a survival factor in TNBC and Claudin-Low tumor cells. In addition, a possible IL-6 inductive activity on TTP expression through STAT3 activation is suggested, but must be proven experimentally.

591. **(**710) **REMODELING OF THE ADIPOSE TISSUE MICROENVIRONMENT AND ANALYSIS OF THE PARACRINE EFFECT IN BREAST CANCER**

Priscila Ayelén Pagnotta[1,2], Sabrina Fletcher[1], Tomás Gonzalez Garello[3], Rubén Dreszman[4], María Luján Crosbie[5], Natalia Santiso[5], Anabela Ursino[5], Celeste Frascarolli[5], Alicia Amato[5], Juan Carlos Calvo[1,2] and Judith Toneatto[1]

1 IBYME – CONICET, 2 Departamento de Química Biológica (FCEN, UBA), 3 IEGEBA – CONICET, 4 Clínica de Microcirugía, 5 Complejo Médico Policial Churruca-Visca

The adipose microenvironment is involved in the progression of breast cancer. The aims of this work were to analyze the remodeling of the tumor-associated microenvironment and evaluate the paracrine action of soluble factors released by adipose tissue (AT). Breast adipose tissue explants from cancer patients (hATT, attached to the tumor -AC- and 2 cm from this -BC-) or normal (hATN) and conditioned media (CM) obtained from them were used. Immunofluorescence microscopy analysis was performed on sections of paraffin-embedded human AT. The intensity of protein expression was analyzed through multiple regression and posterior comparisons with Tukey test (α=0.05). The expression of Plin1 was 5-fold higher for patients with invasive ductal carcinoma (IDC) compared to ductal lobular carcinoma (DLC), being higher in hATT-AC than in -BC with overweight; consistent with Western Blot assay (WB) results. Premenopausal patients presented a 3-fold higher expression of ATGL (IIF) compared to postmenopausal patients, which is consistent with WB results. HSL expression did not show changes. The expression of FABP4 was 3-fold higher in IDC respect to DLC (IIF). FABP4 protein had a tendency to decrease in hATT-AC in those patients with

IDC. CAV1 expression tends to increase in hATT-AC with respect to hATN in IDC. At the paracrine level, CM-hATT: 1-increased the number of lipid droplets and decreased in its size, cell area and triglyceride content; 2-decreased the expression of FABP4 and ATGL and increased of Plin1 and total and p-HSL; 3-increased the browning protein expression in white adipocytes with respect to CM-hATN and Ctrol. These results show that peritumoral adipocytes undergo a remodeling in a subtype of carcinoma dependent manner, promoting lipolysis and the acquisition of beige adipocyte features by paracrine action. The results, together with previous observations, allow us to conclude that these changes alter the function of adipose tissue and favor cancer progression.

592. (713) **MECHANISMS UNDERLYING THE ANTI-TUMORAL EFFECTS OF THE FLAVONOID 2'-NITROFLAVONE IN HUMAN TRIPLE-NEGATIVE BREAST CANCER CELLS**

Vanina Sol Vachetta[1], Viviana Blank[1], Milagros Bustos[1], Mariel Marder[1], María Gabriela Ortega[2], Margot Paulino Zunini[3], María Teresa Elola[1], María Fernanda Troncoso[1]

[1]Instituto de Química y Fisicoquímica Biológicas (IQUIFIB, UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA, [2]Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, [3]DETEMA Centro de Bioinformática Estructural, Facultad de Química, Universidad de la República, Uruguay

Triple-negative breast cancer (TNBC) is one the most aggressive subtypes of mammary tumors. Flavonoids are polyphenolic compounds that cause several antineoplastic effects. We previously described that the synthetic flavonoid 2'-nitroflavone (2'NF) inhibits viability, survival and migration of human TNBC cells. Herein, our aim pointed to find out mechanisms that mediate 2'NF anti-tumoral effects in these cells. We first evaluated MDA-MB-231 cell cycle progression (propidium iodide staining, flow cytometry) upon treatment. 2'NF (5 μM, 72 h) decreased the percentage of cells in G1 phase from 59.30±1.63 (control) to 43.00±.2.29% ($p<0.001$), while cells in S and G2 phase increased from 23.44±1.24 (control) to 31.08±3.34% and 17.26±0.77 (control) to 25.92±2.50%, respectively ($p<0.01$). Regarding apoptosis, 25 μM 2'NF (72 h) decreased viable cells from 95.13±1.07 (control) to 14.15±3.63%, increasing early apoptotic cells from 1.62±1.09 (control) to 77.60±2.94% ($p<0.001$; acridine orange/ethidium bromide staining). Hypodiploid cells increased from 3.4±0.4 (control) to 23.8±2.2% or 45.7±1.1% after 72 h-treatment with 5 and 25 μM 2'NF, respectively ($p<0.001$; propidium iodide staining, flow cytometry). After 72 h-treatment, 5 and 25 μM 2'NF induced PARP-1 cleavage (2.93±0.52 and 2.95±0.14, respectively, control: 1, $p<0.01$; Western blot). In MDA-MB-231 and BT-549 cells (claudine-low TNBC) 25 μM 2'NF decreased α-tubulin expression (0.55±0.06 and 0.39±0.10, respectively, control: 1, $p<0.01$; Western blot). 2'NF (5 and 25 μM) also produced giant multinucleated cells, a typical characteristic of mitotic catastrophe (phalloidin-TRITC/DAPI staining). Besides, molecular docking analysis indicated 2'NF interaction with the active site of PARP-1 (binding energy: -6.5262 kcal mol^{-1}). In conclusion, 2'NF impairs cell cycle progression, induces apoptosis, affects α-tubulin expression and promotes mitotic catastrophe in TNBC cells. Interaction between 2'NF and PARP-1 will be further evaluated.

593. (714) **MICROENVIRONMENT MODULATES TUMORIGENICITY OF BREAST CANCER CELLS DEPENDING ON HORMONE RECEPTOR STATUS**

Priscila Ayelén Pagnotta[1,2], Dana Pinco[3], Magalí Gimenez[3], Sofía Goldberg[3], Sabrina Fletcher[1], Rubén Dreszman[4], María Luján Crosbie[5], Natalia Santiso[5], Anabela Ursino[5], Celeste Frascarolli[5], Alicia Amato[5], Juan Carlos Calvo[1,2] and Judith Toneatto[1]

1 IBYME – CONICET, 2 Departamento de Química Biológica (FCEN, UBA), 3 Escuela ORT Argentina, 4 Clínica de Microcirugía, 5 Complejo Médico Policial Churruca-Visca

There are differences in patients with triple negative breast cancer (TN) and positive hormone receptors and recently the role of the microenvironment in the progression of different breast cancer subtype has become relevant. The aims were: 1) Analyze bioinformatically the expression of key genes in TN, positive estrogen receptors, positive progesterone receptors and in cell line models; 2) Evaluate the effect of soluble factors released by human breast cancer adipose tissue explants (hATT) and normal breast adipose tissue (hATN) on tumorigenesis. The Xena Browser (TCGA Breast Cancer) and GEO (GEO2R; GSE139670) databases were used. The cell lines MCF7, T47D and MDA-MB-231 and conditioned media (CM) from hATT and hATN were used. Adhesion and proliferation were analyzed. There were differential expression and survival probability in genes of pluripotency, cellular interaction, metabolism and signaling pathways, which depend on the expression of the hormone receptors of the patients or the cell lines. A decrease in adhesion was observed in MCF7 incubated with CM-hATT and in MDA-MB-231 treated with CM-hATN and hATT. CM-hATN promoted greater adhesion in MCF7 than MDA-MB-231. Preliminary results suggest that the CM-hATN decreased proliferation of MDA-MB-231. CM-hATT induced a tendency to increase proliferation of MCF7. Preliminary results don't show differences between treatments for T47D. In the MDA-MB 231 cells OCT4 protein expression (Western Blot) was decreased, which was associated with a poor survival probability (Xena Browser data), after incubation with CM-hATT. Also, CAV1 protein expression increased in the MDA-MB-231 incubated with CM-hATN. The tumor microenvironment releases soluble factors that could modulate the gene expression favoring key processes in the progression of the disease, while soluble factors of a microenvironment without cancer could decrease these effects. These biological events depend on the expression of the hormone receptors of the patients.

594. (728) **SYNERGISM BETWEEN TRASTUZUMAB AND 1A-116 RAC1 INHIBITOR IN THE TREATMENT OF HER2-OVEREXPRESSING HUMAN BREAST CANCER CELLS**

Virginia J. Wolos[1], Ivana J. Tapia[1], Marianela Abrigo[1], Elisa D. Bal de Kier Joffé[1,2], Daniel F. Alonso[2,3], Georgina A. Cardama[2,3], Gabriel L. Fiszman[1,2]

[1]Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigación, Departamento de Inmunobiología, Argentina, [2]CONICET, Argentina, [3]Centro de Oncología Molecular y Traslacional (COMTra), Universidad Nacional de Quilmes, Argentina

HER2-targeted trastuzumab monoclonal antibody, combined with chemotherapy, is the treatment of choice for patients with HER2-overexpressing breast cancer. Despite its success, therapy resistance is still a major obstacle in clinical practice. Upregulation of Rac1 activity, a member of the Rho-GTPase family, has been reported to be involved in trastuzumab resistance mechanisms. Thus, the purpose of our study was to evaluate the effects of combining trastuzumab with 1A-116, a specific Rac1 inhibitor, in the treatment of HER2-overexpressing BT-474 human breast cancer cell line. First, by crystal violet assay, we assessed the effect of each drug on the viability of BT-474 cell monolayers after a 72-hour treatment. As shown by concentration-response curves, both trastuzumab and 1A-116 decreased cell viability with relative IC50 values of 0.1 μg/mL and 40.7 μM, respectively. Moreover, under the same experimental conditions, significant levels of synergy between drugs were determined by Combenefit 2.021 software ($p<0.05$). Combined treatment with (1-10) μg/mL trastuzumab and (20-25) μM 1A-116 showed a significantly higher effect on cell viability than each drug alone. To further analyse this effect, we studied trastuzumab and 1A-116 treatment on BT-474 cells cultured as 3D tumour spheroids for 14 days. While IgG (50 μg/mL)-treated control spheroids increased their volume more than threefold, treatment either with trastuzumab (50 μg/mL) or 1A-116 (25 μM) prevented spheroid growth ($p<0.05$). Interestingly, trastuzumab and 1A-116 concomitant treatment induced a significant reduction in spheroid volume compared with spheroids treated with each drug separately ($p<0.05$). To conclude, our results support the interaction previously reported between HER2 and Rac1 signalling pathways. Further studies will help to shed new light on the mechanisms behind the resistance promoted by Rac1 activity and the potential use of Rac1 inhibitors to improve trastuzumab effects.

595. (753) **HISTAMINE H_3 RECEPTOR EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER: THERAPEUTIC OPPORTUNITY WITH PHARMACOLOGICAL INHIBITION**

Mónica A. Táquez Delgado[1], Melisa B. Nicoud[1], Ignacio Ospital[1], Michelle F. Corrêa[2], Gustavo A. Borges Fernandes[2], Daniela Speisky[3], João P. S. Fernandes[2], Vanina A. Medina[1].
1 Laboratorio de Biología Tumoral e Inflamación. Instituto de Investigaciones Biomédicas (BIOMED), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Católica Argentina (UCA), Argentina
2 Departamento de Ciências Farmacêuticas, Universidade Federal de São Paulo, Diadema, SP, Brazil
3 Hospital Británico, Buenos Aires, Argentina

Triple-negative breast cancer (TNBC) accounts for 10-15% of incident breast cancers and lacks specifically targeted and effective therapy, exhibiting the worst prognosis. We have previously reported the expression of the histamine H_3 receptor (H_3R) in benign and malignant lesions, and cell lines derived from human mammary glands. In this work, we aimed at evaluating the expression of H_3R specifically in TNBC samples and cell lines and investigating the antitumoral properties of a series of 10 H_3R antagonists, 1-(2,3-dihydro-1-benzofuran-2-yl)methylpiperazines (LINS01 compounds).[1,2]
Analysis of the TCGA Pan-Cancer Atlas data set showed that H_3R mRNA expression was higher in the aggressive basal-like/TNBC tumors compared with the most favorable clinical outcome luminal subtype ($P<0.01$). To corroborate the bioinformatic analyses, the H_3R status was evaluated by immunohistochemistry in 50 TNBC human samples in relation to clinicopathological parameters. It was detected in around 40% of the specimens and it was verified in TNBC cell lines (MDA-MB-231, 4T1, BT546). Cell viability (celltiter-blue), clonogenic proliferation, cell apoptosis (Annexin-V and TUNEL) and migration were assessed in human MDA-MB-231 and murine 4T1 TNBC cells. Results indicate that compounds LINS01009, LINS01010, LINS01016, LINS01022 and LINS01023 (0.01-100 μM) produced a concentration-dependent inhibition on cell growth. The highest responses were observed for LINS01022 and LINS01023, showing an IC_{50} of 9.9±1.1 and 5.2±1.2 μM for MDA-MB-231 cells, and 11.1±1.3 and 3.4±1.2 μM for 4T1 cells, respectively, in the clonogenic assay. These effects were partially reversed by the selective H_3R agonist (*R*)-alpha-methylhistamine. LINS01016, LINS01022 and LINS01023 (25-50 μM) significantly induced cell apoptosis and suppressed cell migration in both cell lines (ANOVA, $P<0.01$). We conclude that the H_3R is involved in the regulation of TNBC progression, offering novel therapeutic potentials for H_3R antagonists.
[1]Correa et al. *Front Pharmacol* **2017**,*8*,825
[2]Correa et al. *Bioorg Med Chem* **2021**,*30*,115924

596. (757) **LXR ACTIVATION IMPAIRS ESTRADIOL DEPENDENT PROLIFERATION IN HUMAN BREAST CANCER CELLS THROUGH DOWNREGULATION OF GENE EXPRESSION ASSOCIATED WITH DNA REPLICATION AND CELL CYCLE PROGRESSION**

Evelyn Olszanowski[1,2], María Florencia Ogara[1], A. Silvina Nacht[3], Santiago Andrés Rodríguez-Seguí[1,4], Guillermo P. Vicent[5], Adali Pecci[1,2]
[1] CONICET-Universidad de Buenos Aires, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), C1428EHA, Buenos Aires, Argentina.
[2] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, C1428EHA, Buenos Aires, Argentina.
[3] Gene Regulation, Stem Cells and Cancer Program, Center for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona 08003, Spain.
[4] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, C1428EHA, Buenos Aires, Argentina.
[5] Molecular Biology Institute of Barcelona, Consejo Superior de Investigaciones Científicas (IBMB-CSIC), Barcelona, 08028, Spain.

Liver X Receptors (LXRs) belong to the nuclear receptors superfamily of ligand activated transcription factors, whose endogenous agonists are the oxysterols. They play a key role in the regulation of the cholesterol homeostasis, induce the de novo synthesis of triacylglycerides, and counteract pro-inflammatory effects. LXRs are also known to compromise cell proliferation in several cancer models. However, their role in breast cancer (BC) has not been studied in depth and reports are, in fact, contradictory. Here we examined the potential involvement of LXRs in BC cells with special emphasis on their possible crosstalk with the Estrogen Receptor alpha (ERα). To address this objective, we performed colony formation (CFA) and propidium iodide staining assays in MCF-7 cells treated with or without Estradiol (E2) and the LXR agonist, GW3965. Our results showed that GW3965 impared the cell proliferation capacity induced by E2 (CFA: #colonies, Mean±SD: E2 208.7±25.7; E2+GW3965 131.3±23.7, n=3, padj<0.01, ANOVA). With the aim of understanding the molecular mechanisms underlying these functional effects, we performed a bulk RNA-seq experiment in duplicates. The differentially expressed genes between E2 and E2+GW3965 conditions revealed several genes whose expression was affected by GW3965; which are widely enriched in terms associated to DNA replication, cell cycle, G1 to S transition, and Breast Cancer (padj<0.05) including genes such as *PCNA, MCM4, CCND1, POLE3, TOP2A, BRCA1, BRCA2, RAD51, RET, E2F1, E2F2* as well as the ERα (*ESR1*). Interestingly, the presence of GW3965 increased the expression of the Glucocorticoid Receptor (GR) (*NR3C1*), which is consistent with a less proliferative phenotype observed in cells treated with this ligand. Further experiments are necessary to address the mechanism underlying LXR function in BC, but our results point to a functional crosstalk with other steroid receptors, such as ERα and/or GR as putative mechanisms underlying LXR effects.

597. (779). **COMBINATION THERAPY OF PACLITAXEL WITH UVB1: A NEW THERAPEUTIC OPTION FOR AGGRESSIVE BREAST CARCINOMAS**

Ibarra Agustina[1], Clemente Valentina[1], Guevara Josefina A.[1], Alonso Eliana N.[1,2], Quevedo Mario A.[3], Fermento María E.[1,2], Coló Georgina P.[1,2], Fall Yagamare[4], Ferronato María J.[1,2], Facchinetti María M.[1,2], Curino Alejandro C.[1,2].
1. Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) – CONICET, Bahía Blanca, Argentina.
2. Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
3. Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina.
4. Departamento de Química Orgánica, Facultad de Química e Instituto de Investigación Sanitaria Galicia Sur (IISGS), Universidad de Vigo, 36200, España.

Hormone-independent and Triple-Negative (TN) Breast Cancer (BC) are aggressive tumors that are treated with therapies such as anti-HER2 and cytotoxic chemotherapy, respectively. These tumors have bad prognosis and their therapeutic strategies provoke adverse side effects. Therefore, the identification of an alternative approach to treat these BC is needed. Our laboratory studies the antitumor properties of a non-hypercalcemic calcitriol analog called UVB1 that has previously demonstrated antineoplastic effects in different types of cancer. The aim of this work is to evaluate the antitumor effects of UVB1 on aggressive BC cells, either alone or in combination with paclitaxel (PTX). To this end, cell viability was evaluated by crystal violet assays in 4T1 and MDA-MB-231 TNBC cell lines treated with vehicle, UVB1, PTX or combination of drugs. The results show that UVB1 (1000 nM) with low concentrations of PTX display a greater reduction in viability with respect to control and monotherapies in both cell lines (120 and 48 h of treatment, respectively). Combination indexes (CI) obtained by Chou-Talalay method were less than 1, which indicates synergism between UVB1 and PTX. These effects are maintained with a lower UVB1 concentration (1 nM) in both cell lines. Molecular modeling studies, including molecular docking

and molecular dynamics simulations, suggest a cooperative binding mode to VDR of UVB1 and PTX, which in turn elicits a close regulation of the conformational behaviour of the activating factor 2 (AF-2) region of VDR. The combination of UVB1 and PTX strongly favours the AF-2 conformation that resembles the one observed for the natural substrate calcitriol. Altogether, these results suggest the potential combination of a calcitriol analogue with lower doses of conventional chemotherapeutics for aggressive BC treatment.

598. (784) **AQUEOUS EXTRACT OF THE ARGENTINEAN NATIVE PLANT *PROSOPIS CALDENIA* (CALDÉN) INDUCES CYTOTOXIC EFFECTS AGAINST TRIPLE-NEGATIVE BREAST CANCER CELLS**

María Julia Ferronato[1,2], Ana Paula Pedersoli[1], Eliana Noelia Alonso[1,2], Agustina Ibarra[1], Agustina Gutierrez[2,3], Pablo Marinangeli[3,4], Valentina Clemente[1], Georgina Pamela Coló[1,2], María Eugenia Fermento[1,2], Alejandro Carlos Curino[1,2], María Marta Facchinetti[1,2]

1. Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) – CONICET, Bahía Blanca, Argentina. 2. Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina. 3. Laboratorio de Fitotecnología, Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Universidad Nacional del Sur (UNS) – CONICET, Bahía Blanca, Argentina. 4. Departamento de Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.

Plant-originated drugs/formulations are employed as a complementary therapy for treating various human ailments including cancer. Recently, native species from Argentina belonging to genus *Prosopis* have begun to be studied for their beneficial biomedical properties. *Prosopis caldenia* (Pc) is locally known as “Caldén” and it has not been studied from a medicinal point of view. The aim of this work was to investigate the antitumor potential of Pc leaves aqueous extract (PcAE) against MDA-MB-231 and 4T1 Triple Negative Breast Cancer (TNBC) cells. To this end, cell viability was studied by colorimetric crystal violet and MTT assays in cells treated with vehicle or PcAE (10-100-1000-2500-5000 ug/ml). Cell cycle was analysed by flow cytometry in cells stained with propidium iodide. Cell migration was assessed by “wound closure” assays. The results show that PcAE reduced cell viability in a concentration-dependent manner in both cell lines at 48 and 72 hours (h) of treatment ($p<0.001$). Cell cycle analysis demonstrate that PcAE (1000 ug/ml) arrested 4T1 cells at S-phase (48 h: PcAE 49.51 ± 0.24 vs. vehicle 35.92 ± 0.67 %; $p<0.001$ - 72 h: PcAE 41.83 ± 2.55 vs. vehicle 32.75 ± 2.77 %; $p<0.05$). In MDA-MB-231 cells, PcAE (1000 ug/ml) induced S-phase arrest at 48 h (PcAE 43.62 ± 1.15 vs. vehicle 37.19 ± 0.41 %; $p<0.001$) and increased the percentage of cells in sub-G0 population at 72 h of treatment (PcAE 3.78 ± 0.25 vs. vehicle 2.16 ± 0.12 %; $p<0.01$). Regarding cell migration, PcAE decreased the migratory capacity of MDA-MB-231 cells (1000 ug/ml, 16 h, $p<0.001$). These results show, for the first time, the antitumor effects of the active principles present in an aqueous extract of Caldén leaves on TNBC cells. This study highlights the importance of the native species of our country as a potential resource of metabolites with therapeutic implications in cancer.

599. (813) **HEMOXYGENASE-1 GENETIC VARIANTS EFFECTS ON BREAST CANCER PROGRESSION**

Schweitzer Karen, Alonso Exequiel Gonzalo, Mascaró Marilina, Fernández Chávez Lucia, Coló Georgina Pamela, Alonso Eliana Noelia, Ferronato María Julia, Fermento Eugenia, Curino Alejandro Carlos and Facchinetti María Marta.

Laboratorio de Biología del Cáncer, Instituto de investigaciones Bioquímicas de Bahía Blanca (INIBIBB-UNS-CONICET), Argentina

Hemoxygenase-1 (HO-1) is a microsomal enzyme that catalyzes the degradation of the heme group, and its C-terminal truncated form can translocate to the nucleus and perform functions at the transcriptional level. Our laboratory has already shown that HO-1 has antitumoral effects in breast cancer. We have additionally confirmed that the HO-1 truncated form is not enzymatically active. The aim of this work was to study the effect of genetic overexpression of HO-1 variants (full-length form (FL), full-length form without enzymatic activity (H25A) and nuclear truncated form (T-HO1)) on cellular processes related to cancer development, and also the molecular mechanisms through which HO-1 would modulate the cellular processes investigated. To accomplish this goal, we used T47D human breast cancer cell line that was stably transfected with plasmids overexpressing HO-1 variants. To analyse cell behaviour between variants, we performed viability assays and flow cytometry, and to quantify differential protein expression we used immunofluorescence and western blot. We observed significant differences in cell viability between wild-type T47D cells and T47D cells overexpressing HO-1 variants. We found that the wild-type form is more proliferative than the FL-, H25A- and T-HO1-overexpressing forms ($p<0.05$, two-way ANOVA). We also observed that in H25A-overexpressing cells this behaviour results, in part, from an increase in cell death ($p<0.05$, two-way ANOVA). Finally, by immunofluorescence, we observed differences in the number of actin filopodia. So far, these results indicate that the overexpression of HO-1 FL seems to display an anti-tumor role. The behaviour of the H25A and the T-HO1 forms would indicate that the anti-tumor behaviour is the result of HO-1 enzymatic activity and its nuclear role. Future experiments will allow us to understand the role of the different pathways involved between HO-1 variants.

600. (846) **SYNERGISTIC COMBINATION OF PACLITAXEL WITH NOVEL NON-HYPERCALCEMIC CALCITRIOL ANALOG EM1 AGAINST TRIPLE-NEGATIVE BREAST CANCER CELLS: NEW MECHANISM OF ACTION**

Josefina Alejandra Guevara[1], Agustina Ibarra[1], María Julia Ferronato[1], Eliana Noelia Alonso[1], María Eugenia Fermento[1], Georgina Pamela Coló[1], Cristian Alejandro Vitale[2], Evangelina Mascaró[2], Mario Alfredo Quevedo[3], María Marta Facchinetti[1], Alejandro Carlos Curino[1].

1. Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) – CONICET, Departamento de Biología, Bioquímica y Farmacia (UNS), Bahía Blanca, Argentina. 2. Laboratorio de Química Orgánica, Departamento de Química, Universidad Nacional del Sur (INQUISUR), Bahía Blanca, Argentina.

3 Unidad de Investigacion y Desarrollo en Tecnologia Farmaceutica (UNITEFA-CONICET), Facultad de Ciencias Quimicas, Ciudad Universitaria, Universidad Nacional de Cordoba, Cordoba, 5000, Argentina.

Triple-negative breast cancer (TNBC) is currently treated with cytotoxic drugs such as paclitaxel (PTX) since it lacks targeted therapies, although it shows limitations in extending patient survival. The known mechanism of action of PTX is binding to β-tubulin and inducing apoptosis. The vitamin D receptor (VDR) is expressed in different tissues, including TNBC. Calcitriol, its natural ligand, shows antitumor activity, but its usefulness is limited by the hypercalcemia it causes at antitumor doses. Reports suggest that PTX shows synergism when combined with calcitriol. The aim of this work is to combine PTX and non-hypercalcemic VDR analogs synthesized by our group, to study the potential synergism of the calcitriol analog EM1 with the cytotoxic PTX. EM1 in combination with PTX (EM1+PTX), showed a synergistic effect on the viability of 4T1 and MDAMB231 TNBC cell lines ($p<0.001$), CI<1. In contrast, EM1+PTX showed an antagonistic (protective) effect on non-tumor mammary HC11 cells ($p<0.001$), CI>1 (viability assays by crystal violet staining, Chou-Talalay method). In addition, (EM1+PTX) delayed the migration of 4T1 and MDAMB231 cells ($p<0.001$, wound closure assay). Interestingly, synergistic effects were lost when the Vitamin D Receptor (VDR) was silenced in 4T1 cells. Docking and molecular dynamics studies showed a direct interaction of PTX with the VDR by binding to the region near AF-2, stabilizing the active conformation between the VDR and its natural ligands. Cell cycle analysis by flow cytometry showed that the percentage of cells in the sub G0/G1 phase induced by PTX is higher in wild-type than in VDR-silenced cells. These results suggest that combining EM1 with current chemotherapy could increase

the therapeutic effect on the cancer cell and prevent cell damage on normal cells. This differential effect could decrease the adverse effects associated with chemotherapy, through a new mechanism of action never before described for PTX.

601. (879) **RELEVANCE OF NEUTROPHIL EXTRACELLULAR TRAPS IN THE CONTEXT OF HER2+ BREAST CANCER TREATMENT**

Romina Karas[1], Marianela Abrigo[1], Ivana Tapia[1], Virginia Wolos[1], Elisa Bal de Kier Joffé[1,2], Miriam Diament[1], Gabriel Fiszman[1,2].

[1] Universidad de Buenos Aires, Instituto de Oncología Ángel H. Roffo, Área Investigación, Buenos Aires, Argentina.

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Trastuzumab (Tz) and Trastuzumab-emtansine (T-DM1) are therapeutic monoclonal antibodies of choice for patients with HER2-overexpressing breast cancer tumors. However, some patients acquire resistance to these therapies. Tumor-associated neutrophils represent a heterogeneous cell population and their recruitment in the tumor environment may produce and release “neutrophils extracellular traps” (NETs). Although NETs are present in various tumors, their roles in tumor biology have not been clarified yet. Previously, we demonstrated that HER2+ BT-474 human breast cancer cells growing as tumor spheroids activate neutrophils promoting NETs formation, without affecting their growth after 72 hs of coculture.
The aim of this work was to study the role of NETs on Tz and T-DM1 treatment of BT-474 cells in 3D cultures. BT-474 spheroids (500-600um diameter) were incubated with 0.5 x 10^6 neutrophils from healthy donors and were treated with Tz 50ug/mL or with T-DM1 10ug/mL during a week. At the endpoint, spheroid volume was quantified, and viability was determined by trypan blue dye exclusion.
We observed that, while anti-HER2 treatments inhibited the growth and decreased the viability of tumor spheroids, the presence of NETs partially attenuated their cytotoxic effect. Changes in spheroid volumes after treatment were: Tz -9% vs NET-Tz +46.6% ($p<0.01$), T-DM1 +15.1% vs NET-T-DM1 +67% ($p<0.01$). Cell viability shown after treatment (relative to IgG-treated control spheroids) was: Tz 89.7% vs NET-Tz 114.7 ($p<0.05$), T-DM1 26.9% vs NET-T-DM1 229.6% ($p<0.001$). In summary, NETs could be attenuating Tz and T-DM1 cytotoxic effect by inhibiting drug diffusion. Further studies may help to better understand some of the microenvironment roles behind the acquisition of drug resistance in HER2+ breast cancer.

602. (901) **MICROBIOME RELEVANCE IN TUMOR TISSUE OF EARLY BREAST CANCER PATIENTS**

Leonardo Dandeu[1*], Pablo Aguilera[2*], Sofía Sidlik[1], Alberto Penas-Steinhardt[2], Pablo Marenco[3], Martín Vázquez[4], María del Pilar Carballo[3], María Fernanda Alsina[3], Norma A Chasseing[1], Vivian Labovsky[1].

*[1]Instituto de Biología y Medicina Experimental (IBYME)-CONICET, CABA, Argentina, [2]Universidad Nacional de Luján, Luján, Argentina, [3]Instituto de Oncología Ángel H. Roffo, CABA, Argentina, [4]Instituto Héritas, Rosario, Argentina. *First authorship.*

Some microbes are known to be damaging to human health. Breast microbiome composition are poorly understood. In order to study the prognostic relevance of the microbiome in the evolution of early breast cancer, a study was carried out including samples of frozen breast tumor tissues from women with invasive ductal breast carcinoma, clinicopathological stages I/II with a 5-year follow-up minimum (*Hospital Roffo*, n=22). Samples were processed and DNA was extracted using the QIAamp-DNA-Mini-Kit. The bacterial profile was identified studying the region of the 16S rRNA gene using the Illumina Miseq platform. OTUs classification and Alpha and Beta diversity analysis were performed to study their possible associations with the classic parameters of breast cancer and tumoral progression. In this way, taxonomic analysis of these samples indicates that *Proteobacteria* (44.20%) and *Firmicutes* (23.22%) are the most representative phylum followed by *Actinobacteriota* (11.00%) and *Bacteroidota* (9.44%). Difference in microbial abundances (evenness index) has been found between patients with positive sentinel node and negative sentinel node ($p=0.05$) and patients with positive progesterone receptor (PR+) and (PR-) $p=0.02$. Moreover, diversity and richness metabolic pathways were found between patients PR(+) and PR(-) (Shannon index $p=0.03$) and Ki67 marker (Chao1 index=0.02).To conclude, our results provided insight into the possible relationship between difference abundance of certain bacteria, as well as difference richness and diversity of metabolic pathways with the classical prognostic parameters: sentinel node, PR, and ki67. These results open up new paths to study the relevance of the host microbiome with the progression of early breast cancer.

ONCOLOGY VIII *Saturday, November 19, 14-15:30 hr*

Chairs: Hernán Farina - Laura Todaro - Andrea Loaiza Pérez - Patricia Pennisi

603. (17) **PTHrP/MET AXIS IN THE AGGRESSIVE BEHAVIOR OF COLORECTAL CANCER CELLS**

María Belén Novoa Díaz[1], Cintia Birkenstok[2], Pedro Carriere[1], Luis Gomez[2,3,4], Graciela Gigola[1], Ariel Zwenger[5], Natalia Calvo[1], Claudia Gentili[1]

1 Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)- INBIOSUR (CONICET-UNS), Bahía Blanca, Argentina, 2 Departamento de Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina, 3 Departamento de Ciencias de la Salud, UNS, Bahía Blanca, Argentina, 4 Ente Descentralizado Hospital Integrado de la Región Sanitaria 1, Bahía Blanca, Argentina, 5 Centro de Estudios Clínicos SAGA, CEC SAGA, Santiago de Chile, Chile

Met receptor is involved in the progression of colorectal cancer (CRC). Parathyroid hormone-related peptide (PTHrP) is a cytokine from the tumor and its microenvironment associated with the aggressiveness of different types of cancer. Previously, we found in HCT116 cells from CRC that the binding of PTHrP to its receptor (PTHR1) favors chemoresistance to drugs employed in CRC treatment and other events associated with an aggressive phenotype. PTHrP diminished the sensitivity of these drugs through Met. In HCT116 cells xenografts, PTHrP modulates markers expression linked to tumor progression including Met. The aim of this work is to further investigate the relationship between PTHR1, PTHrP and Met in CRC models. Using SU11274, the Met specific inhibitor, and the following techniques: western blot, wound healing assay and monitoring morphological changes we observed the reversal of cell migration ($p<0.05$) and the epithelial-mesenchymal transition (EMT) program ($p<0.01$) induced by PTHrP in HCT116 cells. Also, the effects of the cytokine on the expression of E-cadherin and Snail (both EMT markers) were reverted when the cells were pre-incubated with SU11274. These findings strongly suggest that Met activated by PTHrP participates in events associated to the CRC aggressive phenotype. Interestingly, we found *in vivo* and by immunohistochemical (IHQ) analysis that PTHrP not only enhances Met expression but also its own receptor ($p<0.01$). Finally, by IHQ, we proceeded to perform an observational analysis of human samples to validate the findings obtained by *in vitro* and *in vivo* assays. No correlation was found between the expressions of both receptors (Met and PTHR1) in the tumor samples. However, we found with statistical significance that in less differentiated tumors, Met expression increased ($P = 0.035$), while PTHR1 expression was lower ($P = 0.0496$). In conclusion, we consider that PTHrP/Met axis could have a positive impact on the knowledge of CRC biology.

604. (49) **MIGRATION AND INVASION AS CAPACITATING ABILITES FOR OSTEOSARCOMA METASTATIC SUCCESS: PARENCHYMA AND STROMA INTERTWINED**

Matías Valenzuela Alvarez[1], Luciana M. Gutierrez[1], Juan Bayo[2], María José Cantero[2], Mariana Garcia[2], Marcela F. Bolontrade[1]

1Remodeling Processes and cellular niches laboratory. Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) - CONICET - Hospital Italiano Buenos Aires (HIBA) - Instituto Universitario del Hospital Italiano (IUHI), Potosí 4240, CABA

(C1199ACL), Argentina; 2IIMT – CONICET, Facultad de Ciencias Biomédicas, Universidad Austral, Av. Perón 1500, Pilar (EP B1629AHJ), Argentina.

Migration and invasion are the main tumor attributes that allow to distinguish between primary and metastatic tumors. Osteosarcoma (OS) is the most prevalent bone tumor, mostly affecting pediatric patients. One of its main clinical challenges is rapid metastatic spread, raising the importance of highlighting the cancer hallmarks: migration and invasion. In order to assess the tumor niche contribution to migration, we carried out assays with mesenchymal stem cells (MSC) and microvascular endothelial cells responding to primary and metastases OS cell lines-conditioned media (CM), demonstrating that both stromal cells migrated further towards the primary OS secretome. Afterwards we analyzed OS cell lines SAOS2 and LM7 migration to MSC-CM, resulting in LM7 migrating significantly more to MSC secretome. With these results in mind, we iv. inoculated OS cells into athymic mice, removed the lungs, obtained lungs-CM and challenged MSC to these CM. Surprisingly, MSC migrated significantly more to CMs from the lungs with LM7 cells. Since this difference could be attributed in part to the complexity added by the in vivo lung microenvironment scenario, we analyzed the dataset GSE14359. Of interest, S100A14 and PECAM1 showed significantly higher expression in metastatic samples compared to the primary counterpart, while CXCR4 and IL-1α showed a similar trend. We also demonstrated a homing of MSC in OS lung metastases. Tissue remodeling is fundamental to allow migration from the primary tumor. We analyzed MMP-2/9 expression and activity, demonstrating presence of pro and active MMP-2 and absence of MMP-9. Of relevance, a higher MMP-2 expression is associated with a worse overall survival time in OS patients. Among other members involved in this biological process, cathepsin D was upregulated in metastatic OS cells. Lung disease remains a major OS death cause. Identification of differentially expressed genes would uncover promising markers and therapeutic approaches for OS spread.

605. (110) **NOVEL METHYLATION-BASED BIOMARKERS FOR COLORECTAL CANCER DETECTION**

Cintia C Vaquer [1], Clara García Samartino [1,2], Maria V Bocanegra [1,2,3], Sebastián Arbona [1,4], Rodrigo D Militello [1,2,3], Rodrigo L Ongay [5], Paula Valdemoros [2], Guillermo Sanguinetti [6], Agustín Correa [6], Pablo A Pellegrini [6], Walter R Minatti [7], Emanuel M Campoy [1,2,3].

1 epiliquid S.A.S. 2 Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. 3 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 4 Facultad de Ingeniería, Universidad Nacional de Cuyo. 5 Centro de Diagnóstico y Tratamiento Gastroenterológico y Hepatológico, HIGEA. 6 Unidad de Cirugía Colorrectal, Hospital Español de Mendoza. 7 Servicio de Coloproctología, Hospital Italiano de Mendoza.

Epigenetic marks refer to chemical DNA modifications that do not affect the DNA sequence but can modify gene expression. They are described early in the tumorigenic process and can be used as biomarkers, for example, for early cancer detection. We developed a bioinformatic algorithm that identifies and hierarchies sensitive and specific epigenetic biomarkers. Currently, we are developing a PCR-based technology capable of detecting biomarkers with high sensitivity, either individually or combined in the same reaction. Objective: Identify and detect methylation-based biomarkers in DNA extracted from tumors derived from colorectal cancer (CRC) patients. Methods: We developed a bioinformatic platform capable of implementing an algorithm that identifies methylation-based biomarkers from public databases (TCGA). We constituted an average risk CRC patients prospective cohort, who attended for video colonoscopy and/or CRC resection surgery. We isolated DNA from the tumor and paired colonic normal tissue distant from the lesion. We used a PCR-based technology to detect a well know epigenetic biomarker (SEPT9) for CRC screening and our proprietary biomarkers. Results: initially, we validated the bioinformatic platform since it accurately identified already described biomarkers and we also identified our own panel of new biomarkers. Applying our own technology, we detected SEPT9 methylation in DNA tumor tissue patients from 20 CCR patients. We applied a ROC analysis obtaining an Area Under the Curve (AUC) of 0.98, which means a high sensitive and specific detection. As controls, we used paired colonic normal tissue. Finally, we chose the best ranked biomarkers identified by the bioinformatic platform that were detected in the same samples, either individually or combined as a biomarker panel in the same PCR reaction (AUC=0.995). Conclusion: We identified and detected a panel of new methylation-based biomarkers that might be implemented in colorectal cancer screening.

606. (191) **DIRECT ACTION OF INTESTINAL MICROORGANISMS IN COLORECTAL CANCER CELL LINE**

Juliana Lourdes Bernacchia[1], Alejandra Graciela Palma[1], María Cecilia Lira[1], Francisco Damián Rosa[1], Adriana De Paulis[2], Oscar Laudanno[3], Micaela Lleyda[4], Susana Nowicki[5], Mónica Vázquez-Levin[4], María Fernanda Rubio[1], Mónica Alejandra Costas[1].

1Laboratorio de Biología Molecular y Apoptosis, IDIM-UBA-CONICET, 2Unidad de Gastroenterología, Instituto de Investigaciones Médicas A. Lanari, Facultad de Medicina, UBA, 3Unidad de Microbiología, Instituto de Investigaciones Médicas A. Lanari , Facultad de Medicina, UBA, 4Instituto de Biología y Medicina Experimental (IBYME), CONICET, 5Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE)-CONICET-División de Endocrinología, Hospital de Niños Ricardo Gutiérrez.

Numerous evidence demonstrate the importance of the microbiome mainly due to its immunological action, in the development of multiple diseases, such as colorectal cancer (CRC). In agreement with previous works, bacteria such as *Escherichia coli* (*E.coli*) and *Bacteroides fragilis*, among others, have high prevalence in CRC patients compared to the normal population. In this work we investigate the possible pathways and signaling that may contribute to CRC development that could be affected by direct action of bacteria over colorectal cancer cells, in the absence of additional signals from the immune system. Therefore, we first determined the total amount of genes that were up or down regulated (log FC >1 or <-1), as determined by RNAseq, in experiments where the CRC cell line DLD-1 was infected with the bacteria *E.coli* for 2 h or *Enterotoxigenic Bacteroides fragilis* (ETBF) for 2 to 24 h at MOI: 500 (database GSE130152). Then, we performed an over-representation analysis using the ConsensusPathDB bioinformatic tool. We found that ETBF upregulates 525 genes involved in processes like cell migration, inflammatory response, and the FOXO-mediated transcription of cell cycle genes (at least $p < 0.05$). While *E.coli* upregulates 2249 genes, most of them involved in TNF, NF-kB, MAPK, and inflammatory signaling pathways (at least $p < 0.05$). Interestingly, 231 upregulated and 414 downregulated genes are shared with ETBF as determined by Venn diagrams. Then to confirm the previous suggested incidence of these bacteria in CRC, we analyzed the presence of both, using LDA score (linear discriminant analysis) in colorectal neoplasms from GMrepo database of human gut metagenomes and found that they have LDA>4, thus, strongly involved in CRC.
We conclude, there are signals and pathways independent of the immune system that could be induced directly by bacteria in the epithelial colorectal cells, contributing to CRC.

607. (301) **TOWARDS A BRAND NEW WAY TU UNDERSTAND KIDNEY CANCER: AN UNSUPERVISED MACHINE LEARNING APPROACH**

Leonardo Rafael Romeo1, 2, Matías Nuñez3, Matías Ferrando1, Constanza Matilde López-Fontana1, Rubén Walter Carón1, Flavia Alejandra Bruna1, Virginia Pistone-Creydt1, 4.

1IMBECU – CONICET, CCT Mendoza, Argentina, 2Departamento de Urología y Trasplante Renal, Hospital Español de Mendoza, Argentina, 3Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Materiales Nucleares-Centro Atómico Bariloche, CONICET, Argentina, 4Universidad Nacional de Cuyo, Facultad de Ciencias Médicas Mendoza, Argentina.

Among the different types of cells that surround renal epithelial cells, renal adipose tissue (AT) is one of the most abundant. We demonstrated that human renal adipose tissue from patients with renal tumors (hRAT) regulates the behavior of epithelial cells differently from normal renal adipose tissue (hRAN), through the proteins expression characterization in hRAT *vs* hRAN. In this work, we evaluated: 1) the differential proteins expression as a whole was sufficient to separate healthy patients from patients with kidney cancer, using unsupervised machine learning algorithms (UMLA); 2) the correlation between adiponectin and leptin expression with clinical characteristics of kidney cancer patients. The biological variables evaluated in Hrat (n=21) and hRAN (n=24) were: adiponectin, AdipoR, leptin, ObR, perilipin and ADAMTS1. The proteins expression by the different ATs were analyzed with UMLA algorithms (t-SNE and UMAP). We selected leptin and adiponectin to study the correlation with clinical characteristics of patients with kidney tumors (sex, age, BMI, smoking, tumor grade, size of the lesion, density of AT, and difficulty in the surgical dissection). The SPSS program was used to statystical analysis, taking a significant $p < 0.05$. Considering the total of biological variables evaluated in the different AT fragments, we were able to separate healthy from kidney tumor patients by UMLA projection. A decrease in adiponectin expression was found in patients with a more undifferentiated tumor related with smoking habits ($p<0.05$). Also, there was a positive correlation between leptin, tumor size and difficulty in tumor dissection ($p<0.05$). The parameters that increase the difficulty in dissection are male sex, smoking history, tumor size and the fat striation degree in imaging studies ($p<0.05$). The study of perirenal AT could be useful to both, the prognosis as well as find new therapeutical strategies to control this disease.

608. (331) **COLORECTAL CANCER SCREENING IN BAHÍA BLANCA: A QUALITATIVE-QUANTITATIVE STUDY OF BARRIERS AND FACILITATORS.**

Yamila Schenfeld[1], Joel Schernenco[1], Iñaki Dopazo[1], Manuel Robles[1], Belén Bellando[1], Gonzalo Picardi[1], Lucia Albanesi[1], María del Carmen Esandi[1,2], María Ernestina Reig[3], María Eugenia Esandi[4], Ariana Bruzzone[2]

[1]Departamento de Ciencias de la Salud-UNS, [2]Instituto de Investigaciones Bioquímicas Bahía Blanca- CONICET,[3]Secretaría de Salud. Municipio de Bahía Blanca, [4]Departamento de Economía-UNS

Colorectal cancer (CRC) is a major public health challenge all over the world. There is robust evidence showing that screening with fecal occult blood test (FOBT) reduces CRC- incidence and mortality. However, implementation of these programs faces many difficulties and fails to achieve expected coverage rates due to multiple barriers. Even though, in Argentina, CRC is the second type of cancer in incidence and mortality, its screening remains suboptimal. The aim of this research is to describe access rates to opportunistic CRC screening at a programmatic area (AP2) of the local health system in Bahia Blanca city between 2019 and 2021. It also explores factors that hinder or facilitate this access from the perspective of key actors working at the municipal health system. A hybrid-design, combining quantitative and qualitative techniques, was employed. CRC screening access rates were estimated as the proportion of FOBT performed at AP2 primary health care centers (PHCC) per year upon targeted population. Qualitative assessment was performed combining two theoretical models: the Meta-framework of Implementation Research and the Theoretical Domains Framework of Behavior Change. Thirty five in-depth interviews were accomplished. The accumulative rate of FOBT in the studied period was ~ 4,6%, a low coverage value considering the national programme expectations (40% in 4 years). Barriers most frequently mentioned were: 1. poor access to more complex studies (i.e colonoscopy) of positive FOBT individuals; 2. Lack of population awareness about CRC. Low adherence of the health care team to the screening recommendations as well as poor formal medical training in this topic were also highlighted. Sense of belonging of the population to PHCCs and a positive implementation climate to incorporate screening as a routine practice emerged as facilitators. This work, in addition to relevate the CRC screening in this area, will contribute to the design of strategies to reinforce it.

609. (333) **RSUME IS AN ADVERSE PROGNOSTIC FACTOR IN RENAL CELL CARCINOMA: INVOLVEMENT OF ROS AND CILIA RELATED PATHWAYS**

David Gonilski-Pacin[1], Nicolas Ciancio del Giudice[1], Belen Elguero[1], Eduardo Arzt[1,2]

[1]Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society

[2]Departamento de Fisiología y Biología Molecular y Celular, FCEN, UBA.

RSUME or RWDD3 is involved in tumor angiogenesis, is upregulated by hypoxia and acts as a negative regulator of VHL protein function in normoxia promoting Hypoxia-inducible factor alpha (HIF-α) stabilization. We evaluated RSUME prognostic value in clear cell Renal Cell Carcinoma (ccRCC) based mainly on the dataset (KIRC) from patients in The Cancer Genome Atlas (TCGA). Wilcoxon signed-rank test and One-way analysis of variance (ANOVA) followed by Tukey's test were used to evaluate relationships between clinical-pathologic features and RSUME expression. Univariate and multivariate Cox regression analysis methods were used to evaluate factors contributing to prognosis. The biological function of RSUME was assessed by gene set enrichment analysis (GSEA) and experimentally validated in RCC-786-O cell silenced for RSUME cell lines. High RSUME expression is associated with poor patients' outcome. It is highly expressed in tumor tissues compared with normal tissues ($P = 0.006$, $P = 0.039$, $P = 0.002$, $P = 0.036$, $P < 0.001$) and associates with tumor T ($P = 0.014$) and tumor M ($P = 0.036$) advanced stages and higher extent cysts ($P = 0.005$). Kaplan-Meier curves and log-rank tests showed that high RSUME expression is associated with a short Overall Survival (OS) ($P < 0.001$) and poor Disease-Free Survival (DFS) ($P = 0.021$). GSEA showed the enrichment of several pathways, among them Glycerophospholipid metabolism, Cilium and ROS related pathways in RSUME high-expression phenotype. RSUME association with ROS and Cilium pathways were assess in RCC-786-O cell silenced for RSUME cell lines. By using dichlorofluorescin diacetate (DCFDA) we observed RSUME silencing diminishes ROS levels and also diminishes AURKA, OFD1 and NPHP3, cilia related genes, validating our *in silico* results.
In summary, RSUME high expression predicts poor prognosis in ccRCC and may impact through its action on metabolism, ROS and cilium related pathways. Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11).

610. (417) **FABP1 REGULATES GENE EXPRESSION IN COLORECTAL CANCER CELLS**

Borús Delfina Lucía1, Zadra Giorgia2, Minsky Daniel3, Rodenak Kladniew Boris Emilio1, Corsico Betina1, Scaglia Natalia1.

1 INIBIOLP, UNLP-CONICET, 2 Institute of Molecular Genetics, Italy, 3 Laboratorio Tandar, CNEA.

Correspondance: nscaglia@med.unlp.edu.ar

Fatty acid binding proteins (FABPs) are small cytosolic proteins that reversibly bind fatty acids (FA) and other lipophilic compounds. In humans, ten isoforms have been reported with both overlapping and distinct expression patterns, suggesting differential functions. Two FABPs isoforms are abundant in intestinal epithelial cells: the liver FABP (FABP1) and the intestinal FABP (FABP2). They are thought to be associated with intracellular dietary lipid transport and trafficking towards diverse cellular fates. Their specific functions are, however, still poorly understood. Given the importance of dietary fats in colorectal cancer (CRC) development, we analyzed the role of FABP1 in CRC lipid metabolism. To this end, we used Caco-2 cells, a well-established model of human CRC, to generate a stable FABP1 *knockdown* model using an antisense strategy (named Caco2-FABP1as cells). We have previously shown that FABP1 *knockdown* resulted in extensive changes in lipid metabolism, including a diminished incorporation of exogenous FA into complex lipids, particularly in the phospholipids class. Accordingly, FABP1as cells exhibited a dramatic decrease in proliferation rate. Here we show

that Caco2-FABP1as cells have decreased *de novo* FA synthesis ($p<0.05$), linking this protein with the modulation of endogenous FA metabolism as well. Consistently, FABP1 *knockdown* reduced the level of enzymes related to FA synthesis, suggesting that FABP1 may affect lipid metabolism through the regulation of gene expression. To gain further insights into this aspect, we performed RNA-Seq in Caco2-FABP1as cells and mock transfected control cells. We identified profound changes in Caco-2 cells transcriptome following FABP1 *knockdown* that have not previously reported, setting the stage for an in-depth investigation of FABP1-mediated lipid metabolism rewiring. Although preliminary, our results suggest that FABP1 represents a key transcriptional and metabolic regulator in CRC cells.

611. (435) **ANALYSIS OF RAC1 EXPRESSION IN COLORECTAL CANCER CLINICAL SAMPLES ACCORDING TO MSI/MSS STATUS AND IN VITRO SENSITIVITY OF MSS CELL LINES TO 1A116 RAC1 INHIBITOR**

Jesús Lemos[1], Paula Bucci[1], Florencia Gottardo[1,2], Valeria Segatori[1,2], Daniel E. Gomez[1,2], Daniel F. Alonso[1,2] and Georgina A. Cardama[1,2]

1- Centro de Oncología Molecular y Traslacional (COMTra), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes

2- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Rac1 GTPase has a critical role in the progression of colorectal cancer (CRC), regulating processes related to growth, chemoresistance and immunomodulation. Despite the fact that immune checkpoint inhibitor-based immunotherapy has revolutionized CRC management, only a small subgroup of patients bearing tumors with microsatellite instability (MSI) benefit from this approach. The aim of the present work was to first study RAC1 target expression in microsatellite stable (MSS) or MSI CRC samples using bioinformatics, and second to explore the antitumoral effects of 1A116, a selective RAC1 inhibitor previously developed by our research group, on different murine and human MSS CRC cells. We used the TCGA database and the Gene Expression Profiling Interactive Analysis II (GEPIA2) platform in order to assess differential expression of RAC1 in normal adjacent and CRC tissue, and then evaluate target expression according to MSS, MSI-low and MSI-high tumor status. After confirming that the target of interest is upregulated in CRC versus normal tissue, we observed that RAC1 expression is significantly higher in MSI-low and especially MSS CRC, than in MSI-high tumor implying RAC1 as a possible therapeutic target in patients MSS/MSI-low tumors. In vitro, 1A116 (5-50 μM) impaired tumor cell growth on highly aggressive murine CT-26 and human HT-29 MSS CRC cells. Furthermore, cytostatic activity of the compound was assessed on 3D spheroid growth using the hanging drop method. After long-term exposure to 10 and 25 μM concentrations of 1A116 a significant arrest of CRC spheroid growth was obtained in both treatments. In addition, after exploring 1A116 impact on expression/secretion of cytokines, we observed that the compound seems to modulate key immune and inflammatory mediators. These results lay the groundwork for further preclinical exploration of 1A116 RAC1 inhibitor as a potential therapeutic tool to increase response to immunotherapies in aggressive and refractory MSS CRC.

612. (492) **CHARACTERIZATION OF ADRB2-MEDIATED ANTITUMORAL EFFECTS AND MECHANISMS OF ACTION OF β-BLOCKER PROPRANOLOL IN OSTEOSARCOMA**

Solernó, Luisina M.[1-2]; Sobol, Natasha T.[1-2]; Ferrero, Maximiliano R.[4]; Llavona, Candela[1-2] Capobianco, Carla S.[1]; Bruzzone, Ariana[5]; Gottardo, M. Florencia[1-2]; Garona, Juan[1-2-3].

1. Centro de Medicina Traslacional (Unidad 6), Hospital de Alta Complejidad en Red El Cruce "Dr. Néstor Carlos Kirchner" S.A.M.I.C.

2. Centro de Oncología Molecular y Traslacional (COMTra), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes.

3. Consejo Nacional de Investigaciones Científicas (CONICET), Argentina.

4. Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA), CONICET-Sociedad Max Planck Alemania.

5. Instituto de Investigaciones Bioquímicas de Bahía Blanca, CONICET-Universidad Nacional del Sur.

Osteosarcoma (OSA) is still associated with limited response to standard-of-care therapy and alarmingly elevated mortality rates. Our group recently reported for the first time that PPN, a repurposed β1/2-adrenergic receptor (ADRB1/2) antagonist, was capable of reducing tumor-associated angiogenesis and xenograft aggressiveness using different OSA preclinical models. The objective of this work was to characterize PPN ADRB2-mediated effects and mechanisms of action on OSA growth, migration and response to chemotherapy. After confirming ADRB2 expression by RT-qPCR in MG-63 and U-2OS OSA cells, pro-mitogenic effects of ADRB agonists epinephrine and norepinephrine were associated with downstream activation of MAPK-associated signaling pathways, as evaluated by western blotting. ADRB2 knockdown by transfection with ADRB2-targeting siRNA reduced in vitro aggressiveness of OSA cells and impaired PPN cytostatic activity, confirming target specificity of the drug. As evaluated by flow cytometry, a significant arrest in the G_0/G_1 cell cycle phase of MG-63 and U-2OS cells was observed after 24 h treatment with PPN (50 μM), which was associated with a significant reduction in CCND1 gene expression, a key cell cycle regulator. OSA growth inhibition was not associated with apoptosis induction. β-blockade with PPN inhibited OSA cell chemotaxis, vasculogenic mimicry and capillary-like tube formation on Matrigel® coated substrates. Migration inhibition was linked to blockade of EGF-induced actin reorganization and stress fiber formation. After histological analysis, *in vivo* therapeutic benefits after addition of PPN (10 mg/kg i.p.) to cisplatin-based metronomic chemotherapy (2 mg/kg i.p.) correlated with reduced tumor mitotic index and increased necrosis. All results were significant at $p<0.05$ (t test or ANOVA, GraphPad Prism). We propose PPN as a potential cost-effective co-adjuvant therapy for OSA management. Further translational studies on metastatic disease are in progress.

613. (493) **IMPACT OF DESMOPRESSIN TREATMENT ON LYMPHOCYTE INFILTRATION AND LUNG PRE-METASTATIC NICHE FORMATION BY COLORECTAL CANCER CELLS**

Natasha Tatiana Sobol[1;2;3], Luisina María Solernó[1;2;3], Candela Llavona[1;3], María Florencia Gottardo[1;2;3], Valeria Inés Segatori[1;2;3], Daniel Fernando Alonso[1;2;3], Juan Garona[1;2;3]

[1]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes, [2]CONICET, [3]Centro de Medicina Traslacional (Unidad 6), Hospital de Alta Complejidad "El Cruce"

Metastatic colorectal cancer (mCRC) still stands as a therapeutic challenge in which tumor cell spread to liver and lungs accounts for most of CRC-related mortality. Desmopressin (dDAVP) is a synthetic vasopressin analog and repurposed hemostatic drug in oncology, with reported antimetastatic and angiostatic action in aggressive tumors. Given that the metastatic niche and stroma-tumor cell interactions are crucial steps during the establishment of metastases, our aim was to explore the impact of dDAVP treatment on the pre-metastatic niche formation and the modulation of lymphocyte infiltration in the lung, the second most common site of metastasis in CRC. Firstly, we characterized the kinetics (7, 15 and 21 days) of lung metastases development in syngeneic Balb/c mice after i.v. inoculation of CT-26 CRC cells, confirming massive tissue colonization and more than 30 macronodules/lung (>1mm) at day 21. Furthermore, to asses the impact of CRC cell-secreted factors on the establishment and growth of metastatic cells we injected CT-26 conditioned medium in Balb/c mice prior to tumor cell inoculation for 4 consecutive days, which led to a 3-fold increase in the number of lung metastasis. Interestingly, this phenomenon was completely reverted after dDAVP co-administration at clinically relevant doses (1 μg/kg i.v.). We then studied the impact of dDAVP on the recruitment of inflammatory cells to the lung during the early steps of metastatic colonization in immunocompetent mice by flow cytometry, where i.v. treatment with the peptide significantly increased by 3 times the CD8+ cell population infiltrating the tissue. No significant changes were observed in the CD4+ population nor in the circulating CD4+ or CD8+T

cells (p<0.05, T-test or ANOVA, GraphPad Prism). In addition to its direct cytostatic action, dDAVP antimetastatic activity seems to be mediated by multiple stromal actors involved in the metastatic niche, including lymphocytes and vascular cells.

614. (508) **ANTIPARASITIC AGENT IVERMECTIN SYNERGIZES WITH IMMUNE-CHECKPOINT INHIBITORS IN A REFRACTORY COLORECTAL CANCER METASTATIC MODEL**

Natasha Tatiana Sobol[1,2,3], Luisina María Solernó[1,2,3], Gisele Martinez[1], Candela Llavona[1,3], María Florencia Gottardo[1,2,3], Valeria Inés Segatori[1,2,3], Daniel Fernando Alonso[1,2,3], Juan Garona[1,2,3]

[1]Centro de Medicina Traslacional (Unidad 6), Hospital de Alta Complejidad "El Cruce"; [2]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes; [3]CONICET

Immunotherapies based on immune checkpoint inhibitors (ICIs) have revolutionized colorectal cancer (CRC) management. Unfortunately, therapeutic benefits are limited to patients with DNA mismatch repair (MMR) deficiency. In this regard, the repurposed antiparasitic drug ivermectin (IVM) has been proposed as a co-adjuvant agent in oncology, considering its potential impact on immunogenic cell death (ICD) induction and reversal of drug resistance. Our aim was to explore IVM antineoplastic activity, particularly in combination with αPD-1 based ICI, using the highly metastatic CT-26 model, a murine $KRAS^{mut}$ and MMR-proficient CRC cell line with limited response to immunotherapy and cytotoxic agents. *In vitro*, CT-26 showed a high sensitivity to IVM, obtaining an IC_{50} of 10.9 μM after a 72h-exposure. Treatment with low concentrations of IVM was also associated with a reduction in tumor chemotaxis and colony-formation ability, impaired tumor cell metabolism and an increased secretion of proinflammatory and immunostimulatory molecules, such as TNF-α and IL-6. *In vivo*, immunocompetent syngeneic mice were injected i.v. with CT-26 cells and, after confirming metastatic colonization in the lung by histological studies at day 7, they were subjected to different treatments; Saline vehicle (control group), IVM (10 mg/kg i.p.), αPD-1 (5 mg/kg i.p.) and IVM plus αPD-1. Although IVM and αPD-1 monotherapies reduced the metastatic burden by 85% and 63%, respectively, the combined therapy completely inhibited the formation of metastatic macronodules (>2mm) in the lung. S.c. preimmunization using CT-26 cells treated ex vivo (24h) with a high cytotoxic IVM concentration one week prior to i.v. re-challenge with viable cells led to full protection against CRC lung metastases, postulating ICD as a possible mechanism of action (p<0.05, T test or ANOVA, Graphpad Prism). Repurposed agent IVM seems to increase immunoreactivity and therapeutic response to ICIs in "cold" experimental CRC tumors.

615. (526) **ANALYSIS OF DNA MISMATCH REPAIR SYSTEM STATUS IN COLORECTAL CANCER: AN EXPLORATORY CLINICAL-PATHOLOGICAL STUDY IN AN ARGENTINE COHORT**

Karina Yonamine[1,2], Cecilia Curvale[1,2], Julieta Saenz[1,3], Esteban Vogel[1,3], Raul Matanó[2], Viviana Tassi[3], Natasha Tatiana Sobol[1,4,5], Luisina María Solernó[1,4,5], Candela LLavona[1,4], Maria Florencia Gottardo[1,4,5], Valeria Ines Segatori[1,4,5], Daniel Fernando Alonso[4,5], Juan Garona[1,4,5].

[1]Centro de Medicina Traslacional (Unidad 6), Hospital de Alta Complejidad "El Cruce", [2]Servicio de Gastroenterología; Hospital de Alta Complejidad "El Cruce", [3]Servicio de Anotomía Patológica; Hospital de Alta Complejidad "El Cruce", [4]Centro de Oncología Molecular y Traslacional, Universidad Nacional de Quilmes ,[5]CONICET.

In Argentina, colorectal cancer (CRC) represents a serious public health problem, ranking second in both incidence and mortality. Evaluation of DNA mismatch repair system (MMR) status is highly relevant in CRC due to its prognostic and predictive impact. Regardless of its clinical utility in CRC, implementation of deficient MMR (dMMR) status assessment in our health system has been partial, and its prevalence in our hospital cohorts is unknown. Our aim was to determine the prevalence of MMR deficiency (dMMR) in CRC samples from the Hospital "El Cruce" (HEC) and to integrate the obtained results with other available clinical-pathological data in a customized digital registry based on the "Research Electronic Data Capture" web platform (RedCap). Presence of MMR proteins (MLH1, PMS2, MSH2 and MSH6) was assessed by immunohistochemistry in an ambispective study on FFPE CRC samples obtained from endoscopic biopsies or surgical specimens. As a result, after assessing immunoreactivity for all 4 MMR-related enzymes in 50 clinical cases we observed a 14% prevalence of CRC tumors with dMMR status. The percentage distribution of dMMR type according to affected enzyme/s was: dMMR type 1 ($MLH1^-$ and $PMS2^-$) 43%; type 2 ($PMS2^-$) 29%; type 3 ($MSH2^-$ and $MSH6^-$) 14%; type 4 ($MSH6^-$) 0%; and other types of less prevalent dMMR tumors (in this case $MLH1^-$) 14%. At the descriptive level, for dMMR CRC patients, stage III disease (43%) was the most common diagnosis, and distal colon (71.4%), particularly sigmoid colon (28,6%), was the most prevalent tumor location. Interestingly, poorly differentiated adenocarcinomas were more commonly found in the dMMR group (dMMR 28.6 *versus* pMMR 6.9%). Patient age, sex, chemotherapy regime, disease recurrence, biomarkers, among other clinical-pathological variables, were also integrated in RedCap. MMR status testing could lead to better therapeutic management and risk stratification and should be included in the diagnostic work-up of all CRC cases.

616. (783) **CELLS DERIVED FROM CLEAR RENAL CELL CARCINOMA CAN SURVIVE BETTER THAN CELLS DERIVED FROM RENAL PROXIMAL EPITHELIA TO EXTRACELLULAR ACIDOSIS**

Victor Van Balen, Ana Mechali, Martin Nahuel Amabile, Natalia Beltramone, Claudia Capurro, Gisela Di Giusto, Paula Ford, Valeria Rivarola.

Laboratorio de Biomembranas, IFIBIO Houssay UBA-CONICET.

The association between cell death and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancers, pHe may affect in a different way cancer and normal cells. This study aimed to investigate whether exposure to acidic media altered cell survival in two renal cell models: HK2, derived from normal human proximal epithelial cells, and 786-O, derived from human renal clear cell carcinoma (RCC). We evaluate the effect of acidosis at different exposure times (24-72h) and different HCl concentrations. The percentage of cell death was evaluated with acridine orange–ethidium bromide dyeing followed by fluorescent microscopy. Our results showed that while 786-O cell survival was not affected by up to 72h of 9.6mM HCl exposure (%Cell death, control: 8.1 ± 1.0 vs. 72h Acid: 7.8 ± 1.7, ns n=60), HK2 cell survival decays with even 24hs of this acidification (%Cell death, control: 8.1 ± 0.8 vs. 24h Acid: 24.9 ± 4.4, p< 0.01 n=70). While 786-O cells were even able to resist 72h exposure to 19.2 mM HCl, HK2 cells were not able to survive that acidification (%Cell death with 72h 19.2 mM HCl, HK2= 80.0 ± 2.0, p< 0.001 n=5 vs. control HK2 cells; 786-O = 9.0 ± 1.0 ns n=5 vs. control 786-O cells). These results show that RCC cells adapt to an acidic environment. To test the hypothesis that the NHE1 isoform of the Na+/H+ exchanger could be involved in this acidic adaptation, we inhibited NHE1. NHE1 inhibition (-NHE) rise 786-O cell death (%Cell death after 24h with 9.6mM HCl, +NHE1: 8.0 ± 1.2 vs. -NHE1: 13.8 ± 2.5, p< 0.05 n=15). In conclusion, RCC cell adaptation to acidosis depends on the NHE1 function. Further studies are needed to assess the mechanism of this adaptation.

617. (842) ***ALOYSIA POLYSTACHYA*** **ANTI-CANCER ACTION IN COLORECTAL CANCER**

Alejandra Graciela Palma1, Juliana Bernacchia1, Mileni Soares Machado1, Paola Di Leo Lira2, Valeria Moscatelli2, María Cecilia Lira1, Francisco Damián Rosa1, Iara Castellanos1, María Florencia Quintanilla1, María Fernanda Rubio1, Daiana Retta2, Mónica Alejandra Costas1

1Instituto de Investigaciones Médicas Dr. Alfredo Lanari (UBA-CONICET), Ciudad Autónoma de Buenos Aires, Argentina, 2Cátedra de Farmacognosia-IQUIMEFA, Facultad de Farmacia y Bioquímica (UBA-CONICET), Ciudad Autóno-

ma de Buenos Aires, Argentina

Previously we have shown that extracts from the plant *Aloysia polystachya* (AP) have a cytotoxic action in colorectal cancer cells but its lower in non-tumoral cells. The coactivator of nuclear receptors RAC3 is overexpressed in several types of cancer, like colorectal cancer, but its expression is low in non-tumoral cells. This molecule is co-activator of the Aryl Hydrocarbon Receptor (AHR), a modulator of intestinal balance, whose expression is increased in tumors. The aim of this work was to investigate the molecular mechanisms of AP cytotoxic action and its possible components involved with this effect in colorectal cancer. Since RAC3 expression and AP cytotoxicity are low in non-tumoral cells, and RAC3 is coactivator of AHR and NF-κB, we first decided to analyze if these molecules were involved in AP effect. When colorectal cancer HCT116 cells were incubated with AP extract we found by immunofluorescence assay that the nuclear translocation of these molecules were increased and that AHR and RAC3 co-localized in these cells. Then, we compared the biological activity of two AP plants grown in different parts of Argentina and found that both were capable to induce cytotoxicity in HCT116 cells (1:2 dilution % cytotoxicity: Buenos Aires 46%, $p<0.05$; Misiones 53%, $p<0.01$). We also performed cytotoxicity assays of AP essential oil, which is more pure and concentrated than the extract, and observed that it had a cytotoxic action in these cells, maintaining the biological activity of the extract (98% cytotoxicity with 1:2 dilution, $p<0.05$). Finally, we determined the cytotoxic activity of different fractions of AP extract and found that both hydrophobic and hydrophilic components of AP had an anti-tumor activity in HCT116 cells. From these results we conclude that AP extract cytotoxic effect over colorectal cancer cells could involve AHR and RAC3 and could be through the combination of hydrophilic and hydrophobic components.

618. (871) **ALTERATIONS IN EPITHELIAL CADHERIN AND FXYD5/DYSADHERIN EXPRESSION IN COLORECTAL CANCER PROGRESSION AND METASTASIS**

Micaela Lleyda[1], Sandra Mendez-Brito[1], Juliana Beracchia[2], Mónica A. Costas[2], Mónica H. Vazquez-Levin[1]
1-Laboratorio de Estudios de Interacción Celular en Reproducción y Cáncer (IBYME; CONICET-FIBYME).
2-Laboratorio de Biología Molecular y Apoptosis (IDIM-UBA-CONICET).

Colorectal cancer (CRC) is the most common malignant disease of the digestive tract, and 2nd in incidence/mortality worldwide and in Argentina. Epithelial Cadherin (Ecad) loss has been related to cell adhesion loss, migration and invasiveness. FXYD5/Dysadherin (FXYD5) was found associated with tumor aggressiveness/metastasis in solid tumors and is a negative Ecad modulator. Studies were initiated to characterize Ecad and FXYD5 expression in CRC and their role in tumor progression/metastasis. Transcript expression analysis was done in datasets from colon adenoma and CRC patient's and cell lines (GEO; TCGA). Paired adenomas/normal tissue analysis (GSE8671; n=32) revealed decreased ECad (NT=20,884±3,342; A=16,855±2,082) and increased FXYD5 (NT=1,107±435; A=3,577±1,193) ($p<0.0001$) in adenomas. In line with these findings, lower Ecad and higher FXYD5 was found in early tumors when comparing to normal tissues (GSE32323; 16 pairs, Ecad NT=13.21±0.19 T=12.86±0.32, $p<0.005$; FXYD5 NT=7.65±0.47 T=8.72±0.43, $p<0.005$). Similar results were obtained in other CRC datasets (GSE4107; NT n=10 T n=12; Ecad $p<0.05$, FXYD5 $p<0.05$; GSE9348; NT n=12 T n=70; Ecad $p<0.05$, FXYD5 $p<0.001$). No differences in Ecad and FXYD5 expression were observed with patient gender (female n=30, male n=40; Ecad p=0.9448, FXYD5 p=0.6735) or age (50-69y (n=43), 70-93y (n=27) Ecad=0.1482; FXYD5=0.3671). When comparing MSS (n=13) and MSI (n=77) tumors (GSE24550), MSI showed higher ($p<0.0001$) FXYD5 and similar (p=0.1084 Ecad levels). Overall survival (TCGA) was lower (p=0.0064) in patients with high (5.153 y; n=246) than low (8.334 y; n=351) FXYD5. Moreover, liver metastasis (n=283; GSE159216) also showed higher FXYD5 levels ($p<0.0001$) than tumors (n=105; GSE178120), while Ecad levels were comparable (p=0.24). Ecad and FXYD5 expression was confirmed in HT29 (High Ecad, Low FXYD5) and HCT116 (Low Ecad, High FXYD5) and are currently under study to assess the underlying mechanisms of CRC progression/aggressiveness.

PHARMACOLOGY AND RELATED TOPICS I

Thursday, November 17, 9-10:30 hr
Chairs: Maria Laura Ruiz - Constanza Paz - Adalí Pecci

619. (39) **ANTIBIOTICS FOR OUTPATIENT USE IN AFFILIATES BELONGING TO A SOCIAL SECURITY SYSTEM OF CORRIENTES FOR 6 MONTHS**

María Teresa Rocha, Andrea Verónica Ruchinsky, Isabel Hartman, María Eugenia Horna, Sergio Daniel Morales, Lorena Dos Santos Antola.
School of Medicine. National University of the Northeast

The aim of the study was to characterize the outpatient dispensation of antibiotics to affiliates of the university social security during the first semester of 2021. Observational, descriptive, cross-sectional study of drug use was carried out. Data source: drug dispensing from the institution's pharmacy, selecting the drugs belonging to Group J01 (antibacterials for systemic use) of the Anatomical Therapeutic Chemical classification system (ATC-2021), both monodrugs and rational associations at fixed doses. Variables analyzed: age, sex, pharmacological group, antibiotic prescribed, quantity dispensed. To quantify the consumption of antibiotics, the DHD was used, which is the number of defined daily doses (DDD) of systemic antibiotics per 1,000 inhabitants per day, a standardized methodology recommended by the World Health Organization for drug use studies. A total of 3086 antibiotics were dispensed; 54% corresponded to the female sex. Average age: 42 years; range: 0 to 94 years. Most belonged to the penicillin group (36%), followed by macrolides (24%) and fluoroquinolones (12%). Regarding to the characteristics of the dispensation, a potential consumption of 8.34DHD could be appreciated; that is, 8 per 1000 affiliates of the institution received 1DDD of systemic antibiotics. The most prescribed were amoxicillin associated with clavulanic acid (2.05 DHD), azithromycin (1.74 DHD), amoxicillin (1.10 DHD), clarithromycin (0.62 DHD), ciprofloxacin (0.52 DHD). A predominance in the prescription and dispensation of amoxicillin in association with a beta-lactamase inhibitor was evidenced in our province where there are no data showing bacterial resistance to amoxicillin, which could indicate an overuse of this antibiotic, with possible unfavorable consequences on human health. due to the growing scourge of bacterial resistance.

620. (74) **ICMT ROLE IN THE RESPONSE TO RENAL ISCHEMIC DAMAGE**

Carla María Borini Etichetti[1], María Fernanda Fussi[2,3], Liliana Monasterolo[2,3], María Cecilia Larocca[1], Sara María Molinas[2,3], Javier Girardini[4].
[1] Instituto de Fisiología Experimental de Rosario (IFISE-CONICET-UNR), Rosario, Argentina.
[2] Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario, Argentina.
[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
[4] Instituto de Inmunología Clínica y Experimental de Rosario (IDICER-CONICET-UNR), Rosario, Argentina.
borinietichetti@idicer-conicet.gob.ar

Renal ischemia-reperfusion (IR) is one of the main causes of acute kidney injury (AKI). Rho GTPases, RhoA and Cdc42, act as regulators of various processes that directly impact the ability of the renal epithelium to regenerate. A key regulator of these Rho GTPases is the enzyme Isoprenyl-cysteine carboxymethyltransferase (ICMT), which participates in the post- translational modification of their C-terminus.The aim of this work was to investigate the role of ICMT in the epithelial tubular cell response to ischemic AKI. We previously demonstrated that, in IR rats, the early stage of renal damage is associated with a decrease in RhoA, Cdc42 and ICMT and the stage of marked regeneration is associated with an increment of these proteins. We also demonstrated that Angiotensin II type 2 receptor ac-

tivation by its agonist, C21, prevented renal IR tubular epithelial cell damage in rats. Here, we found that C21 (1 mg/kg/day, i.p.) prevented the decrease in ICMT abundance in Wistar rats submitted to 40 min unilateral renal ischemia + 1 day of reperfusion (-55%, p<0.01, n=6). To simulate ischemia *in vitro*, MDCK cells were exposed to ATP depletion by incubation with antimycin A (10 uM) and 2-deoxyglucose (10 mM). RT-qPCR studies showed that *ICMT* mRNA levels diminished in response to ATP depletion (-55%, p<0.05). After 24 h of ATP restoration, ICMT mRNA decrease was recovered in accordance with the improvement of MDCK epithelial organization (as evaluated by actin and E-cadherin immunofluorescence confocal microscopy), suggesting that ICMT may cooperate to re-establish epithelial integrity. In addition, cell viability test by Trypan Blue exclusion showed that MDCK cells treated with Cysmethynil, an ICMT inhibitor, were more susceptible to ATP depletion (-50%, p<0.01). This effect was prevented by C21 pretreatment. Collectively, our results suggest that ICMT is a relevant factor in the development of IR damage and may be involved in the renoprotective effects of C21.

621. (82) **DISPENSATION OF VITAMINS AS MONODRUGS IN A UNIVERSITY SOCIAL SECURITY INSTITUTE, CORRIENTES 2021**

Joaquín Burgos, María Teresa Rocha, María Eugenia Horna, Sergio Daniel Morales, Lorena Dos Santos Antola.
School of Medicine. National University of the Northeast

The aim of this study was to characterize the dispensation of medicines containing vitamins in monodrugs as active ingredient, in an university social security institute (first semester of 2021). An observational, descriptive, cross-sectional drug utilization study (DUS) was carried out. Data were obtained from the institution's pharmacy. Medications dispensed containing vitamins as monodrugs as active ingredients were included. Variables analyzed: age, sex and the quantity of vitamins as monodrug dispensed. To quantify the dispensation, the Daily Inhabitant Dose (DHD) was used, a unit of measurement recommended by the World Health Organization for the DUS. The DHD determines the number of people exposed per thousand inhabitants to a Defined Daily Dose (DDD) of the active ingredient analysed. The DDD is an statistical measure used for researchs and corresponds to the expected mean maintenance dose of a drug for its indication in adults. A total of 1,962 vitamins in monodrugs dispensations were included. Predominance of female patients (71.66%). Average age: 52 years (range: 0 to 93). Vitamin D 72.65 DHD, vitamin C 32.05 DHD, vitamin E 1.80 DHD, vitamin B12 0.97 DHD and vitamin K 0.13 DHD were dispensed. Regarding to the most dispensed vitamins, vitamin D was dispensed in 77.15% of cases for female patients, with a DHD of 56.49, and vitamin C in 61.32% of cases for female patients, with a DHD of 19.68. This study, where a high dispensation is observed fundamentally of vitamin D and C, points out the need to carry out interventions to determine the health situations of the affiliates that motivated those dispensations. Vitamins are micronutrients and with minimum quantity the recommended daily needs are covered. Its administration for prophylactic purposes has precise indications and; when they are administered for therapeutic purposes, they should be indicated after demonstrating their deficiency, to avoid adverse effects and an unnecessary increase in health costs.

622. **(**94) **USE OF A NEW COMBINATION OF XYLAZINE-MIDAZOLAM FOR RATS SEDATION**

Estefanía Magalí Zeni Coronel[1,2], Marina Soledad Bonanno[1,3], Mariana Seijo[1], Susana Noemí Zeni[1]
[1]Laboratorio de Osteopatías INIGEM-CONICET, FFyB-UBA, Hospital de Clínicas José de San Martín. Ciudad Autónoma de Buenos Aires (CABA), Argentina
[2]Cátedra de Bioestadística, FVet-UBA, Argentina
[3]Cátedra de Histología y Embriología, FO-UBA, Argentina

One of the most used and routinely established anesthetic modalities in rodents, is ketamine (K) combined with benzodiazepines; however, K induces secondary undesired effects, added to that its acquisition in Argentina is difficult due to being considered an abuse drug. To avoid these adverse, bureaucratic and economic obstacles, we evaluated the possibility of replacing ketamine/xylazine (KX) by an anesthesia protocol that could provide a quick and effective surgical level of anesthesia and allow full access to the oral cavity. Wistar rats (n=24) were subject to the application of saline solution 3 times a week and weekly replacement of the periodontal ligature (PL) under a combination of 20 mg X/Kg and 5 mg midazolam (M)/Kg. After 21 days, animals were sacrificed and soft organs (liver, kidneys and duodenum) were extracted for histology and pharmacological combination safety verification. The time for sedation (in seconds) was evaluated and compared against the KX combination. Results: average ± SD (min-max): ataxia 113±60 (62-325); lateral decubitus 164±62 (76-359); foot reflex 306±112 (129-537) and ocular reflex 527±215 (145-1063). Mortality and respiratory distress were not observed. Ataxia marked the beginning of the induction period and was obtained after less than 2±1 min compared to 8.8±4.0 min with KX. After the completion of the dental procedure with XM, foot reflex recovery required 38±14 min and ocular reflex 43±19 min. Sedation lasted: 77±10min versus 20-40 min induced by intramuscular/intraperitoneal K/X (40-90 mg/kg/5-15mg/kg, respectively). Variability in depth of anesthesia was not observed in XM as compared to the marked variability observed with KX combination. Conclusion: Induction to the anesthetic plane was significantly shorter with XM than KX combination. The tested combination appears to be a suitable alternative to replace KX for minor oral procedures, as well as for longer surgical interventions due to its prolonged anesthesia effect.

623. (355) **COMPARATIVE ANALYSIS OF THE BIOLOGICAL ACTIVITY OF DIFFERENT G-CSF ANALOGUES ON MOBILIZATION AND DIFFERENTIATION OF MURINE PLURIPOTENT BONE MARROW CELLS.**

Materazzi L[1,3], Marvaldi C[2], Acebedo M[1,3], Giambalvo Gómez D[1,3], Ferraiolo P[1,3], Mazzey J[1,3], Diez RA[3], Lombardi MG[1,3].
[1]Laboratorio de Oncoinmunología Molecular.[2] Laboratorio de Fisiopatología de la preñez y el parto; [1-2] Centro de Estudios Farmacológicos y Botánicos (CEFyBO)-CONICET, [3]Segunda Cátedra de Farmacología, Facultad de Medicina, Universidad de Buenos Aires.

Granulocyte colony-stimulation factor (G-CSF) is a cytokine that promotes growth and maturation of neutrophil progenitor cells by interacting with a specific cell receptor (G-CSFR). It can also mobilize progenitor cells from the bone marrow into peripheral blood that can be used for hematopoietic reconstitution. In this work, we analysed two recombinant proteins produced by biotechnology based on the G-CSF gene that are used in clinical medicine: lenograstim (L; eukaryotic glycosylation) and filgrastim (F; non-glycosylated). Comparisons between these proteins are limited, but it is generally accepted that they function as full agonists of G-CSFR and have equivalent efficacy in their clinical capabilities. However, conflicting reports have emerged regarding the effects obtained with both forms of G-CSF. Here, we use a cytopenia model induced by a single dose of cyclophosphamide (300 μg/g), in 12-week-old CD-1 mice. After 4 days, the G-CSF analogues were administered or not during 4 days (daily dose:300 μg/g). Then, a blood smear was performed and blood sample were collected. Labelling of peripheral blood mononuclear cells with an antibody against CD117 (c-Kit receptor) showed that F and L are able to significantly increase the quantity of circulating haematopoietic precursors, compared to control(*p<0,05). In accordance, analysis of blood smears revealed the appearance of myeloid progenitors in the peripheral blood of G-CSF analogues treated mice. In addition, L significantly increased the % of neutrophils in the leukocyte formula with respect to control(*p<0,05). Our results indicate that both G-CSF analogues present a comparable capacity to mobilize hematopoietic precursors into peripheral blood, so this effect would not depend on eukaryotic glycosylation. However, L also increased the presence of circulating neutrophils probably reflecting differences in the kinetics of the response; so further studies are required to evaluate the impact on these cell population.

624. (408) **AZILSARTAN AMELIORATES VENTRICULAR HYPERTROPHY BY REDUCING OXIDATIVE STRESS IN OVARIECTOMIZED RATS**

Enzo Cascallares[1], Sofia Ruau[1], Álvaro M. Gutierrez[1], Jorge O. Vélez Rueda[1], Valeria Martinez[1], Verónica C. De Giusti[1]
[1] Centro de Investigaciones Cardiovasculares (CIC-CONICET-UNLP)

Background. Cardiovascular disease is the leading cause of death in postmenopausal women. Estrogen deficiency is associated with increased oxidative stress, which can contribute to left ventricular dysfunction. The novel potent angiotensin receptor blocker azilsartan (AZIL) developed for hypertension treatment, may improve the cardiac function in ovariectomized rats (OVX), by reducing the formation of reactive oxygen species (ROS). In this study, we analyze the effect of AZIL on cardiovascular damage during menopause. *Methods.* Spontaneously hypertensive rats (SHR) (female, 16-weeks-old, 180-200g) were sham-operated or bilateral ovariectomized (SHR-OVX). SHR-OVX rats were randomly divided in 2 groups: no-treated (control), treated with 10 mg/kg/day of AZIL in drinking water for 6 weeks. Systolic blood pressure, cardiac hypertrophy indices and oxidative stress in isolated cardiomyocytes were measured. Results (n=4 per group) were analyzed by ANOVA (*p<0.05). *Results.* Consistent with the lack of estrogen, uterine weight was decreased in SHR-OVX. The ovariectomy increase the systolic blood pressure (SBP) and AZIL prevented such increase (in mmHg, SHR-OVX: 190±5, AZIL: 135±4*). Cardiac hypertrophy parameters were worsened in SHR-OVX whereas the treatment reduced the intraventricular septum in diastole, the posterior wall thickness and the left ventricular mass index. Oxidative stress studies in cardiomyocytes demonstrated that AZIL decreased the ROS production (in % SHR-OVX: 43±2*) as well as lipid peroxidation (in % SHR-OVX: 27±5.2*) and improved the antioxidant system determined by the reduced and oxidized gluthatione ratio (GSH/GSSG: 5.3±0.25*). Our preliminary data suggest that AZIL attenuates cardiac damage caused by oxidative stress provoked by the declined estrogen levels in ovariectomized rats. These findings indicate the possible usefulness of AZIL in the treatment of heart disease in menopausal women.

625. (449) **CROSS-TALK BETWEEN HISTAMINE H2 RECEPTOR AND GLUCOCORTICOID RECEPTOR INFLUENCES GR-REGULATED GENE EXPRESSION AND CELL PROLIFERATION IN ACUTE MYELOID LEUKEMIAS CELLS**

Valeria Torralba-Agu[1], Emiliana Echeverria[1], Carlos Daniel Zappia[1], Federico Monczor[1]
1. Laboratorio de Farmacología de Receptores. ININFA, UBA-CONICET. Argentina.

There are several studies in which histamine and glucocorticoids (GCs) have been administered for the treatment of acute myeloid leukemia separately, but never in combination. We have already reported that histamine H2 receptor (H2r) agonist, amthamine (AMTHA), potentiated glucocorticoid receptor (GR) transcriptional activity using a gene-reporter system transfected in HEK293T cells by a composite mechanism involving cAMP-mediated inhibition and ERK-mediated potentiation. Using the leukemic cell line U937, the aim of the present work was to evaluate if this cross-regulation had any effect on the GR-regulated gene expression and cell proliferation. As expected, treatment of the cells with dexamethasone (DEX) strongly induced GILZ (glucocorticoid induced leucine zipper) gene expression, and this effect was potentiated a 90% by AMTHA (p<0.05), supporting that the phenomenon observed in the heterologous transfection system is conserved in these naive cells. Moreover, when H2r was overexpressed, with the consequent increase in cAMP levels, AMTHA potentiating effect was lost, confirming the inhibitory role of this second messenger. Intriguingly, when cell proliferation was measured, DEX had a dual effect, increasing it by 100 % at low doses (1 to 10 nM) though inhibiting it by 50% at higher doses (1 μM). While AMTHA had no effect per se, when coadministered with DEX, the inhibitory effect on cell proliferation was observed at all GC doses. Consistently, cell treatment with 100 nM DEX induce the expression of GADD45β and p21, as expected by their role in cell proliferation. Considering that GCs could be of clinical use to impede leukemic cell proliferation, the fact that AMTHA helps to achieve the desired DEX effects at lower doses can be of therapeutic relevance. The decrease in the dose of DEX needed to fulfill the therapeutic effect can be useful to avoid the dangerous dose-dependent GCs' side effects.

626. (471) **RELEVANCE OF Hsp70 PHARMACOLOGICAL INHIBITION IN RENAL ISCHEMIA-REPERFUSION INJURY**

Rodrigo Damián García[1], Tatiana Silvina Figueras[2], Valeria Cacciamani[1,2], Andrea Gil Lorenzo[1], Eugenia Benardón[1], Patricia G. Vallés[1,2], Valeria Victoria Costantino[1,2].
[1]Facultad de Ciencias Médicas, UNCuyo.
[2]IMBECU-CONICET.

Acute kidney injury (AKI) as a consequence of ischemia-reperfusion injury (IRI) is a common clinical event that leads to high morbidity, mortality and chronic kidney disease. IRI is also an unavoidable consequence of kidney transplantation. IRI leads to inflammation and cellular injury as a result of activation of innate immunity. TLR4 (Toll-like Receptor 4) is a transmembrane receptor constitutively expressed in epithelial cells of proximal tubules. TLR4 mediates pro-inflammatory and pro-fibrotic pathways. Increased receptor activity on ischemic epithelial cells after IRI leads to apoptotic cell death, inflammation and fibrosis. AKI induces an increase of stress-activated chaperones expression such as Hsp70 (heat shock protein 70). The Hsp70 protective properties in renal IRI are not fully understood and putative modes of protection include anti-inflammatory and anti-apoptotic effects. We propose to evaluate the Hsp70 pharmacological inhibition effect in renal IRI. For this, C57 BL6 wild type (WT) and TLR4 knockout (KO) mice were injected with KNK 437 (Hsp70 expression inhibitor) 48 h before IRI. Uni and bilateral IRI was performed by clamping the renal pedicles for 30 min. Controls underwent the same surgical procedure, but the renal pedicle was not clamped. Mice were sacrificed 1, 2, and 7 days after IRI. Blood samples were collected by cardiac puncture and then nephrectomized to obtain tissue samples. Survival curves at 7 days post-IRI showed that KO mice have higher survival percentage and lower serum creatinine and urea concentrations compared to WT mice. However, in WT and KO mice pretreated with KNK 437 the survival percentage significantly decreased compared to untreated mice. This was accompanied by a significant increase in urea concentration. These results suggest that TLR4 KO mice present improvement in renal IRI, while Hsp70 inhibition impairs it, suggesting a protective role of Hsp70 in IRI possibly by modulating TLR4-mediated cellular responses.

627. (495) **ARE SILDENAFIL DUAL EFFECTS ON MEMORY RELATED TO THE ACTIVATION OF DOPAMINE SYSTEM? IMPORTANCE OF SEARCHING FOR NEW DERIVATIVES WITH RESTRICTED ACCESS TO THE BRAIN**

María Florencia Constantin[1], María Fernanda Ponce Beti[1], Sofía de la Fuente[1], Aida Marcotti[1], Sergio Ribone[2], Mario Alfredo Quevedo[2] and Mariela Fernanda Pérez[1].
[1]Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina, [2]Departamento de Ciencias Farmacéuticas, UNITEFA-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Sildenafil (SILD) is the most clinically phosphodiesterase type 5 (PDE5) inhibitor used for peripheral pathologies, but it is misused without prescription. SILD reaches the brain and is proposed as a cognitive enhancer for pathologies such as Alzheimer's disease. In fact, it improves transmission in the hippocampus (HP), a brain region involved in learning and memory, and also raises dopamine (DA) levels. Coincidently, DA increases may induce memory impairments in some animal models. Nevertheless, SILD central effects are not fully described in healthy conditions. Objectives: 1- to evaluate the effects of SILD on HP-dependent memories, 2- to evaluate the DA dependence of SILD-induced memory deficits, 3- search for SILD derivatives with higher hydrophilicity and preserved activity. Material and Methods: male Wistar rats (50 days old) received a SILD dose (5 mg/kg, i.p.) 2 h before training in novel object recognition-NOR, Barnes-BM, Y-maze-YM or modified YM. Other group

was treated with the DA-D3 receptor antagonist, FAUC365 (3mg/Kg, s.c.), 10 min before NOR training. Memory expression was evaluated accordingly each test. Also, hydrophilic SILD derivatives were identified by in silico methods. Results: SILD reduced NOR ($p<0,05$ unpaired t-test) and modified YM (two-way ANOVA) performance, while no changes were observed in BM or YM. Experiments with FAUC365 are still in progress. On the other hand, molecular docking identified SILD-PDE5 pharmacophoric contacts with some hydrophilic derivatives of SILD described in the bibliography. Conclusions: SILD impairs HP-dependent memory formation, probably by increases in DA activity. These effects reveal the importance of considering the SILD central effects, when it is used under prescription or recreationally by young subjects. Altogether, these results support the experimental evaluation of peripheral-acting SILD derivatives that could avoid these and other possible unwanted central effects.

628. (505) **MONTELUKAST ACTIVATES BK (SLO1) CHANNELS ASSOCIATED WITH THE B4 ACCESSORY SUBUNIT AND EMERGES AS A PHARMACOLOGICAL TOOL TO REDUCE HIPPOCAMPAL EXCITABILITY**

Federico Orsi[1], Juliana Monat[1], Verónica Milesi[1], Jesica Raingo[2], Pedro Martín[1]

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Universidad Nacional de La Plata - CICPBA - CONICET, Buenos Aires, Argentina. [2]Instituto Multidisciplinario de Biología Celular (IMBICE), Universidad Nacional de La Plata CICPBA-CONICET, La Plata, Argentina.

Different β accessory subunits are associated with the large conductance voltage- and Ca^{2+}- dependent K+ channel (BK channel) in a tissue-specific manner. In particular, BK channel/β4 subunits complexes (α/β4) control neuronal excitability in granule cells of the hippocampal dentate gyrus (GCDG) and, β4 deficient mice display neuronal hyperexcitability, a phenotype associated with the temporal lobe epilepsy (TLE). Thus, compounds that increase α/β4 activity are of great interest for improving epilepsy treatment. Then, based on the activating effect of LTB1 on BK channel associated with β1 subunit, we explore the effect of Montelukast (MTK) on this channel heterologously expressed in HEK cells with or without its β accessory subunits (β1, β2, and β4). MTK is a $CysLT_1$ receptor ligand clinically used as an anti-asthmatic drug that could share the affinity properties with the LTB1. Using the patch clamp technique in voltage clamp we found that MTK shifts the activation curves of α/β1 and α/β4 to more hyperpolarized voltages in a concentration-dependent manner (300 nM MTK: $\Delta V_{1/2}$ (α/β1)= -54.4 mV ±8.4; n=6; $p<0,05$ and $\Delta V_{1/2}$ (α/β4)= -36 mV ±13.5; n=5; $p<0,05$) without affecting α/β2 channel and, only at concentrations greater than 1 μM, slightly shifts the homomeric BK channel (without β) curve ($\Delta V_{1/2}$= -10.8 mV ± 3.7; n=3; $p<0,05$). MTK delays α/β4 deactivation process characterized by a longer deactivation τ constant, suggesting that this drug induces open channel stabilization (for 1 μM MTK, at -30mV: τ_{MTK}= 39.3 ± 5.3 ms vs. $\tau_{control}$= 3.6 ± 0.9 ms n= 5, $p<0.05$). Finally, as a promising result, we observed that MTK decreases the number of action potential (AP) spikes in GCDG from mouse brain slices recorded in current-clamp mode (%reduction in the number of spikes induced by 140 pA: 58% ± 8, n=3, $p<0.05$). Our results indicate that MTK directly activates the α/β4 channel and suggest that this effect could reduce DGGC excitability and may be a putative new treatment for TLE.

629. (558) **STUDY OF THE EFFECT OF GLYCEROL (GLY) ON THE SURVIVAL AND MIGRATION OF HEPATOCELLULAR CARCINOMA CELLS TREATED WITH SORAFENIB (SFB)**

Laura G. Chares 1,*, Eugenia Carmeli Barberis 1,*, Nicolás F. Palma 1,2, Verónica I. Livore 1, Magalí Frattini 1, Anabela C. Ferretti 2, Carla G. Comanzo 1,2,3, Lucía Oviedo Bustos 1, Ariel D. Quiroga 1,2,3, María de Luján Alvarez 1,2,3, María P. Ceballos 1.

1. Instituto de Fisiología Experimental (IFISE), Facultad de Ciencias Bioquímicas y Farmacéuticas, CONICET, UNR, Rosario.

2. Área Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario. 3 CAECIHS, Universidad Abierta Interamericana, Rosario.

**These authors contributed equally to this work.*

SFB is used as a first-line therapy for HCC; however, its efficacy is limited and better therapies to prolong survival are still lacking. Combination therapy represents a major research direction for HCC treatment. Our group reported antiproliferative and proapoptotic effects of GLY administration in an *in vivo* model of liver carcinogenesis. Aim: to analyze whether GLY enhances the sensitivity of HCC cells to SFB *in vitro*. Methods: 2D and 3D cultures from Huh7 cells were treated for 72 h with different concentrations of SFB or GLY to obtain dose-response curves. Alternative, cell cultures were treated for 72 h with SFB (2D: 2μM; 3D: 4μM), GLY (2D: 1μM; 3D: 2μM), SFB+GLY, or DMSO (control group). Then, cell viability (2D: MTT; 3D: acid phosphatase; IC50), proliferation (3D: spheroid growth - volume 72h/0h) and migration (2D: wound healing; 3D: spheroid migration) were assayed. Results: SFB and GLY reduced 2D and 3D cell cultures viability in a dose-dependent manner compared with control group. The 3D cell culture was less sensitive to drugs than the 2D cell culture (IC50: SFB 2D: 3.4μM, 3D: 13.7μM[$]; GLY 2D: 2.4μM, 3D: 10μM[$]). In 3D cell cultures, both drugs induced dose-dependent growth inhibition. Cell cultures treated with SFB+GLY showed a greater decrease in viability compared to cultures treated with SFB or GLY (2D: SFB -23%*, GLY -22%*, SFB+GLY -56%*#&; 3D: SFB -16%, GLY -1%, SFB+GLY -42%*#&). Treatment of 3D cell cultures with SFB+GLY decreased spheroid growth compared to individual treatments (SFB -25%*, GLY -11%*, SFB+GLY -35%*#&). SFB+GLY treatment produced the greatest reduction in cellular migration in 2D and 3D cultures. [$]$p<0.05$ vs 2D; *$p<0.05$ vs control; #$p<0.05$ vs SFB; &$p<0.05$ vs GLY. Conclusions: GLY exacerbated the effects of SFB on HCC cellular survival and migration. Additional studies are needed to confirm the relevance of adding GLY during SFB treatment and to elucidate the mechanisms involved.

630. (563) **MULTICELLULAR TUMOR SPHEROIDS (MCTS) AS AN *IN VITRO* MODEL FOR STUDYING SORAFENIB (SFB) RESISTANCE IN HEPATOCELLULAR CARCINOMA (HCC): EFFECT OF THE SIRTUINS 1/2 INHIBITOR EX-527 (EX) ON CELLULAR VIABILITY**

Nicolás F. Palma 1,2, Verónica I. Livore 1, Laura G. Chares 1, Anabela C. Ferretti 2, Carla

G. Comanzo 1,2,3, Marina C. Vera 1,3, María de Luján Alvarez 1,2,3, Aldo D. Mottino 1, María C. Carrillo 1,2, Ariel D. Quiroga 1,2,3, María P. Ceballos 1.

1. Instituto de Fisiología Experimental (IFISE), Facultad de Ciencias Bioquímicas y Farmacéuticas, CONICET, UNR, Rosario.

2. Área Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario. 3 CAECIHS, Universidad Abierta Interamericana, Rosario.

It is essential to count with more relevant preclinical models to study possible chemotherapy strategies for HCC. Tumors are 3D structures and their inner stromal cells contribute to cancer progression and chemoresistance (CR). MCTS composed of cancer and stroma cells provide reliable results *in vitro* since they mimic features of *in vivo* tumors. CR counteracts the efficacy of SFB, a first-line drug used for HCC, and better therapies are still missing. Sirtuins 1/2 promote cancer progression and CR. Aim: to study the effect of EX addition during SFB treatment in MCTS composed of Huh7 or SFB-resistant Huh7 (Huh7-SR) cells together with stromal cells. Methods: Huh7-SR cells were established after incubating Huh7 cells for 6 months with increasing doses of SFB. Resistance was evaluated in 2D cultures (MTT; Western Blot). MCTS of Huh7 or Huh7-SR, endothelial (EA.hy926) and hepatic stellate (LX-2) cells were obtained and treated for 72 h with different doses of SFB, EX, SFB+EX or DMSO (control group). Cell viability was studied (acid phosphatase assay) and IC50 and combination index (CI) values were determined (CompuSyn). Results and discussion: Huh7-SR cells presented with a higher IC50 value for SFB (6.7μM*) compared to Huh7 cells (2.7μM) as well as more PCNA, cyclin D1, P-gp and MRP3 protein levels, confirming SFB resistance. SFB and EX reduced MCTS viability in a dose-dependent manner compared to the

control group. Huh7-SR-formed MCTS were more resistance to SFB than Huh7-formed MCTS (IC50: 11μM* and 7μM, respectively). Different SFB+EX combinations decreased MCTS viability compared to the individual treatments (synergistic effect; CI<1). Huh7-SR-formed MCTS were more viable when the same doses were used (4μM SFB + 40μM EX: -74% and -39%* in MCTS containing Huh7 or Huh7-SR, respectively). *p<0.05. Conclusion: We established a preclinical relevant model to advance with the study of the benefits of incorporating EX to HCC therapy in order to overcome SFB CR.

631. (734) **NUCLEOTIDASE ACTIVITY IN SALIVARY EXTRACELLULAR VESICLES**

[1]Orman B, [2]Barbieri van Haaster M M, [1]Castro Escalante A, [2] Gonzalez DA

Cátedras de [1]Farmacología, [2]Biofísica y Bioestadística. Facultad de Odontología. Universidad de Buenos Aires. Buenos Aires. Argentina.

Objective: to determine i) [ATP] and ATPase activity in whole saliva (WS) and parotid saliva (PS), and in extracellular vesicles (EVs) from both, ii) the contribution of nucleoside triphosphate diphosphohydrolases (NTPDase), ectonucleotide pyrophosphatases (NPP) and Ecto-5'nucleotidase to EVs nucleotidase activity, iii) aquaporin5 (AQ5) in EVs. Methods: WS and PS were obtained from 17 healthy patients. WS was centrifuged for 30' at 5000 rpm to obtain acellular saliva (AS). EVs were isolated by ultracentrifugation (UC) or by Exoquick (EQ). ATPase activity of saliva and EVs (250nM ATP), and salivary [ATP] were measured by luciferin/luciferase technique. EVs nucleotidase activities were measured with 3 mM MgATP, MgADP or MgAMP by released phosphate, with or without inhibitors. Immunolabeling essays in EVs were performed for ectonucleotidases (TEM) and AQ5 (WB). Results were expressed as mean±SEM. Results: [ATP] was 333±65 nM in WS. ATPase activity of WS, AS and PS were 148±28, 33±5 and 12±5 pmol ATP/min/ml (with 250 nM ATP), respectively. In equal condition, ATPase activity of EVs isolated by EQ was 6.1±1.0 pmol and 1.1±0.5 ATP/min/ml for WS and PS, respectively. ATP/ADP/AMPase activities (µmol Pi/min/mg prot) were measured in EVs obtained by UC, with 3 mM ATP: 0.23±0.08, ADP: 0.05±0.01 and AMP: 0.02±0.01. WS ATPase activity decayed to 45%±8% with 0.3 mM βAMP-PCP (ENPP inhibitor) but suramin (NTPDase inhibitor) had no effect. Regarding salivary EVs, only 1 mM suramin reduced the ATPase activity to 50±7%. NTPDasas -1, -2, -3, -8 y ecto-5 nucleotidase antibodies bonded to EVs. AQ5 was also detected. Conclusions: Salivary ATP is hydrolyzed by ectonucleotidases, which also have ADP and AMP as substrates, giving rise to adenosine. Whereas ATP is a proinflammatory purinergic agonist, adenosine has a protective effect. Part of the ATPase activity comes from EVs that are present in both WS and PS. The glandular origin of the EVS is also supported by the detection of AQ5.

632. (764) **INHALED IBUPROFEN-DUO TREATMENT: SECURITY, KINETIC, AND EFFECTS**

Ximena Volpini1, Lautaro Natali1, David Tejerina2, Silvia Farfán4, José Luis Amigone2, Martín Moya3, Melina M. Musri1.

1, Instituto de Investigaciones Médicas Merdeces y Martín Ferreyra (IMMF-UNC/INIMEC-CONICET) Córdoba, Argentina. 2, Laboratorio Central del Hospital Privado de Córdoba, Argentina. 3, Hospital Universitario de Maternidad y Neonatología. Universidad Nacional de Córdoba. 4, Centro de Excelencia en Procesos y Productos de Córdoba, Argentina.

Water-soluble Ibuprofen constitutes a critical strategy for inhalation-based treatments of respiratory diseases. Sodium ibuprofenate amphipathic *Luarprofen* is an inhalable formulation with high lung bioavailability with the ability to inhibit the enzyme Cyclooxygenase, decrease reactive oxygen species concentration, and reduce local airway inflammation in patients. Here, we aim to evaluate the kinetics, security, and effects of *Luarprofen duo (LD, Química Luar®)* formulation in murine models. To this end, we designed administration boxes connected to a nebulizer with a gas exchange compressor. Adult B6 mice of both sexes were nebulized with the vehicle, LD 1X, or LD 1.5X for 20 min. To evaluate the kinetic and pharmaco-security, the procedure was performed 3 times/day for 14 days. Blood and lung concentration of LD, blood gas profile, biomarkers of liver and kidney damage, and lung histology were analyzed immediately after 14 days of daily treatment, and after 14 days of the end of the treatment (resting). We did not find any differences between sexes. High-pressure liquid chromatography showed high LD concentrations immediately in blood after nebulization and progressively decreased until values statistically undistinguishable from controls after 2 h. The drug was also distributed in the lungs, reaching maximum values 2 h after nebulization. LD 1X or 1,5X did not affect the profiles of hemoglobin, arterial gases, and blood acid-base. Also, LD did not cause liver damage, altered kidney function, or increased acute phase protein levels. Importantly, vascular, alveolar, and/or bronchial histological changes were not observed in the lungs. Finally, we evaluated the effects of LD 1X in a model of LPS-induced acute pulmonary inflammation. The drug attenuated the excessive pulmonary infiltration of inflammatory cells and improved the gas exchange. LD would be a novel, safe, and promising therapeutic strategy for inflammatory-lung diseases.

633. (777) **ENDOTHELIN-1 IMPAIRS NOREPINEPHRINE NEURONAL TRANSPORTER IN PC12 CELLS. ROLE OF THE ENDOPLASMIC RETICULUM STRESS**

Fabian A Innamorato Costas[1], Nicolas Ciangreco[1,2], Guadalupe Alvarez[1], Ana Paula Courreges[1], Clarisa Marotte[1], Liliana G Bianciotti[1,3], Marcelo S Vatta[1,2].

[1]Instituto de Inmunología, Genética y Metabolismo, UBA-CONICET, Argentina, [2]Cátedra de Fisiología, Facultad de Farmacia y Bioquímica, UBA, Argentina, [3]Cátedra de Fisiopatología, Facultad de Farmacia y Bioquímica, UBA, Argentina

Neuronal norepinephrine transporter (NET) removes norepinephrine from the synaptic cleft. Several studies show that NET impairment is associated with cardiovascular diseases like hypertension (HT) whereas recent works link endoplasmic reticulum stress (ERE), triggered by misfolded proteins accumulation in the reticulum, with the development of HT. We recently reported that endothelin receptors (ET_A/ET_B) blockade in rats with salt-dependent HT decreases blood pressure and markers of ERE and ERE-dependent apoptosis as well as misfolded NET accumulation in the adrenal medulla. In the present study we aimed to unveil the mechanisms underlying these findings. PC12 cells were exposed to 10mM endothelin 1 (ET-1), 10nM thapsigargin (TG) or 0.1ug/ml tunicamycin (TN) (TG and TN were used as ERE positive controls) and NET and ERE markers expression were assessed by Western blot. Results were expressed as the mean ±SD, and p<0.05 was considered significant. Results showed that ET1, TG and TN increased the expression of non-glycosylated NET vs. glycosylated NET (126%, 172% and 130%, respectively, p<0.05. In addition, ET1 increased the expression of chaperone Bip (binging immunoglobulin protein) (205±47% *vs.* control, p <0.05), proteins of the PERK (protein kinase-like ER kinase) pathway (pPERK: 92±3% vs control, p <0.05; peIF2α/eIF2α: 172± 63% *vs.* control, p <0.05) and ATF6 α (activating transcription factor 6α) (187±39% *vs.* control, p <0.05). Similar findings were observed with TG and TN. These findings suggest that the increase in non-glycosylated NET induced by ET-1 would trigger ERE by the accumulation of this misfolded and non-functional protein and likely others in PC12 cells.

634. (816) **EVALUATION OF THE EFFECT OF *ILEX PARAGUARIENSIS* EXTRACT ON CELL PROLIFERATION**

Maria Rosana Ramirez[1,2], Agostina Guzzonatto[1,2], Ignacio Rintoul[1,3]

[1] Consejo Nacional de Investigaciones Científicas Técnicas (CONICET). [2] Instituto Universitario de Ciencias de la Salud, Fundación Barceló. [3] Facultad de Ingeniería Química. Universidad Nacional del Litoral, Santa Fe, Argentina.

Ilex paraguariensis (Aquifoliaceae) leaves are used for their stimulant, antioxidant, and diuretic activity, presenting as principal components polyphenolic compounds. Previous work reported that *I. paraguariensis* also inhibits the proliferation of oral cancer cells in vitro. Our aim was to study the effect of *I. paraguariensis* extract on cell proliferation in the endometrial stromal cell line (tHESC cells).

For this purpose, tHESC cells were seeded in plates containing culture medium (DMEM-F12, L-glutamine, foetal bovine serum). After approximately 48 hours, the medium was removed from the plates, washed with PBS and the cells were stimulated with *I. paraguariensis* extract (4-400 μg/mL). The corresponding blanks (40 to 400 μg/mL) were then made. Cell proliferation was determined by a colorimetric method (450 nm and at 620 nm in a plate reader). There were 4 replicates per treatment and each experiment was carried out 5 times (n=5). In both cases an analysis of variance (ANOVA) followed by Tukey's test was performed. Results are expressed as the mean per treatment ±SEM, p'0.05 is considered statistically significant (GraphPad Prism). The results showed that *I. paraguariensis* extract at doses of 100-400 μg/ml showed a statistically significant inhibition of cell proliferation compared to basal (p'0.001). It is possible to suggest that *I. paraguariensis* extract requires further studies to determine its efficacy on cell proliferation and safety in, *in vivo* studies.

PHARMACOLOGY AND RELATED TOPICS II

Saturday, November 19, 14-15:30 hr
Chairs: Andrea Cumino - Claudia Bregonzio - María del Carmen Martínez

635. (56) **NEW 1,3-DIHIDROXYACRIDONE DERIVATES: SYNTHESIS AND EVALUATION AS AKT INHIBITORS OF SIGNALING PATHWAY IN SKELETAL MUSCLE CELLS**

Gonzalez A[1,2], Irazoqui AP[1,3], Menéndez CA[2], Steingruber HS[2], Appignanesi GA[2], Buitrago CG[1], Gerbino DC[2].
[1]INBIOSUR (CONICET-UNS), Depto. BByF-UNS, Argentina. [2]INQUISUR (CONICET-UNS), Depto. Química-UNS, Argentina. [3]CIC-PBA, Argentina.

PI3K/Akt signaling pathway is crucial in cell growth, survival, and differentiation, and is also involved in tumorigenesis. This route constitutes an alternative and attractive therapeutic target for the development of new antitumor agents. An interesting strategy applied to the discovery of new drugs consists of the use of so-called "privileged structures" such as acridones (dibenzo-4-pyridones) and their congeners, which represent a biocompatible system against different biological targets. Based on the acridone scaffold, four new prototypes (named 3a-3d) were efficiently synthesized following simple operating procedures and subsequently biologically tested in skeletal muscle (C2C12) and rhabdomyosarcoma (RD) cells. Our results showed no significant change in the number of viable cell (staining with Trypan blue and counted in a Neubauer chamber) when they were treated with the compounds (1 μM) for 24 or 48 hours. Western blot assays revealed that acridone derivatives 3a-3d (0.5 μM) effectively inhibited Akt activation in C2C12 at 24 h, whereas only 3a and 3b compounds at 1 μM were efficient in inhibiting Akt. RD cells showed a different response pattern. These cells treated with 3a (0.5 μM), 3b (0.5 μM) or 3d (0.5 or 1 μM) for 24 h showed significant inhibition of Akt. Furthermore, 3a-3d (1 μM) were highly successful inhibiting Akt phosphorylation at 48 h of treatment. Biological studies revealed that 3b compound could be considered the most promising prototype for the development of new antitumor agents, as it even has a higher inhibitory effect than LY294002, the well-known commercial Akt inhibitor.

636. (60) **COMPARATIVE TEST OF TOXICITY, LOCAL TOLERANCE AND IMMUNOGENICITY, AT REPEATED DOSES, BETWEEN THE REFERENCE PRODUCT OF ETANERCEPT AND THE BIOSIMILAR OF GEMABIOTECH**

Pablo A. Souto, Jorge Mosquera, Virginia Pelagatti, Nestor R. Lago
GEMABIOTECH SAU, Estudios Preclínicos y Clínicos, Buenos Aires, Argentina.

Introduction: Etanercept is an immunomodulator that inhibits tumor necrosis factor alpha (TNF-alpha) widely used in rheumatic diseases. In recent years, the expiration of patents has promoted the development of biosimilar molecules. Comparability trials are needed to show that there are no clinically meaningful differences between the biosimilar and the reference medicine in terms of safety, quality and safety, and efficacy. Objective: To compare the toxicity/safety, local tolerance and immunogenicity between reference medicine (Enbrel®, Pfizer) and the biosimilar (Enerceptan®, Gemabiotech S.A.U.) Methods: 72 adult Wistar rats, divided into 4 groups, received 24 total applications of 50 mg/kg of Enerceptan®, Enbrel® or solution of excipients, twice a week subcutaneously. The fourth group did not received any application and basal values were obtained from this group. A complete anatomo-pathological study of 10 organs was carried out, including macroscopy and microscopy, local tolerance, clinical laboratory, immunogenicity by immunoprecipitation, and detection by immunoradiometric assay. An ANOVA was performed to compare parameters. Results: No significant differences were observed in biochemical parameters. No presence of anti-etanercept antibodies was found in the 3 treatment groups. No significant lesions were observed in the anatomo-pathological study. No signs attributable to toxicity were observed in any of the animals belonging to the three treatment groups. Conclusions: Biosimilarity in toxicity/safety, local tolerance and immunogenicity was demonstrated between the tested biosimilar (Enerceptan®, Gemabiotech) and the reference product (Enbrel®, Pfizer).

637. (61) **PRECLINICAL PHARMACOKINETIC STUDY COMPARING ETANERCEPT INNOVATOR PRODUCT AND THE FIRST BIOSIMILAR DEVELOPED IN ARGENTINA**

Jorge Mosquera[1], Pablo Andrés Souto[1], Virginia Pelagatti[1], Guillermina Forno2, Nestor Lago[1]
[1]GEMABIOTECH SAU, Estudios Preclínicos y Clínicos, Buenos Aires, Argentina, [2]ZELLTEK S.A., Investigación y Desarrollo, Santa Fe, Argentina

Introduction: Gemabiotech etanercept (Enerceptan®) is a biosimilar which was shown to be equivalent to reference etanercept (Enbrel®, Pfizer) in a wide panel of comparative physicochemical studies. Objective: To compare the pharmacokinetic profile of Enerceptan® with the refeence product in rats and monkeys at a single dose. Methods: 20 Wistar rats and 12 *Cebus apella* monkeys received a single subcutaneous dose of 1mg/Kg or 50 mg/Kg of either Enerceptan or Enbrel, respectively. Plasma quantification of etanercept was performed by the sandwich ELISA technique using TNF capture. The AUC0-t was estimated by the trapezoidal rule using non-compartmental methods. Cmax and Tmax were obtained from the Concentration vs. Time curves. AUC0-t, the concentration at the time of the last extraction (168 hours for rats and 384 hours for monkeys) and the elimination rate constant (Ke) were used to calculate AUC0-inf. An ANOVA was performed to compare each evaluated parameter, using means and confidence intervals for the comparison. Results: The pharmacokinetic parameters for Enerceptan and Enbrel, respectively, were the following: (A) Rats: Cmax (ng/ml): 3198.60 and 3257.60; AUC0-168 (h*ng/ml): 222956.46 and 269200.56; AUC0-inf (h*ng/ml): 240226.20 and 297506.44; Tmax (h): 48 and 48. (B) Monkeys: Cmax (μg/ml): 83 and 82.5; AUC0-384 (h*μg/ml): 5584 and 5045; AUC0-inf (h*μg/ml): 5721 and 5195; Tmax (h): 25.3 and 28.8 The pharmacokinetic parameters were within the confidence interval, and no significant differences were observed (p>0.1 ANOVA). Conclusion: The pharmacokinetic behavior of Enerceptan and Enbrel were comparable, supporting the biosimilarity between both products.

638. (95) **COMPUTER-GUIDED IDENTIFICATION OF NEW INHIBITORS OF CLOSTRIDIUM DIFFICILE SPORE GERMINATION**

Franco Caram[1,3], FernandoTrejo[2,3], Pablo F. Pérez[2,3], Alan Talevi[1,3], Carolina Bellera[1,3]
[1] Laboratory of Bioactive Compounds Research and Development (LIDeB), Faculty of Exact Sciences, National University of La Plata (UNLP), La Plata, Buenos Aires, Argentina. [2] Center of Research and Development in Food Cryotechnology (CIDCA), La Plata, Buenos Aires, Argentina. [3]National Council of Scientific and Technician Research (CONICET)

Clostridium difficile (CD) is a Gram-positive anaerobic spore-forming bacteria. It is the main pathogen causing antibiotic-associated colitis and 20 to 30% of cases of antibiotic-associated nosocomial

diarrhea. Meanwhile the global morbidity and mortality rates have been increasing. In this work we developed machine learning models to be used in virtual screening in order to identify new CD spore germination inhibitors. We have compiled 121 molecules tested as inhibitors of CD spores germination, these were divided into three representative groups: training set and two validation groups, using SOMMOC tool. By a combination of feature bagging approximations and forward stepwise we have inferred 1000 ligand-based classificatory models able to recognize inhibitors. Forward, the best individual classifiers model were combined into meta-classifiers and then evaluated by a retrospective screening campaign, against the two validations groups that contained decoys obtained using the LUDe application. The best ensemble model was applied in a prospective virtual screening campaign of DrugBank 5.1.6 (DB), estimating the Positive Predictive Value (PPV) for each compound screened. The training set was balanced with 30 inhibitor compounds and 30 non inhibitors, the validation sets contain, both of them, the same 13 inhibitors, and 24 non inhibitors. The 16 top models with the best performance were combined using the MIN-SCORE operator to improve the predictive capacity and robustness. By analyzing PPV surfaces, a cutoff value of 0.28 was chosen, associated with a specificity of 0.96 and a PPV value of 0.47 for a hypothetical yield of active compounds of 1%.This ensemble model was applied in a virtual screening campaign of DB, obtaining 62 possible hits. As an additional selection criteria, we selected those approved or investigational, obtaining 29 hits. These drugs will be evaluated in *in vitro* assays and those that are most promising will be tested in animal models.

639. (98) **APPLICATION OF IN SILICO DRUG REPURPOSING APPROACHES IN THE SEARCH OF NEW ANTICONVULSANT DRUGS ACTIVE IN THE PTZ KINDLING MODEL**

Estefanía Peralta[1], Maximiliano J. Fallico[1], Denis N. Prada Gori[1], Lucas N. Alberca[1], Carolina L. Bellera[1]
[1] Laboratory of Bioactive Compounds Research and Development (LIDeB), Faculty of Exact Sciences, National University of La Plata (UNLP), La Plata, Buenos Aires, Argentina.

Drug repurposing involves search of new medical uses for already known drugs, including approved, discontinued, shelved and experimental drugs. In this work, we have used machine learning approximations to develop in silico models capable of identifying novel anticonvulsant drugs with protective effects in the PTZ kindling model. For the generation of algorithms, 162 compounds with and without protective effects in the PTZ kindling model in mice were compiled from literature; this dataset was divided into representative training and test sets by clustering technique. Afterwards, linear classifier models were generated in Python. The best classifiers obtained were combined into meta classifiers and validated by retrospective selection experiments. As a result, the dataset was partitioned into a training set of 41 active compounds, 41 inactive compounds, and the test set of 40 active and 40 inactive compounds. In turn, this test set was subdivided into two validation groups that were complemented with 1000 decoys, in order to evaluate the performance of the models obtained. According to this performance, 3000 linear classification models were generated, from which the 7 with the best performance were chosen and combined using the PROM-SCORE operator to improve the predictive capacity and robustness of the obtained models. Using positive predictive surface analysis (PPV), the cutoff value of 0.961 associated with a specificity of 0.979 and a PPV value of 0.20 was chosen for a hypothetical yield of active compounds of 1%. The best ensemble model was applied in a virtual screening of Drug Bank, Sweet Lead and DRH databases. 90 approved drugs were identified as potential protective agents in the PTZ kindling model. The present study constitutes an example of the use of machine learning approximations to systematically guide drug repurposing projects.

640. (122) **COMPARATIVE PHARMACODYNAMICS BETWEEN A PEG-FILGRASTIM BIOSIMILAR DEVELOPED IN ARGENTINA AND THE INNOVATOR PRODUCT IN DIFFERENT DOSES IN A MODEL OF NEUTROPENIC RATS**

Jorge Mosquera, Pablo A. Souto, Virginia Pelagatti, Néstor R. Lago
GEMABIOTECH SAU, Preclinical and Clinical Studies, Buenos Aires, Argentina.

Objective: To determine the similarity between biosimilar Peg-Filgrastim (PEG-NEUTROPINE®, Gemabiotech) and the innovator product (Neulastim®, Roche) in rats with cyclophosphamide (CPO) induced neutropenia. Materials and Methods: Wistar rats (40 males and 40 females) from 6 to 8 weeks of age divided into 4 groups and 8 subgroups were used. Six subgroups received CPO (50 mg/kg) on day 0 intraperitoneally and on day 1 received Peg-filgrastim (Peg-GCSF, Pegylated granulocyte colony stimulating factor) biosimilar or reference subcutaneously at one of the following doses: 0.5 ; 1.0 or 2.0 mg/kg. Two control subgroups were used, one that received only CPO and placebo, and other receiving only placebo. Tail bleeds from day 1 (basal) to day 9 were taken from all animals and WBC count/mm3, WBC differential, and absolute neutrophil count/mm3 was calculated. The values of the biosimilar and the reference drug product were compared using the Student's t-test. Differences with a $p>0.05$ were considered non-significant. Results: A significant increase of white cells and neutrophils was observed in the groups receiving Peg-GCSF compared to the control groups. The neutrophil peak response was achieved on the sixth day for both drugs. At all tested doses, no significant differences between the innovator and the biosimilar were observed. The dose-response curves were similar for both drug products. Conclusion: Based on the analysis of the results, it can be considered that pegfilgrastim behaved similarly to the innovator product from the pharmacodynamic point of view.

641. (123) **PRECLINICAL COMPARATIVE STUDY OF ACUTE TOXICITY, LOCAL TOLERANCE AND IMMUNOGENICITY BETWEEN A BIOSIMILAR PEG-FILGRASTIM DEVELOPED IN ARGENTINA AND THE INNOVATOR PRODUCT**

Pablo A. Souto, Jorge Mosquera, Virginia Pelagatti, Nestor R. Lago
GEMABIOTECH SAU, Preclinical and Clinical Studies, Buenos Aires, Argentina.

Objective: To compare in rats the toxicity, local tolerance and immunogenicity between a biosimilar peg-filgrastim developed in Argentina (Peg-Neutropine®, Gemabiotech) and the innovator product (Neulastim®, Roche). Materials and Methods: Sprague Dawley rats (45 males and 48 females), from 5 to 6 weeks of age, were divided into three groups that received the biosimilar, the reference drug, or placebo. The animals received subcutaneously a single dose of Peg-Filgrastim (Peg-GCSF) (10 mg/Kg) or placebo. Rats were sacrificed at 2, 7 and 14 days post administration. Laboratory analysis, necropsy with complete anatomical-pathological study, histolesivity assays were performed. Anti-G-CSF antibodies were screened by enzyme-linked immunosorbent assay. The results were analyzed by ANOVA, and a Tukey's post-hoc test was used when the difference between the groups was significant ($p<0.05$). Results: Significant differences were found in the groups treated with Peg-GCSF compared to placebo in the weights of the spleens, being maximum at 7 days post-administration due to splenic hematopoiesis in response to treatment. No differences were observed in other organs between the treatment groups and placebo. Mild inflammatory reparative lesions were found in all groups at the injection site. No morphological changes and no toxicity signs were observed. Anti-G-CSF antibodies were not detected in any of the groups. Conclusion: in this single-dose study, acute toxicity, local tolerance and immunogenicity results showed similarity between Peg-Neutropine® and the reference innovator.

642. (299) **ANTIOXIDANT PROTECTIVE EFFECT OF POTATO PEEL POLYPHENOLS**

Filiberti V., Vazquez M., Andreu A.B, Silveyra M.X.
Instituto de Investigaciones Biológicas, IIB-CONICET-UNMdP.

Oxidative stress is associated with many pathologies, such as neurodegenerative diseases, and polyphenols are antioxidants that could prevent this stress. Potato peel waste (PPW) is an abundant leftover

from the potato processing industry. We have previously identified polyphenols in extracts from PPW, such as chlorogenic acid, caffeic acid, and ferulic acid. This study aimed to analyze the antioxidant properties of PPW *in vitro* and evaluate its neuroprotective effect on cell-injured by oxidative stress. First, we compared different solvents to optimize the polyphenolic extraction (ethanol-water 80%, 50%, and water), analyzing the total phenolic content and the antioxidant capacity. We also identified some of the compounds by the DAD-HPLC method. We observed that the compounds and their proportion were different according to the ethanol content of the extracts. However, chlorogenic acid represented 95% of the composition in all of them. To test the neuroprotective activity, we assayed different concentrations of PPW extract *in vitro* oxidative stress model. First, we observed that low concentrations of PPW extracts were not cytotoxic and slightly increased the mitochondrial membrane potential ($\Delta\psi_{mit}$). We also demonstrated that pretreatment with these concentrations of PPW extracts protected injured-oxidative stress cells. Our results showed that PPW polyphenols restored the $\Delta\psi_{mit}$ and ROS levels modified by the injured-oxidative stress and protected cells from cell death. These findings suggest that potato peel waste would be a good source of neuroprotective antioxidant polyphenols to develop a dietary supplement with some impact on human health.

643. (306) **TARGETING THE NAV1.2 CHANNEL TO FIND NOVEL ANTICONVULSANT DRUGS: A STRUCTURE-BASED DRUG REPOSITIONING APPROACH**

Manuel Llanos[1], Nicolás Enrique[2], Maria Laura Sbaraglini[1], Alan Talevi[1], Verónica Milesi[2], Luciana Gavernet[1], Pedro Martín[2].

[1]Laboratorio de Investigación y Desarrollo de Bioactivos (LIDeB) - Universidad Nacional de La Plata, Buenos Aires, Argentina., [2]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Universidad Nacional de La Plata - CICPBA - CONICET, Buenos Aires, Argentina.

Epilepsy is the second most common neurological disease globally, affecting nearly 50 million people worldwide. While pharmacotherapy is the first-line treatment for this pathology, current anticonvulsant drugs (ACDs) fail to control seizures in over 30% of patients. In this regard, our objective was to repurpose existing drugs as safer and better-tolerated ACDs, which overcome the drug resistance problem. To this end, we focused on the voltage-gated sodium channel isoform 1.2 (NaV1.2), a classical target for anticonvulsant drug discovery. Starting from the NaV1.2 Cryo-EM structure, we used Rosetta to sample a conformational ensemble around the initial conformation. Then, a dataset of compounds evaluated against the target (323) was docked on every structure of the ensemble (101), systematically exploring several docking conditions. The final model, able to identify compounds binding to the channel's pore domain, was applied in a virtual screening campaign over the DrugBank database. Three hits: Montelukast (MTK), Cinnarizine (CNZ), and Novobiocin (NVB) were selected for experimental testing by the patch-clamp technique on NaV1.2 channels heterologously expressed in HEK293 cells. MTK and CNZ were the most potent inhibitors, blocking 95.3±4.2% (n=6) and 98.1±1.5% (n=6) of the sodium current at 10 μM concentration, respectively. Moreover, all compounds were able to stabilize the inactivated state of NaV1.2 channels, left-shifting the h-curve by -25.66±3.21mV (n=6), -3.32±0.86mV (n=5), and -30.75±3.00mV (n=5) for MTK, NVB and CNZ, respectively. Additionally, *in vivo* experiments showed that the three candidates presented anticonvulsant effects in three animal models of seizures: the Maximal Electroshock Seizure, 6-Hz psychomotor, and Pentylenetetrazol tests. Altogether, the experimental results validate the high predictive power of the docking model developed, and further support the computer-aided drug repositioning approach as a promising strategy to find novel ACDs.

644. (316) **IN SILICO SEARCH FOR NEW SELECTIVE NAV1.2 BLOCKERS FOR THE TREATMENT OF DRAVET SYNDROME**

Maximiliano J. Fallico[1], Denis N. Prada Gori[1], Manuel A. Llanos[1], Lucas N. Alberca[1], Luciana Gavernet[1], Alan Talevi[1]

[1] Laboratory of Bioactive Compounds Research and Development (LIDeB), Faculty of Exact Sciences, National University of La Plata (UNLP), La Plata, Buenos Aires, Argentina

Dravet syndrome is severe childhood epilepsy, characterized by loss-of-function mutations in NaV1.1 channels. Here, we pursued the computer-aided identification of compounds that selectively block Nav1.2, without blocking Nav1.1, as potential treatments. For that purpose, we resorted to a combination of ligand-based and structure-based models. Two datasets of compounds previously assayed against Nav1.1 and Nav1.2 were compiled from the literature. After data curation, 91 and 167 compounds tested against Nav1.1 and Nav1.2 were kept. 2000 and 4000 linear classifiers based on random subspace exploration of Mordred molecular descriptors were built for Nav1.1 and Nav1.2, respectively. Model ensembles were selectively obtained using the area under the ROC curve in retrospective screens as a selection parameter. The top 12 and 11 models for Nav1.1 and Nav1.2 were combined, in that order, using the MIN operator. Two repurposing databases, DrugBank and Drug Repurposing Hub, were then prospectively screened. Compounds selected as Nav1.2 blockers were subsequently screened by the ensemble of Nav1.1 models, which is used here as an anti-target, to ensure selectivity. 40 selective in silico hits were selected and further analyzed using molecular docking. The candidates were docked into the experimental 3D structure of Nav1.2, and the binding interactions were analyzed. 3 of the resulting hits were acquired and submitted for experimental confirmation in patch clamp. Our results confirmed the predictivity of our hybrid approach to detect new anticonvulsant agents with potential applications as Dravet treatment.

645. (336) **ANTIMETASTATIC AND CYTOTOXIC PROPERTIES AND SAFETY ASSESSMENT OF A NEW COMPOUND BASED ON SILIBININ AND MAGNESIUM(II) CATION**

Juan José Martínez Medina[1], Javier Rodriguez[1], Luciana G. Naso[2]

[1]Universidad Nacional del Chaco Austral - Presidencia Roque Sáenz Peña, Chaco, Argentina.

[2]CEQUINOR-CONICET-CICPBA-UNLP, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Objective: On the basis that metal-chelated flavonoids show better biological effects than flavonoids, we have synthesized and characterized a new coordination compound between silibinin and magnesium(II) cation (Mgsil). The anticancer and antimetastatic activity on the HeLa cell line was determined and coupled with a safety evaluation. Methods: Mgsil has been characterized by spectroscopy techniques. The effects of the compounds on the cell viability were measured by MTT assay. To evaluate the probable mechanism of action, morphological changes, intracellular reactive oxygen species ROS content (using CM-H2DCFDA probe), and mitochondrial membrane potential (MMP) (using DIOC6 probe) were used. The effect of the compounds, at non-cytotoxic concentrations, on adhesion, migration, and invasion was investigated. The safety profile of Mgsil was assessed in terms of mutagenicity (Ames test) and acute toxicity (*Artemia salina* test). Results: The proposed structure for the compound is $[Mg(sil)(OH_2)_2]Cl.3H_2O$. A significant decrease (ca. 70 %) in HeLa cell viability was observed at the concentration of 100 μM of Mgsil after 24 h treatment. Our results were consistent with morphological changes noted: cell shrinkage and nuclear condensation. Silibinin and Mg(II) cation did not induce cell death at tested concentrations. Mgsil was not toxic for HaCat cells (inhibiting 18 % cell viability at 100 μM), showing its specific toxicity against the cervical cancer cells. Besides, it has not mutagenic behavior on *Salmonella typhimurium* (TA98 and TA100) and no toxicity on brine shrimp at this concentration. The complex produced an increment in ROS levels and a decrease of MMP in a dose-dependent manner (ca. 200 and 30 % at 100 μM, respectively) compared to the untreated cells. Mgsil suppressed cell migration, adhesion, and invasion (at 10 μM). Conclusion: Mgsil has an adequate safety profile and is capable of inducing HeLa cell death and of reducing, *in vitro*, cervical cancer metastasis.

646. (339) **REFINEMENT OF THE TECHNIQUE OF DRUG ADMINISTRATION THROUGH THE APPLICATION OF ELECTROSPUN TRANSDERMAL MEMBRANES**

Stella Maris Echarte [1,2]; Dalila Orallo, [1]; Carolina Cámara [1,2]; Anabela La Colla [1,2]; Guadalupe Rivero, [2,3]; Andrea Chisari, [1,2]
[1] Grupo de investigación "Enfermedades Metabólicas" del Departamento de Química y Bioquímica de la Facultad de Ciencias Exactas y Naturales de la Universidad Nacional de Mar del Plata
[2] CONICET
[3] Grupo de Polímeros Biomédicos del Instituto de investigaciones en Ciencias y Tecnología de Materiales (INTEMA)

The controlled release of bioactive agents can be achieved from their encapsulation in a porous matrix in a controlled or sustained manner over time, generally by diffusion. The use of electrospun cellulose acetate membranes as transdermal patches with vitamins E has been reported, and their in-vitro release has been characterized. However, no in vivo studies have been carried out. Polymeric controlled release systems allow evaluating different doses and modulating their release over time, through the alteration of the geometry, architecture, size, surface properties and composition of the membranes. We aimed to prepare and characterization of nanofibrous matrices electrospun cells loaded with vitamin E for *invivo* use as transdermal patches in wound healing in a Wistar rat model. Polymeric solutions of poly(ε-caprolactone) (PCL, 80000Da) at 15-20% w/v were prepared. Different concentrations of Vitamin E were incorporated 1% (A); 6% (B) and 12% (C). The punch technique was used to generate the wound in the middle dorsal part of the rat. A membrane disk was placed directly over the area to be healed.
The efficient incorporation of vitamin E into the membranes was obtained by Agilent 8453 UV-visible absorption spectroscopy, performing a calibration curve with Vitamin E as standard compound at 296 nm. Morphological characterization was performed by scanning electron microscopy (SEM). Regarding the in vivo model, the diameter of healing over time was measured and histological sections of skin were made and stained with the H&E technique. The following results of mg Vit E/g membrane were obtained for (A) 4.90±0.02; (B) 16.86±0.05 and (C) 46.05±0.03. We observed that the diameter of the wound decreases faster in the membranes containing (B) and (C). Regarding histology greater amount of scar tissue is associated higher concentration of vitamin. In conclusion, improvements in the healing process are observed, which demonstrates the effectiveness of the membranes in drug dosing.

647. (440) **OBTAINING SPECIFIC ANTIBODIES AGAINST BLACK MAMBA VENOM USING SILICA NANOPARTICLES AS ADJUVANT**

Federico G. Baudou[1,2], María Herrera[3], Florencia S. Conti[1], Martín Desimone[2,4], Laura Leiva[5], Mauricio de Marzi[1,2]. Guillermo L. Montero[3]
[1]Universidad Nacional de Luján (UNLu), Depto de Ciencias Básicas, Laboratorio de Inmunología, Instituto de Ecología y Desarrollo Sustentable (INEDES), CONICET-UNLu; [2]Consejo Nacional de investigaciones científicas y técnicas (CONICET); [3]Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica; [4]IQUIMEFA-CONICET, Facultad de Farmacia y bioquímica (FFyB, UBA); [5]Laboratorio de Investigación en Proteínas (LabInPro), IQUIBA-NEA (UNNE, CONICET), FaCENA, (UNNE).

Ophidism is a global problem that causes approximately 130 thousand deaths per year. In particular, *Dendroaspis polylepis* (black mamba) has a highly lethal venom (V) due to the presence of Kunitz-type toxins (dendrotoxins) and three finger toxins (3FTXs). Specific antivenom (AV) to avoid fatal accidents are produced by hyperimmunizing animals with V and adjuvants (ADJ). On the other hand, nanoparticles (NPs) have a wide spectrum of application, given their potential therapeutic, immunomodulation and ability to capture antigens and induce specific responses. Hence the interest in associating it with V, replacing classic ADJ such as Freund (F). Here, we generated complexes called nanovenoms (NV) by mixing 10 mg of silica NPs (SiOHNPs, diameter=400 nm, negative charge) and 1 mg of whole V from black mamba for 3 h under stirring. Then, we calculated the subcutaneous (SC) LD_{50} of V and NV in mice (CD-1, 18-22 g, N=5/level) using Probits. In addition, we immunized (5 doses, every 15 days) with V, F-V and NV groups of 5 mice and 3 rabbits (new zealand, 2.8-3.5 kg) (via SC, 5-10 μg V/mouse, 500 μg V/rabbit). At 30 and 60 days we took blood samples and the antibody titter (Ab) was measured in serum for each group by ELISA. At the end, the presence of specific Ab in rabbit crude serum was tested by Western Blot, and the neutralizing potency (ED_{50}) of the lethal activity of V (challenge 1.5 LD_{50} V, intravenous route, IV) was studied. The LD_{50} (μg V/mouse) was V=7.67 (CI=4.76-10.09) and NV=9.95 (CI=2.54-12.16). In mice there was no significant increase in the Ab titter measured by ELISA in the NV group, but in rabbits it was significantly higher compared to F-V ($p<0.05$). Furthermore, the presence of specific Ab for the main V toxins was confirmed. Finally, the ED_{50} (mg V/ml serum) was NV =0.08 (CI=0.03-0.20) and F-V=0.11 (CI=0.06-0.16). Under these conditions, the use of SiOHNPs would serve as potential ADJ in rabbit immunization schemes for mamba AV manufacture.

648. (677) **MOLECULAR BASIS OF ABCB1 INDUCTION BY SORAFENIB (SFB) IN HEPG2 CELLS. ROLE OF INSULIN-LIKE GROWTH FACTOR 2 MRNA-BINDING PROTEIN 1 (IGF2BP1)**

María Bucci-Muñoz, Aldana Magalí Gola, Verónica Inés Livore, Aldo Domingo Mottino, María Paula Ceballos, María Laura Ruiz.
Instituto de Fisiología Experimental (IFISE-CONICET), Fac. de Cs. Bioq y Farm. Universidad Nacional de Rosario (UNR).

IGF2BP1 is an RNA-binding protein and serves as a post-transcriptional fine-tuner regulating the expression of its mRNAs target. Its expression was found to promote hepatocarcinoma (HCC) cell proliferation, migration, invasion and correlates with poor survival and prognosis. Sfb is a first-line therapy used for HCC. Sfb resistance still remains one of the major causes of treatment failure and often involves ABC transporters upregulation, like ABCB1. We showed that IGF2BP1 constitutive levels are involved in regulation of ABCB1 expression, that Sfb induced IGF2BP1 and ABCB1 protein expression and that IGF2BP1 depletion prevented IGF2BP1 and ABCB1 induction by Sfb in HepG2 cells. We aim to elucidate the molecular mechanism of ABCB1 induction by Sfb and the role of IGF2BP1 in this event. HepG2 cells were incubated with Sfb 2μM or DMSO (control; C) for 24h, 48h and 72h. To evaluate IGF2BP1 participation in ABCB1 upregulation by Sfb, IGF2BP1 was transiently knocked down with a siRNA targeting human IGF2BP1 mRNA (IGF2BP1 siRNA) or scrambled (C siRNA) as control. Cells were treated, 24h after transfection, with Sfb 2μM for 48h. IGF2BP1 and ABCB1 mRNA levels were measured by Real Time PCR. Data was presented as mean±SEM, n=3-6, *$p<0.05$ vs C, $^{\#}p<0.05$ vs C siRNA, $^{\pi}p<0.05$ vs C siRNA+Sfb. Statistical analysis was performed using the Student's t test or One-Way ANOVA followed by Newman-Keuls test. Sfb treatment induced IGF2BP1 and ABCB1 mRNA levels at 48h (160±12*%, 136±9*%; respectively) and 72h (175±12*%, 160±11*%; respectively) whereas no changes were observed at 24h. As expected, in C siRNA cells Sfb increased IGF2BP1 and ABCB1 mRNA levels (165±15$^{\#}$%, 210±6$^{\#}$%; respectively). The knockdown of IGF2BP1 prevented IGF2BP1 and ABCB1 induction by Sfb (110±4$^{\pi}$%, 101±3$^{\pi}$%; respectively). Results suggest that IGF2BP1 induction is responsible, at least in part, for ABCB1 upregulation by Sfb. IGFBP1 inhibition could diminish multidrug resistance when ABCB1 substrate drugs are used.

649. (683) **INSULIN-LIKE GROWTH FACTOR 2 MRNA-BINDING PROTEIN 1 (IGF2BP1) INVOLVEMENT IN ABC TRANSPORTERS MODULATION BY PHYTOESTROGENS IN HEPATOCARCINOMA CELL LINES**

Aldana Magalí Gola, María Bucci-Muñoz, Verónica Inés Livore, Aldo Domingo Mottino, María Paula Ceballos, María Laura Ruiz.
Instituto de Fisiología Experimental (IFISE-CONICET), Fac. de Cs. Bioq y Farm. Universidad Nacional de Rosario (UNR).

P-glycoprotein (P-gp/ABCB1) and multidrug resistance associated protein 2 (MRP2/ABCC2) are canalicular export pumps that extrude endo- and xenobiotics out of cells. Previously we reported that genistein (GNT, 1 and 10 μM) induces ABCB1 and ABCC2 in HepG2 cells. It was also demonstrated a link between these transporters and IGF2BP1. However, it has never been tested whether other phytoestrogens like Dadzein (D) or S-Equol (E) modulate ABC transporters in HepG2 cells and whether IGF2BP1 can mediate the regulation of ABC transporters by phytoestrogens. Aim: To evaluate the effect of GNT, D and E on ABCB1, ABCC2 and IGF2BP1 protein expression and the role of IGF2BP1 in ABCB1 and ABCC2 regulation by phytoestrogens. Methods: HepG2 cells were incubated with GNT, D or E (0.1; 1; 10 μM) or vehicle (DMSO) for 48h. As culture medium DMEM/F-12 phenol red free supplemented with charcoal dextran treated fetal bovine serum was used. IGF2BP1, ABCB1 and ABCC2 protein levels were quantified by western blotting. IGF2BP1 was transiently knocked down with a siRNA targeting human IGF2BP1 mRNA (siRNA) or scrambled (SCR) as control. Cells were treated, 24h after transfection, with GNT 1μM for 48h. The efficiency of IGF2BP1 knock down was evaluated by western blotting. Results**:** All results are presented as mean±SEM, n=3, *p<0.05 vs C, #p<0.05 vs SRC. GNT (1 and 10 μM) increased IGF2BP1 (187±30*, 252±39*), D or E did not produced changes in IGF2BP1, ABCB1 or ABCC2. In SCR cells GNT increased IGF2BP1 protein levels (150±17$^{\#}$). The poor knock down of IGF2BP1 efficiency obtained in this experiment (23%) prevented us from performing an appropriate analysis of the role of IGF2BP1 in ABCB1 and ABCC2 induction by GNT in HepG2 cells. Discussion: Of the three phytoestrogens studied only GNT was capable of modulate IGF2BP1 and also ABCB1 and ABCC2 in HepG2 cells. IGF2BP1 involvement in ABCB1 and ABCC2 induction by GNT is currently being tested using small interference RNA strategies.

650. (851) **SYNTHESIS OF MULTIFUNCTIONAL PROBES FOR BORON NEUTRON CAPTURE THERAPY**

Oriana N. Beraldi[1], Cristian Cascardo[2], María Silvina Olivera[3], Cecilia Grissi[2], Hebe Durán[2], Luciana Giordano[1]

[1]Centro de Investigaciones en Bionanociencias (CIBION-CONICET), Ciudad de Buenos Aires, Argentina, [2]Instituto de Nanociencia y Nanotecnología (INN, CNEA-CONICET), Gerencia de Investigación y Aplicaciones, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina, [3]Departamento Coordinación BNCT, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina

BNCT (Boron Neutron Capture Therapy) is an alternative treatment modality aimed to improve the response for tumors resistant to conventional therapies. It consists of the selective incorporation of a ^{10}B enriched compound by cancer cells and neutron irradiation to produce the ^{10}B neutron capture that generates a ^{7}Li and an α particles. Both particles have a range of 5-10 mm, which allows the selective radiobiological effect in tumor cells that have incorporated ^{10}B while sparing normal tissues. BSH ($[B_{12}H_{11}SH]^{2-}$) is a boron cluster, approved for its use in BNCT as a disodium salt. Fluorescence imaging techniques are powerful tools that allow the visualization and analysis of the location of biomolecules due to their high space-time resolution. Boron based fluorophores, borondipyrromethenedifluoride (BODIPY), are widely used for this purpose due to their high photochemical stability, high absorption coefficient, and high fluorescence quantum yield, and also present an intrinsic fluorescence/BNCT duality. Here, we present a new BODIPY-based compound to which a BSH unit was incorporated. A BODIPY was functionalized with maleimide and reacted with BSH to obtain BODIPY-BSH in a total of 4 steps. BODIPY-BSH presents water solubility of at least 0,05mg/μL, and its absorption and emission maximums were located by spectroscopic characterization at 497 and 505nm respectively, with a red-shift of 5nm for both peaks in DMSO. We evaluated cytotoxicity in tumor cells of precursors and BODIPY-BSH. Cytotoxicity was determined by MTT assay. A375 melanoma cells were incubated with different concentrations of these compounds for 24 hours. No cytotoxicity was found up to 1μM. For higher concentrations, significant cytotoxicity was detected at 10μM and 100μM. We conclude that this new compound is a promising agent for BNCT at concentrations below 1μM. Irradiation, incorporation to different tumor cell lines, and microscopic characterization experiments will be carried out in the future.

651. (911) **ANTI-SARS-COV-2 MONOCLONAL ANTIBODIES APPLIED TO DIAGNOSTIC OF COVID-19**

Yanina Hiriart[1], Gastón Ortiz[2-3], Maia Elizagaray[1], Inés Harguindeguy[2], Andrea Crivaro[1], David Romanin[1], María Florencia Ceccacci[3], Sebastián Cavalitto[2-3], Guillermo Docena[1] y Martín Rumbo[2].

1-Instituto de Estudios Inmunológicos y Fisiopatológicos, UNLP-CONICET, 2- Centro de Investigación y Desarrollo en Fermentaciones Industriales Dr. Rodolfo Ertola, UNLP-CONICET, 3-Bamboo biotech SAS

The fast spreading of SARS-CoV-2 worldwide evidenced the need for rapid diagnostic methods. Taking it in account we obtained murine monoclonal antibodies (Mabs) against recombinant nucleocapsid (N) protein of wild type Sars-CoV-2 strain (Wuhan-variant) to be used in immunochemical diagnostic. We selected specific anti-N 5E9, 2A4 and 1B9 hybridoma clones. The Mabs were characterized by SPR and showed Kd values of 7.08 nM, 31 nM and 14.8 nM respectively. Indirect Immunofluorescense assay on Vero cells infected with diferent strains of coronavirus showed that anti-N Mabs recognized wild type and B.1.617.2 (Delta) and Omicron variants. The 59-peptide array spanning the nucleocapsid (N) protein of the USA-WA1/2020 strain from bei RESOURCES was used for epitope mapping by competitive ELISA. All Mabs recognized a single peptide each. With the aim of developing a rapid antigen test using lateral flow technology, one Mab was selected and policlonal antibodies against N protein were obtained from chicken. The lateral flow test was validated comparing the results obtained by PCR in 233 nasopharyngeal swabs and its sensitivity and specificity were 82.6% and 90.7%, respectively. In conclusion, we established a valid rapid antigen test that allows the detection of most relevant SARS-CoV-2 variants for COVID-19 diagnostic. This offer laboratory-independent results at the point of care in a cheaper and faster way than PCR.

REGENERATIVE MEDICINE AND NANOMEDICINE I

Thursday, November 17, 9-10:30 hr

Chairs: Esteban Fiore - Gustavo Yannarelli - Daniela Olea

652. (68) **IMT504 ENHANCES THE CONTRIBUTION OF GLAST+ WNT1+ BONE MARROW STROMAL PROGENITORS WITH HEPATOCYTE- AND ENDOTHELIAL-LIKE CELLS IN THE FIBROTIC LIVER**

Maximiliano Borda[1], María José Cantero[3], Esteban Fiore[3,4], Juan Bayo[3,4], Romina Sierra[1], Sofía Gómez Bustillo[2], Agustina Abalo[1], Gianlucca Giardelli[1], Alejandro Montaner[2,4] & Jorge B. Aquino[1,4]

[1]CONICET-Universidad Austral, Instituto de Investigaciones en Medicina Traslacional (IIMT), Developmental Biology & Regenerative Medicine Laboratory, [2]Instituto de Ciencia y Tecnología Dr. César Milstein. CABA, Argentina, [3]CONICET-Universidad Austral, Instituto de Investigaciones en Medicina Traslacional (IIMT), Gene Therapy Laboratory, [4]Consejo de Investigaciones Cientificas y Técnicas, CONICET

Objectives: To evaluate the effect of IMT504 on fibrogenesis and the contribution of bone marrow stromal cells (BMSCs) subpopulations with hepatic tissue we used *GLAST^{CreERT2};Rosa26^{Tom}* mice. Materials and methods: Liver fibrosis was established by thioacetamide applications during 8 weeks, and animals were s.c. treated with 1 or 3 doses of IMT504. Liver fibrosis degree was determined by red Sirius staining and the phenotype of Tom+ analyzed by immunofluorescence. For *in vitro* assays, Tom+ and Tom- BMSC subfractions were separately incubated with IMT504 for 2 hours and their proliferative and motility capacities were analyzed by flow cytometry and Boyden

chamber. Results: IMT504 treatment was found to dose-dependently ameliorate liver fibrosis and to enhance hepatocyte proliferation. Application of 1 dose of IMT504 was shown to increase the incidence of hepatocyte-like Tom+ cells and endothelial-like Tom+ cells, an effect that was further significant in animals which received 3 doses of this oligonucleotide. Consistently, IMT504 induced the proliferation and motility of Tom+ BMSCs, with minimal effects on the Tom- fraction. Conclusions: IMT504 was found to reduce collagen deposition in a mouse liver fibrosis model and to enhance the contribution of Tom+ BMSCs with parenchymal and endothelial liver cells, likely through the induction of proliferation and migration of GLAST+ Wnt1+ bone marrow progenitors.

653. (89) **RESORBED ALVEOLAR BONE RECOVERY BY TREATMENT WITH PTH 1-34 AT LOW DOSES IN EXPERIMENTAL PERIODONTITIS**

Bonanno Marina Soledad[1,2], Zeni Coronel Estefanía Magalí[1,3], Seijo Mariana[1], Bidevich Nicolas[4], Avendaño María Eugenía[5], Preliasco Mariana[4], Davison Ricardo Mario[4], Zeni Susana Noemí[1]

[1]Laboratory of Metabolic Osteopathies. Institute of Immunology, Genetics and Metabolism (INIGEM), CONICET, School of Pharmacy and Biochemistry (FFyB), Buenos Aires University (UBA). [2]Department of Histology and Embryology, FOUBA. [3]Department of Biostatistics, FVetUBA. [4]Dental career, FNRN. [5]Diagnostic Imaging I, Dentist Faculty, UNCuyo.

We compared two low doses of PTH for the recovery of alveolar bone loss by experimental periodontitis. Periodontitis was induced in 18 female Wistar rats (221±15g); 96 hours later, rats were divided and treated by injection into the gingival sulcus: G1: saline solution (SS), G2 and G3 with 0.2 or 0,4 μg PTH 1-34/Kg/3 times a week. A control group (C) (n=5) without ligature was injected with SS. After 21 days, serum was extracted to determine CrossLapp (CTX), osteocalcin (OCN) and PTH by ELISA. Animals were euthanized and hemimandibles (HM) and tibias (T) were extracted for histomorphometry (percent bone volume: BV/TB% and periodontal space height: PSA). Results (C, G1, G2 y G3 respectively. Mean±SD, different letters indicate $p<0.05$): BV/TV% T 45.7±5.4; 41.3±5.4; 46.6±2.7; 44.1±3.0. BV/TV% HM 486±5.6^{b}; 36.4±4.4^{a}; 43.8±1.7^{ab}; 48.7±6.5^{b}. AEP (μm) 168.3±24.2^{a}; 634.2±125.3^{c}; 686.6±71.7^{c}; 354.1±43.6^{b}. CTX (pg/mL) 47.0±15.3^{a}; 71.7±15.7^{b}; 45.4±10.7^{a}; 42.8±9.8^{a}. OCN (ng/mL) 2.4±0.0^{a}; 2.7±0.0^{c}; 2.5±0.1^{a}; 2.6±0.0^{b}. PTH (pg/mL) 17.7±0.5; 16.7±1.2; 13.5±3.9; 13.7±3.5.Tibia BV/TV% did not evidence systemic effects by PTH treatment. Periodontitis increased bone remodeling while PTH treatment decreased bone resorption. G3 showed an increase in OCN levels without changes in CTX levels along with a significant improvement in hemimandible BV/TV% and a high recovery of AEP when compared to C group. Conversely, G1 cannot reach the values of C. Levels of PTH did not change with periodontitis induction or pharmacological treatment. Conclusions: intermittent administration of PTH in the high low dose tested decreased the progression of periodontal disease without inducing systemic effects suggesting an additional successful treatment for periodontitis. Grants of CONICET and UNRN.

654. (111) **CHANGES IN HUMAN AMNIOTIC EPITHELIAL STEM CELLS APOPTOSIS DURING THEIR HEPATIC DIFFERENTIATION**

Rodrigo Riedel1, Antonio Pérez-Pérez2, Nataly de Dios1, Luciano Pérez1, Mariana Jaime3, Roberto Casale3, Víctor Sánchez-Margalet2, Cecilia Varone1, Julieta Maymó1.

1Biological Chemistry, IQUIBICEN, CONICET-FCEN, UBA, Ciudad Autónoma de Buenos Aires, Argentina; 2Medical Biochemistry and Molecular Biology and Immunology, Sevilla University, Sevilla, Spain; 3Maternity, Posadas Hospital, Buenos Aires, Argentina.

Amniotic epithelial stem cells can be isolated from the human placenta at term. They express embryonic stem cells markers, and they are pluripotent. Moreover, they do not express telomerase, they are not tumorigenic, and they have immunosuppressive properties. These characteristics position hAECs as ideal candidates for regenerative medicine. Hepatic failure is one of the major causes of morbidity and mortality worldwide and the available treatments have several obstacles. Recently, hAECs have been spotlighted as an alternative source of hepatocytes because of their potential for hepatogenic differentiation. This work aimed to assess the changes in hAECs apoptosis during hepatic differentiation. Previously, we have demonstrated that hAECs efficiently differentiate to hepatic-like cells, by applying a specific hepatic differentiation (HD) protocol. We found that HD medium enhances proliferative capacity of hAECs. In this way, we showed that HD significantly increases PCNA expression, measured by Western Blot. We have analyzed the expression of some key apoptosis proteins (Caspase-8, Caspase-3, PARP-1) by qRT-PCR and Western blot. We have also evaluated p53 expression by immunofluorescence. We found a significant reduction in cleaved Caspase-3 and PARP-1 during hAECs HD. Moreover, Caspase-8 expression significantly diminishes in control hAECs while HD treatment prevented this effect. Additionally, we have showed a significant decrease in p53 nuclear localization during hAECs HD, evaluated by immunofluorescence. Finally, we determined that HD treatment decreases the apoptotic nucleus number, measured by DAPI staining. Our results suggest that our hepatic differentiation method not only induces proliferation and survival of hAECs but also reduces apoptosis rates, improving their quality and quantity for an eventual future transplant.

655. (126) **CYTOTOXICITY SCREENING AND ENHANCED ANTI-CANCER ACTIVITY OF ESSENTIAL OILS-LOADED NANOPARTICLES AGAINST A549 LUNG AND HCT-116 COLON CANCER CELLS**

María A. Castro[1], Juan Girotti[1], Sebastián Cisneros[2], Sonia Viña[3], Rosana Crespo[4], Guillermo R. Castro[5,6], Cecilia Yamil Chain[2], Germán Islan[7], Boris Rodenak Kladniew[1].

1 INIBIOLP (UNLP-CONICET CCT La Plata). Fac. de Cs. Médicas, UNLP, 2 Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), CONICET-UNLP, 3 CIDCA, Fac. de Cs. Exactas, UNLP – CCT La Plata, 4 Instituto de Farmacología Experimental de Córdoba (IFEC-CONICET), UNC, 5 Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC, 6 Nanomedicine Research Unit (Nanomed), Center for Natural and Human Sciences (CCNH), Universidade Federal do ABC (UFABC), Santo André, Brazil, 7 Laboratorio de Nanobiomateriales, CINDEFI, Fac.de Cs. Exactas, UNLP (CCT La Plata).

brodenak@med.unlp.edu.ar

Essential oils (EOs) from aromatic plants present several pharmacological activities including anti-cancer effects. EOs are low-cost, mostly non-toxic, and widely used in medicine, however, they show low bioavailability and are exposed to facile degradation by light, volatilization, and oxidation. Here, we designed EO-loaded solid lipid nanoparticles (SLN/EO), as a biocompatible system to deliver and improve EOs anticancer activity. Two cell models, A549 lung and HCT-116 colon cancer cells, were exposed for 24 h to 0-500 μL/L of eight different EOs obtained by hydrodistillation from leaves of local plants. Cell viability (MTT) was evaluated and IC50 were calculated. *Lippia alba* (EO2) and *Clinopodium nepeta* (EO3) were the two most active EOs in both cell lines. SLN containing EO2 (SLN/EO2) or EO3 (SLN/EO3) were prepared by hot melted-ultrasonication method. The morphology, size, z-potential (z-pot), and polydispersity index (PI) were determined by DLS and TEM. SLN/EOs showed spherical shape, sizes of 140-150 nm with narrow distribution (PI< 0.3), and negative z-pot (-5 to -13 mV). EO2 encapsulation decreased IC50 from 275 and 145 μL/L to 131 and 122 μL/L whereas EO3 encapsulation reduced IC50 from 205 and 200 μL/L to 66 and 134 μL/L in A549 and HCT-116 cells, respectively. SLN/EO3 and A549 cells were selected for the following experiments. The encapsulation efficiency (EE) and release of EO3 from SLN were measured by UV-vis spectrometry. The EE of EO3 was high (96.8%) and a controlled release was observed at acidic (5.0) and neutral (7.4) pH conditions. Cell death (Trypan Blue) and cell migration (Wound Healing) were evaluated. Encapsulation of EO3 increased cell death (from 1.3 and 3.4% to 12.6 and 16.0% at 50 and 100 μL/L, respectively,

p<0.05) and cell migration inhibition (p<0.05). Moreover, SLN/EO3 up to 100 μL/L was non-toxic in normal lung fibroblasts. Our results suggest that SLN/EO3 is a promising bioactive tool for lung cancer treatment.

656. (149) **ANALYSIS OF THE EXPRESSION AND MODULATION OF THE HSA-MIR-216/217 CLUSTER IN HUMAN PLURIPOTENT STEM CELLS AND ITS DIFFERENTIATED NEURAL PROGENY**

Rodríguez Varela MS[1], Ferriol S[1], Mucci S[1], Isaja L[1], Sevlever GE[1], Scassa ME[1], Romorini L[1]

[1.] Laboratorios de Investigación Aplicada a Neurociencias - INEU (FLENI- CONICET)

Human pluripotent stem cells (hPSCs) have the potential to differentiate into a wide range of specialized cells. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression that have been shown to be required for key biological processes such as differentiation. Many of them have been described as key regulators throughout neural development. However, knowledge on miRNA-based regulation during neuronal differentiation from hPSCs is still at its dawn. We have previously identified by RNA-Seq that the hsa-miR-216/217 cluster is highly expressed in hPSCs-derived neural stem cells (NSC) and neurons (NEU), without measurable expression levels in hPSCs. Given this expression profile, we aim to study the role of this cluster in hPSCs and its differentiated neural progeny. *In silico* target genes and gene ontology analysis suggested that this cluster may regulate genes involved in NSC and NEU proliferation. By modulating the expression levels of miR-217-5p in hPSCs and NSC with exogenous molecules (mimic/inhibitors), we found that over-expression of miR-217-5p in hPSCs increased the percentage of G1 phase population. In contrast, we did not observe differences in the cell cycle distribution in NSC-transfected with exogenous molecules that inhibit or overexpress miR-217-5p. Additionally, by a cell viability assay, we determined that miR-217-5p induction did not affect hPSCs viability. Finally, we analyzed by RT-qPCR the expression levels of putative miR-216/217 cluster target genes (*WEE1, RREB1, COL4A4, ANLN, GRIA3, SIRT1 and CYCLIN D1).* We observed that miR-217-5p over-expression decreased *OCT-4* and *GRIA3* mRNA expression levels in human embryonic stem cells and *WEE1*, *SIRT1* and *CYCLIN D1* mRNA expression levels in human induced pluripotent stem cells. Our results suggest that the upregulation of miR-217-5p accompanied by downregulation of genes governing stemness and proliferation may participate in the regulation of the early neural specification of hPSCs.

657. (222) **CARDIAC KERNEL TRANSCRIPTION FACTOR NKX2-5 IS REGULATED BY A NOVEL SUPER-ENHANCER REGION IN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES.**

María Agustina Scarafía, Nicolás Posteguillo, Sheila Castañeda, Guadalupe Amin, Belén Palma, Alan Möbbs, Joaquín Smucler, Carolina Colli, Lucía Moro, Ariel Waisman, Alejandro La Greca, Santiago Miriuka.

Laboratorio de Investigaciones Aplicadas a Neurociencias (LIAN), Instituto de Neurociencias (INEU) Fleni-CONICET.

The decisions to commit towards a cardiac cell fate are taken early during development. Defects in the cardiac program entail complications in cell specification and may lead to congenital heart diseases. Known as the cardiac kernel, *TBX5*, *GATA4* and *NKX2-5* are key players of the gene regulatory network governing cardiac differentiation. However, the mechanisms involved in their regulation and the decision making process of cardiac commitment are still not clear. RNA-seq results of human induced pluripotent stem cells (hiPSC) differentiated to cardiomyocytes (CM) revealed 2.208 regulated genes (p-adj<0.05). Among these, the primate-specific long non-coding RNA LINC881 is actively transcribed from a cardiac Super-Enhacer (SE) and we corroborated by qPCR that it was 88 times upregulated during early stages of cardiac commitment (between days 3 and 4 of the differentiation protocol). To study their role in cardiogenesis, we knocked-out (KO) the region comprising the promoter and first exon of LINC881 and most of the region predicted to be part of the SE. KO hiPSC lines were capable of differentiating into CM, however kernel gene *NKX2-5* was significantly downregulated compared to wild type (WT) lines (p<0.01). Rescue of LINC881 in KO lines using a doxycyclin-inducible system did not restore NKX2-5 to WT expression levels, though cells were still capable of differentiating to CM. Although we cannot exclude the participation of LINC881 in this mechanism or other aspects of cardiac commitment at this point, the results presented here indicate that LINC881 alone is insufficient to restore NKX2-5 expression levels and that the SE would be necessary for the regulation of NKX2-5, playing a central role in human cardiac development. Further experiments on the binding dynamics of transcription factors to this SE will aid in clarifying the underlying mechanisms of NKX2-5 regulation, contributing to our understanding of the onset and progression of heart diseases.

658. (223) **ISOLATION OF A NEW POPULATION OF *LACTICASEIBACILLUS CASEI* BL23 EXTRACELLULAR VESICLES BY HYDROSTATIC FILTRATION?**

D'Antoni Cecilia L [1,2], Domínguez Rubio A Paula [1,2], Moretton Marcela A [3,4], Piuri Mariana [1,2], Pérez Oscar E [1,2]

1- Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 2- Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (IQUIBICEN), Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. 3- Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Tecnología Farmacéutica I, Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. 4- Universidad de Buenos Aires, Instituto de Tecnología Farmacéutica y Biofarmacia (InTecFyB), Argentina.

Microbiota-derived extracellular vesicles (EVs) are naturally produced delivery systems with potential therapeutic purposes. The traditional method to isolate EVs is ultracentrifugation (UC). However, this method has many disadvantages, like EV lysis and high rates of contamination with bacterial proteins. The aim of this work was to compare the alternative method hydrostatic filtration (HF) for the isolation of EVs released by *Lacticaseibacillus casei* BL23 with the UC traditional method1. To this end, a 48 h-*L. casei* BL23 culture was centrifuged at 4500 g, and the cell-free supernatant was filtered using a 0.45 μm pore size. The filtered supernatant was poured inside a cellulose ester dialysis membrane (MWCO=1000 kDa). After 24h, the filtrate was dialyzed in PBS for 3 h. Dialyzed samples were concentrated using a Centricon ultrafiltration system (MWCO=100 kDa). We compared SDS-PAGE protein profiles of samples obtained by UC, by HF, and by HF followed by UC. For each sample, we determined the particle size distribution. The protein profile was similar among samples. The size distribution for samples obtained by HF (n=4) showed a bimodal distribution (peaks: 15.9 nm, 91.1 nm) unlike the resuspended pellet obtained by UC, in which a single size population was observed (peak: 62.3 nm). A bimodal distribution was also observed in the UC supernatant after HF (peaks: 17.06 nm, 84.46 nm). By using transmission electron microscopy (TEM), the presence of the larger size EV population was confirmed, and there were elements with a diameter below 30 nm. HF allowed for the isolation of two populations, while a single population is recovered in the UC pellet and one population remains in the supernatant. We described here the presence of a small population that may have implications in the development of natural or engineered nanoparticles for the delivery of small RNAs, proteins and drugs, with promising applications in nanomedicine.

659. (230) **INFLUENCE OF THE ISOLATION METHOD ON THE CHARACTERISTICS AND FUNCTIONAL ACTIVITY OF MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES**

Ricardo Malvicini[1], Maria Cecilia Sanmartin[1], Anna Maria Tolomeo[2], Diego Santa-Cruz[1], Maurizio Muraca[2], Natalia Pacienza[1], Gustavo Yannarelli[1].

[1] Laboratorio de Regulación Génica y Células Madre, Instituto de Medicina Traslacional, Trasplante y Bioingenieria

(IMeTTyB), Universidad Favaloro-CONICET, Buenos Aires, Argentina.
[2] Laboratory of Extracellular Vesicles as Therapeutic Tools, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy.

Extracellular vesicles (EVs) isolation methods are based on different physicochemical properties and may result in the purification of distinct EV populations. Objective: we compared two different isolation methods suitable for producing clinical-grade mesenchymal stromal cells-derived EVs, ion exchange chromatography (IEX) and ultrafiltration (UF), and we evaluated their impact on the composition and functional properties of the EVs. Methods: EVs were purified from the conditioned culture medium using an anion exchange resin (IEX) or Amicon filters (UF, 100kDa cut-off). We assessed nanoparticle size and distribution by NTA and tRPS, and morphology by TEM. We also measured protein, lipid, and total RNA concentration, and immunophenotyped both EV populations by flow cytometry (Macsplex assay). Moreover, immunomodulatory activity was tested using a standardized macrophage polarization assay and T cell stimulation assay. Results: We found that IEX and UF yielded a comparable amount of total particles (7.43E+10 vs. 7.04E+10) with similar size and distribution (mode 96nm vs. 97nm and 94nm vs. 101nm by tRPS and NTA, respectively). In addition, a similar amount of proteins (559±126μg vs. 479±83μg) and lipids (16±0.21μg vs. 18±0.29μg) was obtained with the two procedures. However, IEX yielded a 10-fold higher RNA quantity than UF (p<0.01). MSC-EVs isolated from IEX and UF were positive for the exosomal markers CD9, CD63, and CD81, and showed a comparable surface marker expression pattern. Finally, both populations showed similar immunomodulatory activity *in vitro* as they prevented the acquisition of the M1 phenotype in LPS stimulated macrophages and inhibited the acquisition of the activation markers CD69 and CD25 on T cells. Conclusion: MSC-EVs isolated by IEX and UF displayed similar physicochemical, phenotypic and functional characteristics. In our conditions, EVs presented an important anti-inflammatory activity, which may be helpful for regenerative therapies.

660. (268) **EFFECTS OF THE ADDITION OF TERIPARATIDE TO A BOVINE BONE GRAFTING MATERIAL ON THE REPAIR OF CRITICAL-SIZED BONE DEFECTS IN RATS. PRELIMINARY RESULTS**

Pellegrini G[1,2], Bonanno MS[1,5], Zeni Coronel EM[1], Orzuza R[4], Avendaño ME[1,3], Zeni SN[1]
[1]Laboratory of Metabolic Osteopathies. Institute of Immunology, Genetics and Metabolism (INIGEM), CONICET, School of Pharmacy and Biochemistry (FFyB), Buenos Aires University (UBA); [2]Division of Periodontics, Columbia University, College of Dental Medicine; [3]Diagnostic Imaging I, School of Dentistry, UNCuyo; [4]Department of General Biochemistry and Oral Biology, FOUBA; [5]Department of Histology and Embryology, FOUBA.

We previously determined the osteoconductive effect of a bovine bone graft manufactured in Argentina [Synergy Bone Matrix, Odontit Implant Systems, Argentina (SBM)], on the bone healing process in an experimental model in rats. Several studies have shown that intermittently administered PTH has an anabolic effect on cancellous and cortical bone. On these bases, we hypothesized that the addition of the synthetic PTH analog, Teriparatide (TPT), to SBM could accelerate bone regeneration. The aim of the present experimental study was to evaluate if the addition of TPT to SBM presents any additional beneficial effect versus the use of SBM alone. Thirty adult male Wistar rats of 333±39.22 (n=6/group) and a critical-size bone defect (CSD) was created in the medial aspect of both tibiae. All CSDs received one of the following treatments for 30 days: G1: control without treatment; G2: SBM (Lot No: E11121216); G3: SBM mixed with 20 μg of T (Osteofortil, BioSidus, Argentina); G4: injection of 0.125 μg/day of TPT and G5: SBM mixed with 20 μg of TPT and SC injection of 0.125 μg/day of the same drug. The animals were sacrificed 45 days after the beginning of the experiment. We analyzed serum PTH, P1NP and CTX (ELISA). At the end of the study, the tibiae were evaluated with X-ray microtomography (μCT). Serum PTH levels (pg/mL) at the end of the study were 4.6±1.2a; 11.3±2.3b; 16.4±0.7c; 17.9±0.8c and 19.1±0.5c, respectively, P1NP levels (pg/L) at the end of the study were 2.24±0.24a; 2.63±0.16c; 2.54±0.37c; 2.43±0.41b; 2.48±0.45b; respectively. Preliminary μCT results showed: G1 no bone defect repair; G2: poor repair; G3: repair; G4: overgrowth of bone tissue around the bone callus and G5: repair without bone overgrowth. Our findings showed that the addition of TPT to SBM mixture accelerated the bone repair process while the daily injection did not produce any additional improvement in the bone regeneration exerted by SBM.

661. (302) **BIOCOMPATIBILITY STUDY OF POLYMERIC MATRICES WITH APPLICATION IN BONE TISSUE ENGINEERING: EVALUATION OF ANGIOGENIC CAPACITY**

Angel Hurtado Cuba[1], Luz Torres[1,2], Tamara Oberti[2], Juan M. Fernandez[1]
1.- Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral (LIOMM) UNLP-CIC
2.- Grupo Macromoléculas, INIFTA, UNLP-CONICET

Bone Tissue Engineering (BTE) is an interdisciplinary science that uses materials to generate matrices that support cells and promote bone repair. Due to its key role in skeletal formation and repairing, we study the angiogenic capacity evaluating the proliferation of rat aortic smooth muscle cells (ASMC) and EA.hy926 endothelial cells on a family of hydrogels (HG). The HG is a polymer network of poly 2-hydroxyethylmethacrylate (pHEMA) cross-linked with ethylene glycol dimethylacrylate (E) with and without Alginate (Alg). The E concentration was modified (1, 0.5 and 0.25%) to analyzed this effect over final biomaterials properties. The angioinductive capacity of the bone marrow progenitor cells (BMPC) grown on the HG was evaluated using the culture media after 0 and 7d to analyze the migration of ASMC and EA.hy926 (cell monolayer wound method). We found that proliferation after 48h (MTT technique) of the ASMC on the HG was greater in pHEMA-Alg-1%E (125±2) and in pHEMA-alg-0.5%E (117±2) compared to pHEMA without alginate (100%±3%), but not in pHEMA-alg-0.25%E (99±3). After 48 h of culture, the proliferation of EA.hy926 cells on pHEMA-alg-1%E, 0.5%E and 0.25%E were: (141±2), (123±3) and (118±1), respectively, largest than pHEMA (100%±2%). We found an increase in the migration of ASMC when using the conditioned medium of the BMPC cells grown on pHEMA-Alg-1%E (211±4) and on pHEMA-alg-0.5%E (180±5) but not in pHEMA-alg-0.25%E (106±3) compared to pHEMA without alginate (100%±3%). Regarding the EA.hy926 cells, an increase in migration was observed when pHEMA-Alg-1%E (138±4) and pHEMA-alg-0.5%E (128± 2) was used but not pHEMA-alg-0.25%E (102±3) compared to pHEMA without alginate (100%±2%). Although we previously demonstrated that the 3 HG containing Alg could be good candidates for BTE, only those containing 1 and 0.5% E produce an increase in the angiogenic capacity necessary for bone repair.

662. (312) **THE PAN TGF-β INHIBITOR BRECEPT ENCODED BY A LENTIVIRAL VECTOR AMELIORATES METABOLIC SYNDROME IN A MURINE MODEL**

Carolina A Cámara[1], Anabela La Colla[1], Tania M Rodriguez[2], Stella Maris Echarte[1], Ricardo A Dewey[2,3], Andrea N Chisari[1]
1Departamento de Química y Bioquímica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, 2Laboratorio de Terapia Génica y Células Madre, INTECH (CONICET-UNSAM), 3 Rad Bio S.A.S

Metabolic syndrome (MS) is a growing public health problem closely related to insulin resistance (IR) and obesity and is also associated with an increased risk of developing non-alcoholic fatty liver disease, cardiovascular diseases, and type II diabetes. Nowadays, treatments for MS patients are limited. Molecular signals between the liver and adipose tissue could regulate lipid storage in adipose tissue. The TGF-β pathway is linked to hepatic steatosis and liver fibrosis. Brecept (Br), a pan TGF-β inhibitor, was developed by fusing TβRII-SE, a novel soluble TGF-β type II receptor splice variant, to the Fc portion of human IgG (TβRII-SE/Fc). We aimed to study the effect of lentiviral-mediated liver overexpression of Br (Lv-Br) in

a rat model of MS induced by Western Diet (WD). We compared three groups: control diet, WD, and WD+Lv-Br that received an intrahepatic injection of the lentiviral vector encoding Br at week 10. At week 18, we observed lower glycemia ($p<0.05$) and IR ($p<0.01$) in the Lv-Br group than in the WD group by insulin tolerance test. In week 21, animals were sacrificed. In liver tissue stained with H&E and Masson's Trichome, we observed in the WD+Lv-Br group, compared with the WD group, a remarkably decreased microvesicular steatosis and inflammatory infiltrate in the portal triad (PT). Furthermore, we found that fibrosis in the central vein (CV) and PT was significantly reduced ($p<0.001$). In adipose tissue stained with H&E, we observed hyperplasia ($p<0.01$) and a tendency to atrophy in adipocytes, comparing the WD+Lv-Br group with the WD group. Finally, in transmission electron microscopy liver sections, we observed an important decrease in lipid droplet number/field ($p<0.001$), mean lipid droplet area/field ($p<0.001$), endoplasmic reticulum hypertrophy, and lower swollen mitochondria in the WD+Lv-Br group than in the WD group. Therefore, these results suggest that liver overexpression of Br exerts a beneficial effect against MS induced by a WD in rats.

663. (422) **CHARACTERIZATION OF MOLECULAR MARKERS OF MATURATION IN HIPSC-CMS AS A MODEL FOR CARDIOREGENERATIVE THERAPIES. PRELIMINARY RESULTS**

Julia María Halek[1], Joaquín Smucler[2], María Agustina Scarafía[2], Alberto Crottogini[1], Mariano Nicolás Belaich[3], Santiago Miriuka[2], Alejandro La Greca[2], Paola Locatelli[1].
[1]IMeTTyB, Universidad Favaloro, CONICET. [2]LIAN, INEU Fleni-CONICET, [3]Universidad Nacional de Quilmes

Our objective was to characterize the molecular markers of adult cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) maintained in culture to induce temporal maturation, for its use as *in vitro* models in cardiac regeneration. Cardiovascular disease is the leading cause of morbidity and mortality worldwide. In this context, hiPSC-CMs represent a promising model for the design and study of therapeutic strategies. Considering its immature fetal phenotype and the lack of scientific consensus about the time in culture to consider them adult, it is necessary to generate and characterize hiPSC-CMs from long-term cultures. Objective: To characterize the temporal expression of molecular maturation markers to define the culture duration necessary to obtain hiPSC-CM with an adult phenotype, for its further use in the assessment of cardioregenerative gene therapies. Methodology: hiPSC were cultured and differentiated into cardiomyocytes. Samples were obtained at different times in culture (21, 40, 50, and 70 days), RNA was obtained and gene expression analysis of cardiomyocyte molecular markers was assessed through RT-qPCR. Results: as time in culture increased, we observed a rise in expression level of oxidative metabolism genes, such as *PPARGC1A* and *COX6A1*, and a decrease in the expression of cell cycle genes such as *CDK1, CCND2, CCNA2, CCNB1* and *AURKB*. We also observed an increase in expression levels of *RYR2* gene, which encodes the adult CM ryanodine receptor. Conclusion: The expression pattern of molecular markers in 70-day cultures of hiPSC-CMs is consistent with the adult cardiomyocyte phenotype. We conclude that 70-day cultures of hiPSC-CMs provide an appropriate model to study cardiac regeneration therapies in adult hearts.

664. (423) **UNRAVELLING THE MECHANISMS OF HUMAN AMNIOTIC MEMBRANE PROAPOPTOTIC EFFECT IN CELULLULAR HEPATOCARCINOMA MODELS**

Luciano A. Pérez[1], Rodrigo Riedel[1], Nataly De Dios[1], Antonio Pérez-Pérez[2], Mariana Jaime[3], Víctor Sánchez-Margalet[2], Cecilia L. Varone[1] and Julieta Maymó[1].
[1]Biological Chemistry, IQUIBICEN, CONICET-FCEN, UBA, Ciudad Autónoma de Buenos Aires, Argentina
[2]Medical Biochemistry and Molecular Biology, Sevilla University, Sevilla, Spain
[3]Maternity, Posadas Hospital, Buenos Aires, Argentina.

The stem cells, and particularly the placental stem cells, have called the focus of attention for their therapeutic potential to treat different diseases, including cancer. There is plenty evidence about the anti-tumoral effects of the human amniotic membrane given by their antiproliferative, antiangiogenic and proapoptotic properties. The amnion and its cells both secrete unknown factors and physically interact with tumor cells. Liver cancer is the fifth cause of cancer in the world, with a poor prognosis and survival. Alternative treatments to radio- or chemotherapy have been searched. A few studies have demonstrated the antitumoral effects of the amniotic membrane and their stem cells, but little is known about the molecular and cellular mechanisms involved We have previously demonstrated that the amniotic membrane conditioned medium (AM-CM) inhibits hepatocarcinoma cells proliferation and survival. The aim of this work was to study the apoptotic mechanisms involved in hepatocarcinoma cells death after treating them with AM-CM. First, we have observed that AM-CM induced both early and late apoptosis process, in HepG2 and Huh-7 hepatocarcinoma cells, evaluated by Annexin V/PI staining and flow cytometry analysis. In this way, we have also detected late apoptosis occurrence in HepG2 cells treated during 72 h with AM-CM, determined by DNA fragmentation assay. When analyzing the intrinsic pathway, we have observed that AM-CM treatment promoted an increase in Bax/Bcl-2 ratio and in cytochrome c expression, measured by Western blot and immunofluorescence. The extrinsic pathway was also analyzed. Thus, we determined by Western blot a decrease in total Caspase-8 and Bid expression, in HepG2 cells treated during 24 and 72 h with AM-CM. Our results position amnion-derived stem cells as emerging candidates in anticancer therapy.

665. (432) **MESENCHYMAL STROMAL CELLS EXPRESSING GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR BY *IN VITRO* TRANSCRIPTION MRNA INHIBITED TUMOR GROWTH IN MICE**

María José Cantero[1], Juan Bayo[1], Esteban Fiore[1], Luciana Dominguez[1], Barbara Bueloni[1], Catalina Atorrasagasti[1], Guillermo Mazzolini[1], Mariana Malvicini[2], Mariana García[1].
1- Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Facultad de Ciencias Biomédicas, CONICET-Universidad Austral, Buenos Aires, Argentina.
2- Laboratorio de Inmunobiología del Cáncer, IIMT, Facultad de Ciencias Biomédicas, CONICET-Universidad Austral, Buenos Aires, Argentina.

Introduction: Mesenchymal stromal cells (MSCs) have been used as carriers of anti-tumoral agents due to their capacity to home to tumors. It has also been demonstrated that the granulocyte macrophage colony-stimulating factor (GM-CSF) reduced tumoral growth by improving the antitumor immune response. On the other hand, it has been reported that low doses of doxorubicin (dox) may induce immunogenic cell death improving the recruitment of immune cells. The aim of this work was to determine the therapeutic effect of MSCs expressing GM-CSF in combination with low doses of doxorubicin (dox) in a murine model of hepatocellular carcinoma (HCC). Methods: GM-CSF expressing MSCs were obtained by transfection of *in vitro* transcribed mRNA (IVT mRNA). GM-CSF production was determined by ELISA. MSC expressing GM-CSF (MSC/GM-CSF) were characterized by migration assay and surface markers evaluation by flow cytometry. Murine model of HCC was developed by subcutaneous inoculation of HCC cells Hepa129 (1×10^6 cells) in C3H mice. Then, once tumors reach ~40 mm^3 mice were treated with PBS (control group), dox 5 mg/Kg (dox group) or dox 5 mg/Kg + MSC/GM-CSF (dox/GM-MSC group) at a dose of 1×10^5 cells expressing 0.045 μg/ml of GM-CSF by intratumoral (i.t.) injection. Tumor growth was measured three times a week. Results: ELISA of conditioned media of MSC/GM-CSF indicates that engineered MSCs are able to produce high levels of GM-CSF. In addition, migratory capacity and surface markers of MSCs were not modified by GM-CSF expression. Interesting, treatment with dox/GM-MSC not only reduced tumor growth but also expanded survival of mice in comparison with other groups ($p<0.05$ Dunnett's multiple comparisons test). Conclusions: This finding suggests that combination of MSC/GM-CSF with dox could be a new therapeutic strategy for HCC.

666. (442) **QUAKING KNOCK OUT INDUCES AN ALTERED PLURIPOTENT STATE WITHOUT COMPROMISING CARDIAC MESODERM COMMITMENT**

Carolina Colli[1] lAmin Guadalupe [1] l Sevlever Federico [1] l María Agustina Scarafia [1] l Alan Miqueas Möbbs [1] l Lucia Natalia Moro [1] l Ariel Waisman [1] l Alejandro Damián La Greca [1] l Santiago Gabriel Miriuka [1]

1. Laboratorio de Investigación Aplicada a la Neurociencia, FLENI - CONICET

Circular RNAs (circRNA) were found to participate in the differentiation of human pluripotent stem cells (PSC). They originate from backspliced junctions during processing of pre-mRNAs, producing covalently-closed stable molecules. Formation of circRNA depends on several factors, including the activity of the RNA binding protein Quaking (QKI). QKI5 increases during epithelial-to-mesenchymal transition (EMT), and therefore it could be necessary for the first stages of cardiac differentiation. To test this, we aimed to generate and characterize QKI knock out (KO) lines in PSC. For generating the KO cells (QKI-KO) we targeted a genomic region comprising the promoter region and first exon of QKI gene using CRISPR, affecting expression of relevant isoforms (5/6/7). We corroborated the success of our strategy by PCR and Sanger sequencing on genomic DNA and by RTqPCR and Western blot to confirm the absence of QKI expression. Next, we studied the pluripotent state of QKI-KO cells by assessing their proliferation and differentiation capacities. We observed that, while wild type (WT) and KO cell lines were morphologically indistinguishable, QKI-KO cells showed an increase in cells in S phase compared to WT (51,3%±1.9 vs 41,1%±1.4), demonstrated by measuring EdU incorporation by flow cytometry. Differentiation to cardiac mesoderm was apparently not affected in QKI-KO cells as evaluated by expression analysis of EMT markers (*EOMES*, *MIXL1*, *PDGFRα*) 24, 48h and 72h after addition of CHIR. However, pluripotency marker genes (*OCT4*, *SOX2* and *NANOG*) were not downregulated after CHIR addition, indicating that QKI-KO cells might have an altered exit from pluripotency. In summary, we successfully generated a KO cell line for QKI that evinces increased proliferation rates, sustained expression of pluripotent markers during early EMT and differentiates into cardiomyocytes. Further experiments will be directed to characterizing the different cardiac cell types obtained compared to WT.

667. (443) **ALTERNATIVE: QUAKING KNOCK OUT INDUCES AN ALTERED PLURIPOTENT STATE WITHOUT COMPROMISING CARDIAC MESODERM COMMITMENT**

Carolina Colli lAmin Guadalupe l Sevlever Federico l María Agustina Scarafia l Alan Miqueas Möbbs l Lucia Natalia Moro l Ariel Waismanl Alejandro Damián La Greca l Santiago Gabriel Miriuka

1. Laboratorio de Investigación Aplicada a la Neurociencia, FLENI - CONICET

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668. (452) **PHYSICO-CHEMICAL STUDIES OF CROTOXIN ISOLATED FROM RATTLESNAKE VENOM ADSORBED TO SILICA NANOPARTICLES FOR POTENTIAL USE IN THE PRODUCTION OF NEW ANTIVENOMS**

Florencia S. Conti[1], Exequiel Giorgi[1, 2], María Eugenia Díaz[1, 2], Mauricio De Marzi[1, 2], Juan Pablo Rodríguez[3], Federico G. Baudou[1, 2].

[1]Universidad Nacional de Luján (UNLu), Depto. de Ciencias Básicas, Laboratorio de Inmunología, Instituto de Ecología y Desarrollo Sustentable (INEDES), CONICET-UNLu; [2]Consejo Nacional de investigaciones científicas y técnicas (CONICET); [3]Laboratorio de Investigaciones Bioquímicas de la Facultad de Medicina (LIBIM), Instituto de Química Básica y Aplicada del Nordeste Argentino (IQUIBA-NEA),Universidad Nacional del Nordeste, Consejo Nacional de Investigaciones Científicas y Técnicas (UNNE-CONICET), Corrientes, Argentina.

The accident produced by venomous snakes, ophidism, is a globally neglected health problem. In Argentina, *Crotalus durissus terrificus* (southern rattlesnake) is one of the species that generates the most accidents. Its venom (V) contains mainly crotoxin (CTX), responsible for the high lethality. On the other hand, the use of nanoparticles opens a range of possibilities within the medical field given its potential therapeutic and immunomodulatory use. Therefore, the interest in transporting V toxins in the production of new antivenoms (AV).
In this work, we isolate CTX by fast protein liquid chromatography (FPLC) and generate nanovenoms (NVs), complex formed by CTX adsorbed to silica nanoparticles (SiNPs) of 150 nm in diameter of both charges. For this, 1 ml of CTX was mixed under stirring with 10 mg of SiNPs (+/-). The charge and size were corroborated by potential Z and DLS (dynamic light scattering) respectively. NVs were also microphotographed by Transmission Electron Microscope (TEM) and analyzed by Fourier Transform Infrared (FT-IR). Additionally, we studied the enzymatic activity exerted by CTX present in NVs by hemolysis radial test. The results obtained by FPLC allowed to isolate CTX, the maximum peak (11th fraction), then purified and concentrated by dialysis and lyophilized (2 mg/ml PBS 10 mM). Through TEM we can observe on NVs the protein halo corresponding to the presence of CTX, and the FT-IR spectra obtained show the peak (abs 510 cm^{-1}) corresponding to H-S-H bounds characteristic of CTX. Finally, it was found that NVs retain their enzymatic activity thanks to the halo produced in the hemolysis radial test. The use of these complexes studied here at the physical-chemical level, where the activity of CTX adsorbed to NVs is also preserved, it allows us to continue perfecting their possible use as adjuvants in the production of new generation AV.

REGENERATIVE MEDICINE AND NANOMEDICINE II

Friday, November 18, 14-15:30 hr

Chairs: Julieta Maymo - Leonardo Romorini

669. (454) **POLYMERIC MICELLES CO-LOADED WITH PACLITAXEL AND HISTAMINE AS A NEW STRATEGY TO IMPROVE CONVENTIONAL CHEMOTHERAPY FOR BREAST CANCER**

Melisa B. Nicoud[1], Ignacio Ospital[1], Mónica A. Táquez Delgado[1], Jennifer Riedel[2], Pedro Fuentes[2], Ezequiel Bernabeu[2], Marcela A. Moretton[2], Roxana Rubinstein[1], María Jimena Salgueiro[3], Paolo Lauretta[1], Diego A. Chiappetta[2], Vanina A.

Medina[1,3]
1 Laboratorio de Biología Tumoral e Inflamación. Instituto de Investigaciones Biomédicas (BIOMED), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Católica Argentina (UCA), Argentina, 2 Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Tecnología Farmacéutica I, Buenos Aires, Argentina, 3 Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Física, Buenos Aires, Argentina

Breast cancer is now the most commonly diagnosed cancer and the leading cause of cancer death in women worldwide. Triple negative breast cancer (TNBC) is the most aggressive subtype, associated with the worst prognosis. Non-specific chemotherapeutic drug paclitaxel (PTX) is used as the standard regimen, but its poor solubility and several associated adverse effects conditioned its clinical use. The aim of this work was to improve the chemotherapeutic response of PTX by developing new nanomicellars polymeric formulations. Micellar systems were developed with the biocompatible polymer Soluplus® (S), surface decorated with glucose residues (SG) and co-loaded with histamine (HA, 5 mg/mL) and PTX (4 mg/mL). Their physicochemical characterization includes the determination of micellar size and its distribution, zeta potential and physical stability. We evaluated antitumor activity in human MDA-MB-231 and murine 4T1 TNBC cells. Cytotoxicity and apoptotic assays showed that HA-PTX co-loaded micelles exhibited better antitumor activity compared to free PTX and Genexol® (commercial micellar-based PTX-nanoformulation) and single treatments loaded micelles in both cell lines ($P<0.05$). HA-PTX co-loaded SG micelles significantly reduced proliferative capacity and cell migration while increasing reactive oxygen species production in TNBC cells. Interestingly, histamine reduced PTX-cytotoxic effects on HBL-100 non-tumorigenic breast cells. In a murine model of TNBC developed in BALB/c mice with 4T1 cells we found that HA-PTX loaded SG micellar system reduced tumor weight and neovascularization, and modulated subpopulations of immune cells including $CD8^+$ cytotoxic T lymphocytes and NK cells in the tumor draining lymph nodes and in the spleen. We conclude that co-encapsulation of PTX with histamine might improve the chemotherapeutic effects of PTX.

670. (490) **DEVELOPMENT AND CHARACTERIZATION OF SOLPULUS® NANOMICELLES ASSOCIATED WITH IgG AGAINST SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* VIRULENCE FACTORS**

Girón Daniel[1], Gomez Fernando[1], Amaral María Marta[1], Chiappetta Diego[2], Moretton Marcela[2], Sacerdoti Flavia[1].
1. Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
2. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Tecnología Farmacéutica I, Buenos Aires, Argentina. UBA-CONICET.

Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin producing *Escherichia coli* (STEC), a foodborne pathogen responsible for the development of Hemolytic Uremic Syndrome (HUS). Several STEC virulence factors are involved in the colonization of the intestinal epithelia, including Intimin (Int) and EspB. We propose that specific antibodies (IgG) coupled to nanomicelles (NMs) can be used as a tool for the treatment of STEC infections and prevention of HUS. The aim of this work was to couple Soluplus® NMs with IgG against Stx2, Int and EspB, characterize them and evaluate their functionality against STEC. IgG-Stx2, IgG-Int, IgG-EspB were purified from hyperimmune bovine colostrum by affinity column. Soluplus® NMs were coupled with IgG (NM-IgG-Stx2, NM-IgG-Int, NM-IgG-EspB) by mixing 4 mg/ml of IgG with 1% NMs in PBS. The morphology and hydrodynamic diameter of the NMs with or without IgG were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). NM-IgG-Stx2 neutralization capacity was evaluated on HGEC and Vero cells. The capacity of NM-IgG/Stx2/Int/EspB to modify the growth behavior of STEC and the ability to inhibit bacterial adhesion to HCT-8 was evaluated. No significant differences between NMs hydrodynamic diameter and NM-IgG were observed (71.30 ± 2.10 and 92.38 ± 18.51 nm respectively, $p>0.05$). TEM analysis revealed circular particles with a diameter of approximately 100 nm for both NMs and NM-IgG. NM-IgG-Stx2 preserved the neutralization capacity of the toxin, in a dose dependent manner, compared to IgG ($p>0.05$). Finally, no inhibition of STEC growth nor inhibition of bacteria adhesion *in vitro* was observed with IgG nor NM-IgG/Stx2/Int/EspB. We proposed that IgG assemble efficiently on the corona of the NMs without a significant size, shape and IgG functionality change. These results suggest that the inhibitory strategy against STEC may be improved involving other adherence virulence factors.

671. (507) **CHARACTERIZATION OF HUMAN AND OVINE MYOFIBROBLASTS TO BE USED AS AN IN VITRO MODEL OF CARDIAC REPROGRAMMING**

Francisco Stefano Cimbaro[1], María del Rosario Bauzá[1], Alejandro J Simonin[2], Araceli Castro[1], Alberto José Crottogini[1], Mariano N Belaich[2], Fernanda Daniela Olea[1].
1Instituto de Medicina Translacional, Transplante y Bioingeniera - Universidad Favaloro – CONICET, Argentina.
2-Universidad Nacional de Quilmes, Argentina

Objectives: several strategies have been proposed to induce reverse left ventricular remodeling secondary to ischemic cardiomyopathy. One of them is to promote the conversion of myofibroblasts to cardiomyocytes through gene therapy-mediated cardiac reprogramming . As an initial step to obtain an in vitro model for reprogramming assays, the aim of this study was to obtain cultures of human and ovine myofibroblasts, characterize them and evaluate the efficiency of transduction of an ad-hoc baculoviral vector. Materials and Methods: human and ovine left ventricle explants were cultured during 14 days, letting myofibroblasts migrate and attach to the dish. Grounded cells were replated and cultured for three passages. To characterize if these cells were fibroblast, gene expression of Col1a1, Col1a2, Col3a1 and FSP1 (by RT-qPCR) and labeling for vimentin and troponin T (by immunocytochemistry) were assessed. In order to optimize the transduction efficiency, the cells were infected at different MOI (0, 50, 100, 200 and 400) with a baculovirus encoding GFP gene and the percentage of fluorescent cells was measured at 24 hours by flow cytometry. Results: human cells from the explants showed increased expression of fsp1, Col1a1 and col1a2 genes with respect to control human cells, while ovine cells only showed increased expression of Col3a1 with respect to ovine control cells. Furthermore, both cell types were positive for vimentin and negative for troponin T. Optimal transduction efficiency was observed at MOI 100 in both human (83,22%) and ovine (94,39%) cells. Conclusions: cultured human and ovine cells extracted from heart explants expressed genes of collagen and were positive to vimentin and negative to Troponine T demonstrating to be myofibroblasts. Furthermore, these cells were efficiently transduced, indicating that they can be used as an in vitro model to study reprogramming by gene manipulation.

672. (633) **EXTRACELLULAR VESICLES PRODUCED BY PROBIOTIC BACTERIA: CHARACTERIZATION AND VISUALIZATON BY HIGH PERFORMANCE MICROSCOPY TECHNIQUES**

Ana Paula Domínguez Rubio[1,2], Cecilia L. D'Antoni[1,2],Cecilia Samaniego[3,4], Carla Spagnuolo[3,4], Mariana Piuri[1,2], Oscar E. Pérez[1,2]
1 Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 2 Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. 3 Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 4 Centro de Investigaciones en Hidratos de Carbono, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

Extracellular vesicles (EVs) are composed of a lipid bilayer with cytosolic components such as DNA, RNA and proteins. Bacterial nanosized EVs have been proposed to be involved in signaling between probiotic bacteria and their mammalian hosts. The aim of this study was to analyze the EVs produced by *Lacticaseibacillus casei* BL23 (47±3nm) and *Bacillus subtilis* 168 (142±14nm) by Atomic Force Mycroscopy (AFM), Transmission Electronic Microscopy (TEM), Confocal Laser Scanning Microscopy (CLSM) and Super-resolution Microscopy (SRM). *L. casei* BL23 was grown in MRS medium at 37°C for 48h and *B. subtilis* 168 was grown in BHI medium at 37°C under 200rpm agitation for 18h. Cultures were spun at 4,000g for 25 min at 4°C. The supernatant was filtered, concentrated using a 100kDa filter and centrifuged at 110,000g for 2h at 4°C. The pellet was resuspended in PBS for AFM and TEM, or labeled with CFSE or boroxol for CLSM and SRM. Topography of EVs obtained by AFM showed EVs with spherical shape morphology for both strains. To elucidate details regarding shape and ultrastructure, TEM images were analyzed. *L. casei* BL23 EVs showed bilayered membranes and an electron-dense luminal content; consistent with the notion that vesicles contain bioactive cargo such as proteins or nucleic acids However, *B. subtilis* 168 EVs did not always show a central electron-dense core. Both techniques confirmed the size distribution of these EVs (n=3, p>0.05). CLSM images of EVs stained with CFSE showed an identical signal to EVs shedding from the bacteria, but EV size resulted smaller than the theoretical resolution of the microscope. By SRM we observed spherical EVs, with the certainty that the fluorescence comes only from a single EV. The results contribute to the characterization of bacterial EVS. The expression and encapsulation of biomolecules into EVs could represent a scientific novelty with applications in food, nutraceuticals and clinical therapies.

673. (648) **TRANSFERRIN BIOCONJUGATION OF CONJUGATED POLYMER NANOPARTICLES FOR SELECTIVE PHOTODYNAMIC THERAPY OF GLIOBLASTOMA**

Matías Daniel Caverzán[1], Paula Martina Oliveda[2], Rodrigo Emiliano Palacios[1], Carlos Alberto Chesta[1], Luis Exequiel Ibarra[2]

[1]Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Rio Cuarto y CONICET, Río Cuarto, Argentina, [2]Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Rio Cuarto y CONICET, Río Cuarto, Argentina,

Glioblastoma (GBM) is the most aggressive brain tumor. New therapies are proposed such as Photodynamic Therapy (PDT) that combines light, oxygen and photosensitizers (PSs) to eradicate tumor cells through massive generation of ROS. We recently developed conjugated polymer nanoparticles (CPN) as new efficient PSs. The main objective of the present work was to decorate CPN surface with holo-transferrin (Holo-Tf) that recognize transferrin receptor 1 (TfR1) which is overexpress in GBM cells and endothelial cells from the blood-brain barrier. CPN were synthetized by nanoprecipitation method using conjugated polymer F8BT, two type of stabilizer polymers (poly(styrene-co-maleic anhydride) PSMA and polystyrene-polyethylene glycol-carboxyl PSPEG) and porphyrin PtOEP; and afterwards, protein bioconjugation was achieved by carbodiimide reaction using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Different proportions of coupling reagents, CPN and Holo-Tf were tested in order to determine the most optimal coupling reaction efficiency. Purification of unbound Holo-Tf was achieved by size exclusion centrifugation. Holo-Tf decorated CPN were characterized by DLS (size and Z potential) and gel-electrophoresis mobility. PSMA was able to contribute to the development of narrow size CPN (25 nm) with Holo-Tf. Using Human Protein Atlas, TfR1 expression was investigated in different cell lines including GBM to later validate receptor expression using flow cytometry immunophenotyping. Finally, cell uptake in GBM cell lines was evaluated comparing unconjugated CPN with Holo-Tf decorated CPN by flow cytometry and PDT efficacy by MTT assay. GBM U87MG and MO59K cells expressed more TfR1 than T98G and cell uptake was effective at low concentration (6,25 ug/mL) and different incubation times (30 min, 1 and 4 hs) using Holo-Tf decorated CPN. After PDT, Holo-Tf CPN improved cell death in TfR1-overexpressing GBM cells. CPN have risen as promising nanosystem platform to eradicate aggressive forms of cancers with selectivity of recognition of biomolecules.

674. (672) **ANGIOGENIC EFFECT OF *MEIS1* OVEREXPRESSION IN INFARCTED SHEEP HEART**

López AE[1], Bauzá MDR[1], Simonin JA[2], Scharn A[1], Castro A[1], Silvestro C[1], Crottogini A[1], Belaich MN[2], Olea FD[1], Locatelli P[1]

[1] Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB- Universidad Favaloro- CONICET), [2] Laboratorio de Ingeniería Genética y Biología Celular y Molecular Área Virosis de Insectos, Universidad Nacional de Quilmes.

Angiogenesis plays an essential role in post-myocardial infarction cardiac regeneration by supplying blood perfusion to the infarcted heart and guiding cardiomyocyte (CM) migration. There is controversy regarding the pro or anti-angiogenic role of the transcription factor meis1. Our previous *in vitro* results in rat indicate that meis1 is an angiogenic gene. Objectives: To inject the adult ovine infarcted heart with a baculoviral vector overexpressing *meis1* (Bv.Meis1), assess its effects and the expression of angiogenic genes. Methods: 12 young adult sheep with acute myocardial infarction (AMI) received 10 intramyocardial injections of 0.2 ml in the infarct border zone containing Bv.Meis1 or Bv.Null. Seven days after AMI, the animals were euthanized, and tissue samples of the injected infarct border zone were harvested. Angiogenic gene expression was assessed by RT-qPCR. Tissue samples of the infarct border zone underwent immunohistochemistry to quantify capillary and arteriolar densities. Results: Angiogenic gene expression such as *angiogenin, igf and hgf* were increased in infarcted sheep treated with Bv.Meis1 (*angiogenin* fold increase: 2.547±1.760 Bv.Meis1 vs. 1.257±0.8104 Bv.Null, $p<0.05$, *igf* fold increase: 2.086±1.558 Bv.Meis1 vs. 0.8445±0.4802 Bv.Null, $p<0.05$ and *hgf* fold increase: 3.165±2.630 Bv.Meis1 vs. 1.104± 0.7338 Bv.Null, $p<0.05$). Microvascular densities at 7 days post-treatment showed that capillary and arteriolar density increased in Bv.Meis1 group (1545± 372.1 capillaries/mm^2 and 15.54± 7.47 arterioles/mm^2, $p<0.05$) with respect to Bv.Null group (1234± 345.2 capillaries/mm^2 and 11.52± 5.55 arterioles/mm^2, $p<0.05$). Data was analyzed with t test and Mann Whitney´s test. Conclusion: The treatment of infarcted sheep with Bv.Meis1 suggests an angio-arteriogenic role of this transcription factor, as previously shown in rats. Further experiments are needed to elucidate meis1 mechanism of action.

675. (708) **DERMATAN SULFATE/CHITOSAN NANOMATERIALS AS A COMBINATORY STRATEGY WITH 5-FLUOROURACIL IN COLORECTAL CANCER**

[1]Blachman Agustín, [1]Birocco Ariadna, [1]Curcio Sofía, [2]Rodríguez Jésica A., [2]Barredo-Vacchelli Gabriela R., [3]Moya Sergio, [2]Camperi Silvia A., [1]Calabrese Graciela C.

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Ciudad Autónoma de Buenos Aires, Argentina; [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Nanobiotecnología (NANOBIOTEC) UBA-CONICET, Ciudad Autónoma de Buenos Aires, Argentina; [3]Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, Spain.

We reported the synthesis of a new polyelectrolyte complexes based on dermatan sulfate (DS) and chitosan (CS) (PECs) charged with the antiinflammatory peptide IRW; being able to deliver IRW to injured endothelial cells through CD44 receptor. This receptor plays a key role in initiation and progression of colorectal cancer (CRC). The aim of the present work is to study the interaction of PECs with CRC cells and evaluate the effect of the combinatory treatment between PECs and the chemotherapeutic agent 5-Fluorouracil (5-FU). The nanoformulation is synthesized by ionotropic gelification and characterized by Dynamic Light Scattering. HCT116 and Caco-2 CRC cell lines are employed. The interaction of FITC-PECs with both cell lines and their internalization, after 2, 4 and 6h, are studied by Flow Cytometry and fluorescence microscopy. Finally, the effects

of PECs and 5-FU combination on the metabolic activity of CRC cell lines is addressed by MTT assays. PECs displayed a single population of 442(±43) nm, with a size distribution of 0.366 (±0.031) and a Zeta-Potential of +37(±1) mV. No toxicological endpoints were detected with PECs (10 µg/mL of DS). After 4 hours of incubation the interaction and internalization of the nanosystems was observed for cells, reaching 62.44(±8.73)% and 24.87(±4.36)% for HCT116 and Caco-2, respectively; confirmed by fluorescence microscopy. This interaction decreased in the presence of antiCD44 antibodies prior to the addition of PECs ($p<0.05$). Pretreatment of both cell lines with the nanoformulation followed by 5-FU treatment, showed a significant decrease in the metabolic activity compared with 5-FU alone ($p<0.05$). In conclusion, PECs interact and are internalized by CRC cells. Hence, pretreatment with the nanoformulation followed by 5-FU exerted greater effects in the metabolic activity than the employment of the chemotherapeutic agent alone, paving the way to the possibility of applying this combination in CRC treatment.

676. (712) **CHROMATOGRAPHYC SCALABLE METHOD TO ISOLATE ENGINEERED EXTRACELLULAR VESICLES DERIVED FROM HUMAN UMBILICAL CORD PERIVASCULAR CELLS FOR THE TREATMENT OF LIVER FIBROSIS**

Luciana M. Domínguez[1], Milagros Albornoz, Juan Bayo[1], Agostina Onorato[1], Ma. José Cantero[1], Bárbara Bueloni[1], Catalina Atorrasagasti[1], Mariana García[1], Gustavo Yannarelli[2], Esteban Fiore[1], Guillermo Mazzolini[1].

[1] Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral CONICET, Buenos Aires, Argentina

[2] Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro-CONICET, Buenos Aires, Argentina

Introduction: We previously demonstrated that extracellular vesicles (EV) mediate the therapeutic effect of human umbilical cord perivascular cells (HUCPVC) over-expressing hIGF1 on liver fibrosis in mice. Aim: To apply a scalable method by affinity chromatography to isolate engineered HUCPVC-derived EV loading therapeutic genes for liver fibrosis therapy. Methods: EVs were isolated by ion exchange chromatography from supernatants of HUCPVC infected with adenoviruses codifying for IGF1 (EV-IGF1) or *green fluorescent protein* (EV-GFP). EVs morphology was analyzed by electron microscopy, markers (CD9, CD63, CD81), and IGF1 cargo expression by flow cytometry (FC), and ELISA. Antifibrotic effect of EVs was determined in experimental mice model of liver fibrosis (thioacetamide for 8 weeks). The treatments were administered on week 6 (groups: saline, EV-IGF1 or EV-GFP, 3 doses, 15 mg/dose/mice every 5 days). Collagen deposition was measured by Sirius red staining; immunohistochemistry for PCNA proliferating cells, and aSMA for activated Hepatic Stellate Cells (HSC) was performed. *In vitro* effects of EVs on HSC pro-fibrogenic genes expression (CFSC-G2 cell line) were evaluated by qPCR. Results: EV-IGF1 isolated by chromatography show a typical and homogeneous morphology, and were CD9, CD63 and CD81 positive. Increased IGF1 levels on EV-IGF1 determined by ELISA and FC indicate its loading on EV. *In vivo* treatment with EV-IGF1 resulted in a further amelioration of collagen deposition ($p<0.001$) and aSMA levels in liver tissue in comparison with controls, confirming its antifibrotic effect. Consistently, an increase of PCNA+ cells after EV-IGF1 ($p<0.0001$) administration shows the induction of liver regeneration. *In vitro* incubation of CFSC-G2 cells with EV-IGF1 resulted in downregulation of Col1a2, and aSMA expression ($p<0.001$ vs. DMEM) demonstrating a reduction of HSC activation. Conclusion: The scalable chromatographic method retained antifibrotic and pro-regenerative effects of engineered EV and emerges as an alternative for the treatment of liver disease.

677. (749) **CHEMICAL AND DOSE-DEPENDENT EFFECT OF TITANIUM AND SILICA NANOPARTICLES OVER MACROPHAGES**

Exequiel David Giorgi[1,2], Sofía Genovés[2], Sofía Diaz[2], María Eugenia Diaz[1,2], Martin Desimone[3], Mauricio De Marzi[1,2]

(1)Instituto de Ecología y Desarrollo Sustentable (Universidad Nacional de Lujan-CONICET), (2) Universidad Nacional de Luján, Departamento de Ciencias Básicas, (3) Universidad de Buenos Aires, IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica (FFYB)

Nanoparticles (NPs) are particles below 100 nm of diameter and they can generate different biological effects depending on their chemical composition, size, shape and morphology. Our objective is to compare the effect of silica and titanium dioxide NPs over a mouse macrophage cell line (RAW 264.7), evaluating cell metabolic activity and nitrites expression. Titanium dioxide NPs were provided by Evonik (AEROXIDE TiO2 P 25). As colloidal silica model we used LUDOX®. Size and shape of the NPs were analyzed by TEM and microimages were processed by ImageJ software. NPs were also analyzed by FTIR and DLS. Mouse macrophage cells (RAW 264.7) were cultured with different concentration (25-500 μg/mL) of NPs for 24-96 h. Cell supernatants were analyzed by Griess reaction to measure nitric oxide expression in macrophages supernatants culture. Cell metabolic activity was determined by MTT assay. A significant decrease on cell metabolic activity in presence of LUDOX® was observed, showing a major effect at higher NPs concentrations (500, 250 and 100 μg/mL) and longer times ($P<0.001$). A cytotoxic effect was observed at 72 h ($IC50= 91.4 \pm 1.1$ μg/mL; $R^2= 0.9805$). On the other hand, titanium dioxide NPs generated an increase of metabolic activity at the higher concentration (500 μg/mL) at 24 h in comparison with the control ($P<0.01$). After that, metabolic activity diminished at the same level that control cells. Despite of this effect, there were no differences of nitrites expression between treated cells and control cells.

As conclusion, a chemical and dose-dependent effect was observed in cells treated with NPs. In the case of LUDOX®, there was a cytotoxic effect but in the same concentration rate, titanium dioxide NPs generated an increase of metabolic activity in macrophages but without enhancing nitric oxide expression.

678. (789) **IN VIVO STUDIES OF A 3D BIOMATERIAL BASED ON CHITOSAN AND POLYFUMARATE FOR BONE REGENERATION**

María Laura Lastra[1], María Silvina Molinuevo[1], María Susana Cortizo[2], Ana María Cortizo[1]

[1]Laboratorio de Investigación en Osteopatías y Metabolismo Mineral (LIOMM), Dpto. Cs. Biológicas, Fac. de Cs. Exactas, UNLP-CIC PBA, La Plata, Bs. As., Argentina. [2]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Dpto. de Química, Fac. Cs. Exactas, UNLP,CONICET- CCT-La Plata, CC 16, Suc. 4. Argentina

Regenerative medicine uses different strategies to stimulate and support tissue regeneration after injury, due to the limited self-healing capacity of certain tissues. In our group, we are intereste in the developement of compatibilized biomaterials based on natural and synthetic polymers. We have previously designed a biomaterial of chitosan combined with a fumaric polymer. The crosslinked biomaterial between fumarate-copolymer and chitosan could be molded in microspheres and a porous scaffold [Lastra et al., Colloids and Surfaces B: Biointerfaces 196 (2020)]. The objective of this work was to evaluate tissue regeneration capacity of the microspheres compared to the porous scaffold using an in vivo bone regeneration model. *Methods*: Biomaterial was obtained from a mixture of a hydrolyzedcopolymer of vinyl acetate and diisopropyl fumarate and chitosan. The microspheres were obtained by water/oil emulsion and the porous scaffold by lyophilization. A 2 mm diameter circular craniotomy was performed on each parietal bone of WKAH/Hok rats. Sterilized materials were placed in the right defect and the left lesion was left without material as a control. After 30 days, the animals were sacrificed; the parietal bone was dissected and processed for bone histomorphometric analysis. *Results*: we found that the porous scaffold was less efficient for bone tissue repair than microspheres ($p<0.01$). Although no differences between control condition and microspheres were found. Additionally, morphological analysis showed a good integration between bone tissue and the biomaterial, with cells invading both porous scaffold and microspheres. *Conclusion*: particulate biomaterials, such as microspheres could be a useful model for bone tissue regeneration. This material showed osteointe-

gration and support cell growth. In a future study it should be desirable to evaluate the biomaterial as a cell carrier to improve bone tissue regeneration.

679. (790) **DERMATAN SULFATE/CHITOSAN POLYELECTROLYTE COMPLEXES AS DRUG DELIVERY PLATFORM TOWARDS CD44+ BREAST CANCER CELLS**

Birocco Ariadna[1], Blachman Agustín[1], Curcio Sofía[1], Goldberg Micaela[1], Moya Sergio[2], Calabrese, Graciela[1].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Ciudad Autónoma de Buenos Aires, Argentina; [2]Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, Spain

Breast cancer (BC) remains one of the major causes of death worldwide. Our research group reported a novel nanoformulation, based on polyelectrolyte complexes (PECs) of dermatan sulfate (DS) and chitosan (CS); which are internalized by the injured endothelium, through the specific interaction between DS and CD44 receptor. The expression of CD44 by BC cells is often associated with more invasive and proliferative capabilities. The aim of the present work is to study the interaction of PECs with CD44+ BC cells, to evaluate their potential for drug delivery. PECs were obtained by ionotropic gelification. Their hydrodynamic diameter (Dh), size distribution (PDI) and Z-Potential was measured by Dynamic Light Scattering. MDA-MB-231 and MCF-7 cell lines were incubated with FITC labeled PECs (10µg/ml DS) for different time periods to study interaction by flow cytometry and fluorescence microscopy. Cell proliferation and cell migration were evaluated by MTT, propidium iodide and wound healing assays after 24h of PEC incubation. PECs displayed a single population of 595,8(±23,5)nm, with a PDI of 0.249(±0,026) and a Z-Potential of +42,7(±0,6) mV. After 4 h of incubation, the nanoformulation interacted with both cell lines(74.3(±6.3) and 23,9(±6.3), respectively). The interacting cell populations were mostly CD44 positive, and this interaction partially decreased in the presence of anti-CD44 antibody. The higher interaction with triple-negative MDA-MB-231 cells prompted us to analyze their cellular responses in the presence of PECs: they did not induce proliferation, or alter the rate of cell migration after 24h. The obtained results showed that PECs interact with BC cells, and suggest that the nanoformulation could be a promising platform for the delivery of cytostatics or anti-migratory drugs for BC therapy.

680. (791) **AMORPHOUS SILICA NANOCARRIERS: A NOVEL CHEMOTHERAPEUTIC APPROACH FOR TRIPLE NEGATIVE BREAST CANCER**

Ibarra Agustina(1), Clemente Valentina(1), Busso Marina B. (2), Barrientos Anabel (3), Fermento María E. (1,4), Alonso Eliana N. (1,4), Coló Georgina P. (1,4), Facchinetti María M. (1,4), Ferronato María J. (1,4), Agotegaray M. (2), Curino Alejandro C. (1,4).
(1) Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) – CONICET, Bahía Blanca, Argentina.
(2) Instituto de Química del Sur (INQUISUR), CONICET – Departamento de Química, Universidad Nacional del Sur, Bahía Blanca, Argentina.
(3) Servicio de Patología del Hospital Interzonal General de Agudos Dr. José Penna, Av. Láinez 2401, 8000 Bahía Blanca, Argentina.
(4) Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.

Triple negative breast cancer (TNBC) is a heterogeneous group of tumors with difficult clinical management, due to the lack of the molecular targets to the conventional chemotherapeutics. Nanotechnology represents a strategy to overcome this problem employing nanocarriers that guide drugs to the tumoral site. Previously, we have reported the synthesis, characterization and viability effects on TNBC cell lines of two novel amorphous silica nanoparticles (NPs), Si@NH_2 and Si@FA, the last modified with folic acid. The aim of this work is to continue with these preclinical studies, elucidating their mechanism of action and performing biocompatibility assays. Transmission electron microscopy (TEM) was used to examine the morphology of NPs. Cell cycle was analysed with propidium iodide (PI) staining in MDA-MB-231 cells after 24 and 48 h of treatment (NPs at 500 μg/mL). Apoptotic cells were quantified by Annexin V-FITC + PI staining. Histological evaluation of the organs of an in vivo assay that has been previously reported was performed. The haemolytic effect on red blood cells was evaluated by determination of free haemoglobin on plasma obtained from the treatment of blood exposed to 10-500 μg/ml of each NP. The results show that TEM micrographs revealed monodisperse spherical shape for NPs, with biocompatible size. Both NPs induced cell cycle arrest in G0/G1 phase at 24 h ($p < 0.001$) and cell death at 48 h of treatment. The percentage of cells in early apoptosis was higher in Si@FA treated cells with respect to control ($p < 0.01$). The histological analysis of the internal organs did not demonstrate differences in mice treated with NPs in respect with control mice. Neither NPs induced haemolysis since free haemoglobin was quantified as less than 10 mg/dl. Altogether, these results suggest the potential use of Si@FA as nanocarrier for TNBC treatment. Further studies are in course to evaluate the incorporation of conventional chemotherapeutics.

681. (795) **TUNING OF A BIOMIMETIC ARTIFICIAL SKIN VIA 3D BIOPRINTING**

Mikele Amondarain[1], Ilia Villate Beitia[2], Antonella Lombardi[1], Joaquín Smucler[1], José Luis Pedraz[2,3,4], Santiago Gabriel Miriuka[1], Carlos Daniel Luzzani[1].
[1]Laboratorio de investigación Aplicada a la Neurociencia, FLENI-CONICET, [2]Laboratory of Pharmaceutics, NanoBioCel Group, Faculty of Pharmacy, University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain, [3]Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), [4]Institute of Health Carlos III, Vitoria-Gasteiz, Spain, Bioaraba, NanoBioCel Research Group, Vitoria-Gasteiz, Spain.

Bioprinted skin tissue has the potential for helping drug screening, formulation development, clinical transplantation, chemical and cosmetic testing, as well as basic research. Here we evaluated three-dimensional pieces with biocompatible materials composed of two layers, one of them containing fibroblasts and the other keratinocytes. On the one hand we analyse mechanical characteristics of bioinks, and on the other we evaluated cells viability and morphology, and their activity within the printed pieces after seven days of culture. Fibroblasts and keratinocytes were included in bioinks. We combined in different proportions alginate, gelatin and fibrinogen until obtaining the most favorable bioink for the cells. BIO X CellInk bioprinter was used to print the pieces. Cell viability was evaluated with the LIVE/DEAD Cell Imaging Kit. We used confocal microscopy to evaluate cell morphology and Scanning Electron Microscopy (SEM) to analyse the porosity of bioinks. We found that the addition of fibrinogen to the base-bioink that we had already tested of alginate and gelatin increases post-print cell viability and in the case of fibroblasts, they acquire the typical fusiform morphology. Immunohistochemistry showed that fibroblasts are active as they were observed to be secreting collagen. In conclusion, we found that the combination of fibrinogen with alginate and gelatin improves the extracellular environment allowing fibroblasts to remain active, secreting indispensable substances that favour cell viability and leading to cell proliferation.

682. (801) **A COMPARISON STUDY OF THREE MESENCHYMAL STEM CELLS-DERIVED EXTRACELLULAR VESICLES SOURCES FOR EXPERIMENTAL THERAPY IN LIVER FIBROSIS**

Luciana M. Domínguez[1]; Milagros Albornoz[1]; Juan Bayo[1]; Ma. José Cantero[1]; Bárbara Bueloni[1]; Catalina Atorrasagasti[1]; Celeste Biani[2], Carlos Luzzani[2], Mariana García[1]; Gustavo Yannarelli[3]; Esteban Fiore[1]; Guillermo Mazzolini[1].
[1] Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral CONICET, Buenos Aires, Argentina

[2] *Laboratorio de Investigación Aplicada a Neurociencia (LIAN), CONICET, FLENI, Buenos Aires, Argentina.*
[3] *Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro-CONICET, Buenos Aires, Argentina*

Introduction: Liver cirrhosis involves chronic damage, wound healing and fibrogenic processes. Mesenchymal stem cells (MSC)-derived extracellular vesicles (EVs) are an interesting therapeutic option for regenerative medicine. Aim: To compare EVs derived from different clinical relevant sources of MSC as therapeutic tool for liver fibrosis. Methods: EVs were isolated by ion exchange chromatography from supernatants of adipose tissue MSCs (ASC-EV), induced pluripotent stem cells-derived MSCs- (iMSC-EV), and umbilical cord perivascular cells (HUCPVC-EV). EVs isolation was confirmed by protein (BCA) and CD63 (ELISA) quantification and markers expression (CD9, CD81) by flow cytometry. *In vitro* effects of EVs on hepatic stellate cells (HSC) pro-fibrogenic genes expression (Col1A2 and α-SMA) were evaluated in CFSC-G2 cell line by qPCR. Antifibrotic effect of EVs was determined in experimental mice model of liver fibrosis (thioacetamide for 8 weeks in BALB/c mice). On week 6, ASC-EV, iMSCs-EV and HUCPVC-EV were i.v. injected every 5 days for a total of 3 doses. At week 8, animals were sacrificed, and liver samples analyzed. Collagen deposition was measured by Sirius red staining; immunohistochemistry for PCNA proliferating cells, and α-SMA (α-smooth muscle actin) for activated HSC was performed. Results: The three MSC source produce significant amount of EVs with typical surface markers CD9, CD63 and CD81. *In vitro* HSC incubation with EVs from different MSC sources down-regulates in similar levels the Col1a2 ($p<0.01$ vs. DMEM) and α-SMA ($p<0.01$ vs. DMEM) pro-fibrogenic genes expression. *In vivo* treatment with ASC-EV, iPSC-EV and HUCPVC-EV decreased collagen deposit and α-SMA levels in liver tissue. In addition, EVs from different sources induce the hepatocellular proliferation when compared with vehicle. Conclusion: Our results show that EVs derived from ASC, iMSC and HUCPVC have similar anti-fibrotic and pro-regenerative potential, and make them attractive therapeutic tool to decrease liver fibrogenesis.

683. (807) **GENERATION OF MICROPATTERNED CIRCULAR COLONIES OF PLURIPOTENT STEM CELLS AND SPATIAL CHARACTERIZATION OF SPECIFIC MARKERS OF THE THREE GERM LAYERS IN RESPONSE TO BMP4**

Smucler J., Möbbs A.M., Sevlever G.E., Miriuka S.G., Luzzani C.D., La Greca A.D., Waisman A.
Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Instituto de Neurociencias (INEU) - FLENI-CONICET

Human Pluripotent Stem Cells (hPSC) have the capacity to self-renew and differentiate in vitro into specialized cells. While the spatial distribution of cell populations is a key variable in regulating cell fate, common cell culture practices generate colonies of different geometries in an aleatory manner. Control over colony spatiality affects cell-cell interaction and specific signaling pathways that can influence pluripotency and differentiation. Circular colonies recapitulate part of the biophysical cues present in the embryonic disc before gastrulation. Furthermore, when hPSCs are cultured in these conditions, activation of BMP4 signaling induces a geometric-specific differentiation that resembles early gastrulation. In this work, we developed a device capable of generating functionalized surfaces of extracellular matrix (ECM) proteins in various shapes and sizes for hPSCs culture, called micropatterns. A Laminin-coated surface was treated with UV-C light through circular opaque patterns of 1000 um diameter. Light denatures the ECM protein in specific regions, in a stencil-like way, leaving only small delimited portions of functional laminin where cells can adhere. Our laboratory studies mesodermal lineage differentiation using BMP4. We thus analyzed BMP4 effects on micropatterned cell colonies as a validation of the device generated. Cells were cultured in circular colonies of 1000 μm for 24 hours and then treated with BMP4 for 42 hours. Immunofluorescence was performed against SOX2 (ectoderm), SOX17 (endoderm) and TBXT (mesoderm). Using quantitative bioinformatic analysis, we found a positive center for SOX2, a middle ring for TBXT, and an outer ring for SOX17. We observed the spatial distribution of key factors within the colony consistent with the distribution on the germinal disc.
The device generates cellular micropatterns that have features of the embryonic disc in vitro, resulting in a novel tool to create gastruloids in a fast and reproducible way.

684. (814) **EFFECT OF SPARC EXPRESSION IN MESENCHYMAL STEM CELLS AND ANALYSIS OF THEIR POTENTIAL FOR WOUND REGENERATION**

Antonella Lombardi[1], Mikele Amondarain[1], Marcela N. García[2], Alejandro La Greca[1], Joaquín Smucler[1], Gustavo E. Sevlever[1], Santiago G. Miriuka[1], Carlos D. Luzzani[1].
[1]LIAN-INEU-FLENI-CONICET.
[2]Cátedra de Citología, Histología y Embriología-Facultad de Ciencias Médicas-UNLP.

Mesenchymal Stem Cells (MSCs) are widely studied for their interesting cell biology and broad-ranging clinical potential. To date, there has been a rapid expansion of both preclinical and clinical studies using the MSC-derived secretome with the advantages of a cell-free system. The potential for therapeutic effects of this secretome could be optimized by pre-conditioning MSC with small molecules, biological agents, and biomaterials, or by genetically modifying them.
Wound regeneration is a complex process that requires cell migration, the development of an inflammatory environment, angiogenesis, granulation, tissue formation, re-epithelialization, and extracellular matrix (EM) remodeling. MSCs and their secretome have an active role in this process. SPARC, also known as osteonectin, is one of the many proteins secreted by MSCs. This protein is known to be involved in EM remodeling and assembly as well as in the regulation of cell migration and proliferation processes. In view of this, the aim of this work was to study whether the modification of SPARC expression in MSCs affects their secretome composition and their regenerative capacity. For this purpose, we used a lentiviral system to obtain SPARC knockdown cells (SPARC-KD-MSC). After characterizing them and validating that they kept their identity as MSCs, we used both wild type (WT) and SPARC-KD-MSC conditioned medium (CM) in wound healing *in vitro* assays with human skin keratinocytes (HaCat). We observed that wound closure was slower when using SPARC-KD-MSC CM than with WT CM. We also analyzed changes in the HaCat cell cycle distribution and no differences between conditions were found. These results suggest that SPARC may be playing a role in the HaCat wound migration process. In the future, we plan to deepen this analysis in order to characterize the role of SPARC in the wound healing process *in vitro* and *in vivo*, as well as to test whether its overexpression increases the regenerative capacity of MSCs.

REPRODUCTION I

Wednesday, November 16, 14-15:30 hr
Chairs: Vanesa Hauk - Verónica White

685. (30) **ALTERED COLLAGEN DEPOSITION AND MIR-199 EXPRESSION IN PLACENTAS FROM GDM PATIENTS: EFFECT OF A DIET ENRICHED IN EXTRA VIRGIN OLIVE OIL**

Dalmiro Gomez Ribot[1]; Esteban Díaz[2]; María Victoria Fazio[2]; Hebe Lorena Gómez[2]; Carlos Gresta[2]; Evangelina Capobianco[1]; Alicia Jawerbaum[1].
1. Laboratorio de Reproducción y Metabolismo. CEFYBO-CONICET. Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
2. Hospital General de Agudos Dr. Pirovano.

Introduction: Gestational Diabetes Mellitus (GDM) is a pregnancy pathology that induces placental alterations, including fibrosis. MiR-199 participates in the regulation of profibrotic processes in different tissues. Besides, miR-199 negatively regulates LDL receptor (LDLR) and Clathrin (CLTC), genes involved in lipoprotein endocytosis. Previously, we found that a maternal diet enriched in Extra Virgin Olive Oil (EVOO) reduces proinflammatory markers in term placentas from women with GDM. Aim: To evaluate if a diet supplemented with

EVOO regulates collagen deposition and the expression of miR-199 and its target genes LDLR and CLTC in term placentas from GDM women. Methods: Healthy (Control, C) and women with GDM, enrolled between 24-28 weeks of pregnancy, were recommended a standard diet for pregnancy. A group of women with GDM followed a diet enriched with 36 mL/day of EVOO (GDM-EVOO group). After delivery, placentas were obtained (n=15 per group). Collagen deposition was evaluated by Masson's staining; and miR-199, LDLR and CLTC expression was determined by qPCR. Results: Masson's staining revealed that collagen deposition was increased in the villi (17%, $p<0.01$) and fetal vessels (7%, $p<0.05$) in the GDM group compared to C, alterations prevented by the diet enriched in EVOO. In the GDM group, miR-199 levels were 37 times greater than in C ($p<0.01$), an alteration prevented by the EVOO-enriched diet. LDLR and CLTC expression were reduced in the GDM group compared to C (69% and 80% respectively, $p<0.05$), alterations prevented by the diet enriched in EVOO. Conclusion: Our results showed an increased deposition of collagen both in the villi and fetal vessels of the placenta. Along with these alterations, miR-199 levels were increased in the placenta from women with GDM and possibly related to the decreased expression of LDLR and CLTC, which may affect lipoprotein endocytosis. The EVOO-enriched diet prevented these alterations, possibly improving placental function.

686. (57) **THE ENVIRONMENTAL POLLUTANT 3-METHYLCHOLANTHRENE MAY ACCENTUATE THE ADVERSE EFFECTS OF OVERNUTRITION ON REPRODUCTIVE DEVELOPMENT**

Jeremias Pablo Flores-Quiroga, María Agustina Meneghini, María Florencia Heinecke, Verónica White, Alicia Graciela Faletti.
Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), Facultad de Medicina, Universidad de Buenos Aires.

The aim of this study was to assess whether exposure to the environmental pollutant 3-methylcholanthrene (3MC), considered as an obesogen, may enhance the adverse effects caused by overnutrition on reproductive development. To this end, prepubertal male rats fed standard or cafeteria diet were exposed to vehicle or a combination of 3MC (0.1 mg/kg) and α-naphthoflavone (αNF, 80 mg/kg) three times per week for 40 days. The study consisted of five experimental groups: controls (SDV), overweight rats (CDV), 3MC-exposed overweight rats (CD3MC), 3MC+αNF-exposed overweight rats (CD-3MCαNF), and αNF-exposed overweight rats (CDαNF). At 61 days of age, blood, bone marrow and spermatozoa were obtained. Sperm count (millions of spermatozoa/ml), motility (percentage), morphology by staining with Giemsa, the presence of DNA fragmentation in lymphocytes by the comet assay, and metabolic profile were examined. CDV rats had higher body weight (387±7 g; $p<0.05$) than SDV rats (349±10 g), but 3MC and αNF reduced this increase to SDV values ($p<0.05$). No differences were found in the cholesterolemia, glycemia and triglyceridemia levels. However, and compared with SDV rats (37±1), all CD rats showed lower sperm counts (CDV: 16±1; CD3MC: 13±2; CD3MCαNF: 15±3; CDαNF: 13±1; $p<0.001$). Also, CD groups showed a decrease in the motility percentage compared with SDV rats (15-50%). Furthermore, the CD3MC group showed a greater decrease in the number of motile spermatozoa (34±2; $p<0.001$) and a higher tail DNA content (2.7±0.7; $p<0.05$) compared with CDV rats (53±3; 0.9±0.2; respectively), but αNF prevented this effect (52±2; 0.26±0.01, respectively). No differences were found in the morphology between all groups. All groups exposed to αNF (43.0±0.2 days; $p<0.05$) showed early puberty compared with SDV rats (44.2±0.2 days). These results indicate that overnutrition can make a developing organism more vulnerable to an environmental pollutant with obesogenic features, at least at the reproductive level.

687. (58) **FETAL PROGRAMMING OF ALTERED DECIDUALIZATION IN THE OFFSPRING OF DIABETIC RATS**

Cintia Romina Gatti, Sabrina Lorena Roberti, Lautaro Recchia, Romina Higa, Alicia Jawerbaum
Laboratory of Reproduction and Metabolism. CEFYBO-CONICET. School of Medicine, University of Buenos Aires, Argentina.

Introduction: Diabetes mellitus is a metabolic pathology that leads to placental, fetal and offspring alterations. Little is known regarding putative alterations in the uteri of the offspring of diabetic rats, and if maternal diets enriched in olive oil can prevent these alterations. Aim: To evaluate the expression of prolactin (PRL) and fatty acid-binding protein 4 (FABP4), key proteins in decidualization, and 4-Hydroxynonenal (4-HNE) levels, a pro-oxidant marker, in the decidualized uteri of prepubertal diabetic rat offspring fed or not with an olive oil-enriched diet. Methods: Mild pregestational diabetic rats were obtained by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic rats received a 6% olive oil-enriched diet or a standard diet from day 1 of pregnancy until parturition. The offspring were fed a standard diet and the uteri of the offspring (F1) evaluated at 30 days postnatal, after decidualization induction with PMSG (50 UI) and hCG (50 UI). *Prl1* and *Fabp4* were evaluated by RT-qPCR, PRL and FABP4 levels by Western blot and 4-HNE by immunohistochemistry. Results: The decidualized uteri of diabetic rat offspring showed reduced *Prl1* mRNA levels (0.38 fold-change) and PRL protein levels (47%) compared to controls ($p<0.05$). *Fabp4* mRNA (1.88 fold-change) and FABP4 protein levels (142%) were increased in the decidualized uteri of diabetic rat offspring compared to controls ($p<0.05$). These alterations were partially prevented by the maternal diet enriched in olive oil. The decidualized uteri of diabetic rat offspring showed an increase 4-HNE levels (35% $p<0.05$ vs. Control group), an alteration prevented by maternal a diet enriched in olive oil. Conclusions: Key markers of decidualization and pro-oxidant environment are altered in the decidualized uteri of diabetic rat offspring at a prepubertal stage, alterations that may affect their reproductive capacity and which can be partially prevented by a maternal diet enriched in olive oil.

688. (67) **ENDOCANNABINOID SYSTEM, TRANSPORT OF NUTRIENTS AND CELL DEATH IN PLACENTAS-DERIVED FETAL GROWTH RESTRICTION.**

Julieta Aisemberg1, Carolina Marvaldi1, Manuel Luis Wolfson1, Ana María Franchi1.
1Centro de Estudios Farmacológicos y Botánicos-CEFyBO-UBA-CONICET, Bs As, Argentina.

The endocannabinoid system (ECS) is a lipid signaling system that includes endogenous ligands (AEA, 2-AG), receptors (CB1, CB2, TRPV1), and biosynthetic (NAPE-PLD) and hydrolysing (FAAH) machineries. Its role has become significantly important to multiple pregnancy events, including implantation and placentation. Alterations in placental ECS may impair pregnancy success. The aim of the current study was to use a mice model of dexamethasone-induced fetal growth restriction (FGR) in order to investigate whether ECS alterations in the placenta might be occurring. Moreover, as FGR can occur via impaired transport of key nutrients to the fetus, we further characterized the effects of dexamethasone on the expression on the placental glucose transporter 1 (GLUT1). Given that placental insufficiency results in FGR associated with placental apoptosis, we sought to investigate apoptosis in these placentas. Pregnant mice received a subcutaneous injection of vehicle or dexamethasone (8 mg/kg) from gestational day 14 to 15. Dam subsets were euthanized at day 14 to 16 of pregnancy for placenta assessment via western blot, qPCR and FAAH activity. The results were analyzed with one-way ANOVA and Tukey test ($p<0.05$). Dexamethasone (FGR) modulated FAAH and CB2 expression by downregulating protein levels in placentas on gestational day (gd) 16 ($p<0.05$). CB1, TRPV1 and NAPE expression and FAAH activity did not change significantly. FGR did not alter the expression of GLUT1 protein in placentas on gd 16. Caspase 3 protein levels significantly decreased in placentas on gd 16 after dexamethasone treatment ($p<0.05$). AIF and XIAP mRNA expression did not change in FGR placentas. FGR compromised placental growth and function. These findings suggest a dysregulation of endocannabinoid signaling in restricted placentas. Furthermore, dexamethasone treatment might alter trophoblast turnover. It could be interesting to examine the role of the ECS in placentas of FGR pregnancies.

689. (77) **PERINATAL EXPOSURE TO GLYPHOSATE OR A GLYPHOSATE-BASED FORMULATION CAUSES LONG-TERM EPIGENETIC ALTERATION ASSOCIATED WITH IMPLANTATION FAILURES**

Ailín Almirón[1], Dalma B. Cadaviz[1], Virginia Lorenz[1, 2], Florencia Doná[1], Jorgelina Varayoud[1, 2], María Mercedes Milesi[1,2]

[1] Instituto de Salud y Ambiente del Litoral (ISAL), UNL – CONICET, Argentina. [2] Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas (FBCB) – UNL, Argentina.

Glyphosate (Gly) is the active ingredient of glyphosate-based herbicides (GBH); the most globally used pesticide. We have shown that perinatal exposure to either Gly or GBH induces implantation failure in rats. Here, we investigate whether this alteration is associated with aberrant uterine expression of leukemia inhibitory factor (*Lif*) gene, a key marker of endometrial receptivity, and we explored epigenetic mechanisms of transcriptional regulation. Pregnant rats (F0) were exposed to Gly or GBH through food, in a dose of 2 mg of glyphosate/kg/day, from gestational day (GD) 9 until weaning. Sexually mature F1 females became pregnant and uterine samples were collected on GD5 (preimplantation period). The mRNA expression of *Lif* was evaluated by RT-qPCR. To analyze the methylation status of *Lif*, enzyme-specific restriction sites were searched *in silico* in the regulatory regions of the gene and assessed using the methylation-sensitive restriction enzymes-PCR technique. To determine changes in histone post-translational modifications, histone acetylation (H3Ac and H4Ac) and methylation at lysine residues (H3K27me3) along the different regulatory regions of *Lif* were assessed by chromatin immunoprecipitation (ChIP) assays. Gly and GBH exposure decreased the mRNA expression of *Lif*. Moreover, an increase in DNA methylation was detected in Gly- and GBH-exposed rats, compared with the control. In most of the regions analyzed, ChIP data showed increased levels of H3Ac in GBH group, but decreased acetylation levels in Gly group vs control. Regarding H4Ac, no significant changes were detected. In addition, Gly and GBH groups showed higher level of H3K27me3 in one of the regions analyzed, compared to the control. In conclusion, perinatal exposure to Gly or GBH downregulates the expression of *Lif* at the receptive stage, associated with epigenetic disruption of the regulatory regions of the gene. These alterations could account for the Gly- and GBH-induced implantation failures.

690. (168) **MOUSE OVARIAN DISTURBANCES CAUSED BY A HIGH FAT DIET: THE EFFECT OF METFORMIN ON OVARIAN FUNCTION RESTORATION**

Candela Velazquez[1], Noelia Carnovale[1], Mariela Bilotas[1], Rocío Marinoni[1], Yamila Herrero[1], Valeria Pomponio[1], Katherine Prost[2], Gabriela Meresman[1], Fernanda Parborell[1], Dalhia Abramovich[1]

[1]Instituto de Biología y Medicina Experimental-CONICET (IByME-CONICET)

[2]Hospital Interzonal General de Agudos Pedro Fiorito, sector de Endocrinología

Introduction: Female reproductive performance is affected by metabolic deregulations. Nowadays, many women are overweight, so understanding the impact of a high fat diet (HFD) is imperative. Folliculogenesis takes place in the ovary and depends on a correct blood supply, therefore, alterations of angiogenesis may have consequences. Metformine (MET) is a hypoglycemic drug administrated in diabetic type 2 patients that was shown to improve ovulation and pregnancy rates in women. The mechanisms underlying these effects are still unclear. Objective: To analyze the effects of HFD and MET on metabolism and reproductive function in female mice. Methodology: 21d.o. female C57BL/6 mice were fed with a HFD or a control diet for 16 weeks. One HFD and one control group received MET the last four weeks. Mice were weighted once a week. Serum, gonadal and visceral fat were obtained. Estrus cycle and metabolic parameters were evaluated. Ovaries were isolated to perform WB and histology. One-way-ANOVA was used for statistical analysis. Results: HFD-mice had higher body weight, glycemia, GTT, HOMA-IR, t-cholesterol, P_4 and adipose tissue. MET improved GTT, HOMA-IR and decreased adipose tissue and P_4. The estrus cycle was shorter in HFD and the number of cycles/14d was increased. However, anovulatory stages were longer in HFD. MET had no effect on estrous cycle. The follicular dynamic was altered in HFD and MET improved it, raising the percentage of corpora lutea and primary follicles. Atretic follicles were higher on HFD with no effect of MET. Ovarian VEGF and DNA damage were elevated while periendothelial cell area was decreased in HFD. MET reversed these alterations and improved the 3β-HSD increase and the periendothelial cell area decrease found in HFD. Conclusion: HFD affects metabolism, hormonal profile, estrous cycle, angiogenesis and folliculogenesis. Changes in ovarian VEGF may be one of the possible causes of the observed disturbances. MET improves some of these alterations.

691. (201) **PLASMA CELL-FREE DNA AS A POTENTIAL TOOL FOR OBSTETRIC OUTCOMES PREDICTION: QUANTIFICATION IN PREGNANT AND NONPREGNANT WOMEN**

Paula J. Cepeda[1,2*], María E. Racca[1,2*], María M. Milesi[1,3], Jorgelina Varayoud[1,3], María A. Cardozo[1,2,5], Enrique H. Luque[1,3], Mónica Muñoz-de-Toro[1,4], María F. Rossetti[1,2], Jorge G. Ramos[1,2]

*1 Instituto de Salud y Ambiente del Litoral (ISAL), CONICET-UNL, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 2 Departamento de Bioquímica Clínica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 3 Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 4 Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 5 BLUT Laboratorios, Santa Fe, Argentina, *These authors contributed equally to this work.*

Cell-free DNA (cf-DNA) was associated with adverse obstetric outcomes prediction. We studied cf-DNA levels variability in plasma (pl-) samples to evaluate its utility as a potential biomarker. Pl-samples from first (1st), second (2nd) and third (3rd) trimester pregnant (P, N=59) and nonpregnant (NP, N=24) women were used. Cf-DNA was obtained using QIAmp DNA blood mini kit (QIAGEN). Actin-β (*ACTB*) gen was quantified by real-time quantitative PCR using an *ACTB* standard curve. For PCR assays, 1000 *ACTB* standard copies were added to samples as an internal control. Cf-DNA level was expressed as *ACTB* copy number and means±SD were statistically compared between groups. A correlation study between cf-DNA level and the gestational age (GA) at the delivery has been done. Cf-DNA increased in P respect to the NP group (2391±1388 vs 1506±446 *ACTB* copies, respectively; $p<0.001$). In P, there was a cf-DNA increase in the 2nd (3047±2017 *ACTB* copies, $p<0.05$) and 3rd (3748±1133 *ACTB* copies, $p<0.05$) trimester with respect to 1st (1851±528 *ACTB* copies). A pre-partum pl-sample showed 26000 *ACTB* copies. Three P women developed gestational hypertension (GH): a 1st trimester pl-sample showed 3607 *ACTB* copies and two 3rd trimester pl-samples showed 1687 and 1543 *ACTB* copies. High 1st trimester cf-DNA level correlated negatively with the GA at delivery ($p=0.08$). Our results support a pl-cf-DNA increase throughout pregnancy, peaking at term. We showed a change in cf-DNA level from 1st to 2nd and 3rd trimester. Cf-DNA levels in GH cases were out of mean±SD for the corresponding trimester. Finally, a high 1st trimester cf-DNA level was correlated to early GA at delivery. Cf-DNA appeared to be related to GH cases and GA at delivery, supporting it as a promising biomarker in obstetric outcomes prediction. Future studies will focus in evaluate cf-DNA in a greater number of P women and in evaluate its utility for prediction of different obstetric outcomes.

692. (252) **THE ABSENCE OF CB1 RECEPTOR REDUCES FAAH ACTIVITY IN AN LPS-INDUCED MURINE MODEL OF PRETERM DELIVERY**

Carolina Marvaldi, Julieta Aisemberg, Ana M Franchi, Manuel L Wolfson.

Preterm delivery (PTD) is the leading cause of morbi-mortality in

neonates. The endocannabinoid system (eCS) is one of several signaling pathways involved in physiopathology of reproduction. It comprises the main endogenous ligand anandamide (AEA), receptors CB1, CB2 and TRPV1, biosynthesizing enzyme NAPE-PLD and the catabolizing enzyme FAAH. Several studies have shown that FAAH is the key player in regulating AEA levels. Previous works from our lab have demonstrated that maternal LPS administration altered FAAH activity in deciduas and peripheral blood mononuclear cells (PBMC), resulting in embryo resorption. In our LPS-induced murine model of preterm delivery, we observed that the eCS of both the decidua and PBMC is involved in triggering a PTD. We also demonstrated that CB1-KO mice presented a lower PTD rate when compared to WT mice. Therefore, the aim of this study was to investigate whether the lack of CB1 receptor produces an imbalance in the decidual eCS, we well as its effects in deciduas exposed to an inflammatory model of PTD. We studied whether LPS treatment altered the eCS in the deciduas from CB1-KO mice on day 15 of gestation. We observed that LPS treatment diminished CB2 receptor protein levels in these mice ($p<0.05$). No differences were found on TRPV1 receptor protein levels. Regarding the enzyme that synthesizes AEA, we observed no differences in NAPE-PLD protein levels between treatments or genotypes. However, we observed that CB1-KO deciduas presented a lower activity of FAAH when compared to WT deciduas ($p<0.05$). The same pattern of response was found in control mice and LPS-treated mice. In summary, CB1-KO mice in day 15 of gestation present a decreased basal activity of FAAH in the deciduas while the maternal treatment with LPS reduces the protein levels of CB2 in these tissues. Taken together, our results suggest that the lack of CB1 receptor results in a dysregulation of other components of the decidua eCS, which might impact the physiology of this tissue.

693. (281) **ADVANCED MATERNAL AGE IN A RAT MODEL INDUCES ALTERATIONS IN EMBRYO DEVELOPMENT AND DECIDUAL ACTIVATION OF FOXO1**

María Laura Leonardi, Cintia Romina Gatti, Irune Pirrone, Alicia Jawerbaum, Romina Higa
Laboratory of Reproduction and Metabolism. CEFYBO-CONICET. School of Medicine, University of Buenos Aires, Argentina.

Background: Pregnancy at Advanced Maternal Age (~35 years, AMA) is associated with obstetric complications and neonatal adverse outcomes. Studies in animal models of AMA showed that these complications may be related to decidualization defects. The decidua is essential for a correct implantation, placenta and embryo development. FoxO1 is a transcription factor that plays a role in decidualization and embryo development by modulating genes related to oxidative stress and extracellular matrix remodeling. FoxO1 can be inhibited by phosphorylation. Aim: To evaluate reproductive alterations in a rat model of AMA during organogenesis stage. In decidua of AMA rats analyze lipoperoxidation, FoxO1 activation and the expression of its target genes *Mmp2* (involved in extracellular matrix remodeling) and *MnSOD* (antioxidant response). Methods: 3-month-old (Control) and 10-month-old (AMA) Wistar rats were mated with young males. At day 12 of pregnancy, uterine morphology and embryo developmental parameters were measured. In the decidua, lipoperoxidation (TBARS), FoxO1 phosphorylation status (Western Blot) and mRNA levels of *Mmp2* and *MnSOD* (qPCR) were measured. Results: AMA rats showed an increase in macroscopic uterine anomalies (41%, $p<0.01$) and embryo resorption rate (44%, $p<0.001$) as well as a decrease in the number of viable embryos (24%, $p<0.01$) and crown-rump length (11%, $p<0.001$). Lipoperoxidation was increased (40%, $p<0.05$), P-FoxO1 levels were reduced (63%, $p<0.05$) and total FoxO1 was unchanged in the decidua of AMA rats. Decidua of AMA rats showed higher mRNA levels of *Mmp2* (1.65 fold change, $p<0.05$) and no changes in the *MnSOD*. Conclusion: AMA induced alterations in the pregnant uterus and embryo development during organogenesis stage in the rat. Decidua from AMA rats showed an increase in oxidative status together with an increase in active FoxO1 and its target gene *Mmp2*. Reproductive anomalies in AMA may be related to alterations in decidual metabolism and remodeling.

694. (309) **PI3K AND MAPK SIGNALING PATHWAYS ARE INVOLVED IN LEPTIN SURVIVAL EFFECT IN PLACENTAL CELLS UNDER HYPOXIC CONDITION**

Salinas Sebastián[1], Riedel Rodrigo[1], Jaime Mariana[2], Casale Roberto[2], Maymó Jullita[1], Varone Cecilia[1], de Dios Nataly[1]
1. Departamento de Química Biológica FCEN-UBA, Instituto de Química Biológica IQUIBICEN, CONICET, Buenos Aires, Argentina
2 Hospital Nacional Profesor Alejandro Posadas, Buenos Aires, Argentina

Leptin promotes proliferation and survival of trophoblastic cells, and prevents cellular stress in trophoblastic cells. Leptin is incremented in preeclampsia, as a compensatory response to hypoxia or oxidative stress present in placental cells. As a model of hypoxia we use $CoCl_2$ treatment that stabilizes HIF-1α transcription factor, involved in hypoxia response. In the present study, we evaluated MAPK and PI3K signaling pathway, on leptin actions after $CoCl_2$ treatment. We used Swan-71 cells, a first trimester cytotrophoblast human cell line and human term placental explants. Both cell models were treated with 100 µM PD98059 (PD) and 50 nM Wortmannin (Wort) pharmacological inhibitors of MAPK and PI3K respectively, combined with $CoCl_2$ in the presence of leptin. A Caspase-8 and Cytochrome C expression was determined by WB. Apoptotic DNA fragmentation was determined by the DNA ladder assay, cell proliferation was analyzed by Ki67 expression determined by IF and cell counter, and finally, MTT assay was used to evaluate cell survival. Leptin diminished Caspase-8 and Cytochrome C expression in placental explants under simil-hypoxic condition; the presence of Wort, completely reverted leptin effect on Cytochrome C levels (1.4 ± 0.04). Leptin prevented $CoCl_2$ apoptosis in placental explants determined by DNA ladder assay and this effect was blocked with PI3K signaling pathway inhibitor. On the other hand, cell proliferation was diminished after $CoCl_2$ treatment, analyzed by the expression of Ki67 and cell counting. Leptin significantly reversed this effect and Wort blocked leptin proliferative effect in Swan-71 cells (0.84 ± 0.34) under hypoxia stress. Similar results were obtained when analyzing leptin enhanced cytotrophoblast cell survival that was diminished by PD or Wort inhibitors. All these results suggest that HIF-1α stabilization has negative effects on cell survival; and leptin protects trophoblastic cells from the hypoxic condition involving MAPK and PI3K pathways.

695. (381) **STRESS DURING PREOVULATORY PERIOD ALTERS PATTERN EXPRESSION OF ADHESION MOLECULES INVOLVED IN LYMPHOCYTE INFILTRATION IN DAIRY CATTLE**

Etchevers Lucas[1,2], Renna María Sol[1,2], Belotti Eduardo Matías[1,2], Diaz Pablo Uriel[1,2], Rey Florencia[1,2], Ortega Hugo Hector[1,2], Salvetti Natalia Raquel[1,2], Amweg Ayelén Noelia[1,2].
[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET) Universidad Nacional del Litoral (UNL), Argentina. [2]Facultad de Ciencias Veterinarias, UNL, Esperanza, Santa Fe, Argentina.

Proper expression pattern of adhesion molecules must take place both in the circulating leukocytes and in the activated endothelium for leukocyte infiltration into the ovary. These infiltrated leukocytes act by secreting regulatory molecules necessary for ovulation. The aim of this study was to analyze the effect of stress during the preovulatory period on the expression of adhesion molecules in circulating T- and B-cells and in the endothelium of vessels of the medulla and the dominant follicle. Holstein cows with regular estrous cycles without reproductive disease were included. For stress induction, animals (AG; N=7) were challenged with 100 IU of adrenocorticotropin every 12 hours for 4 days before ovulation when ovariectomy was performed (day 18). Control group (CG; N=5) received saline solution in the same schedule. Blood samples were taken on days 15 and 18 for leukocyte isolation. Expression of CD62-L, CD11b and CD44 was evaluated by flow cytometry in T- and B-cells and ovarian expression of VCAM-1 and PECAM-1 was analyzed by immunohis-

tochemistry. A Generalized Linear Model was used to analyze the results. In T-cells, expression of CD44 and CD62-L was lower in the AG ($p<0.05$) than in the CG. Also, the expression of CD44 and CD11b decreased over time ($p<0.05$). The percentage of T-cells expressing CD44 and CD62-L decreased in the AG ($p<0.05$) and over time ($p<0.05$). In B-cells, CD11b expression was lower in AG than in the CG ($p<0.05$). Also, the percentage of B-cells expressing CD11b was lower in the AG than in the CG ($p<0.05$) and the percentage of B-cells expressing CD44 decreased over time ($p<0.05$). In the dominant follicle endothelium, VCAM-1 expression was lower in the AG than in the CG ($p<0.05$) while PECAM-1 expression was similar ($p>0.05$). Our results present novel insights into the effect of stress during the preovulatory period on the expression of adhesion molecules necessary for proper leukocyte migration into the ovary for successful ovulation.

696. (396) **ALTERED PROLIFERATION AND APOPTOSIS IN PREOVULATORY FOLLICLES IN COWS GESTATED UNDER HEAT STRESS CONDITIONS**

Ormaechea N[1], Notaro US[1], Chiaraviglio JA[1], Cattaneo Moreyra ML[1], Ortega HH[1,2], Rey F[1,2], Salvetti NR[1,2], Rodriguez FM[1,2].

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET) Universidad Nacional del Litoral (UNL), Argentina. [2]Facultad de Ciencias Veterinarias, UNL, Esperanza, Santa Fe, Argentina.

Environmental conditions experienced by pregnant cows can affect the fertility of their daughters through changes at the molecular level in the ovary during development. In fact, the ovarian follicular reserve is established during the fetal life and many factors can affect the future fertility of the offspring. The aim of the study was to analyze the expression of proliferation and apoptosis markers in preovulatory follicles of cows gestated under heat stress in different stages of pregnancy. Holstein cows (n=20) gestated under different environmental conditions during their *in utero* development were used. Ovarian samples were obtained by ovariectomy and determination of proliferation (PCNA) and apoptosis (Caspase-3; BCL-2; BAX) in granulosa and theca interna layers of preovulatory follicles was done by immunohistochemistry. Gestation was divided into two periods (P1: 0-150 days; P2: 151 days-birth); and three trimesters (T1: 0-90 days; T2: 91-180 days; T3: 181-birth days) in which the exposure to high Temperature-Humidity Index (THI) was calculated. Positive associations between PCNA expression in theca interna and THI during all gestation and particularly in the second trimester were observed. Caspase-3 expression in granulosa cells shown positive association with THI in the second period of gestation. Also, positive associations between expression of BCL-2 in theca cells and high THI during all gestation, and in the first period and second trimester of gestation were observed. Moreover, the expression of BAX in theca interna were positively associated to high THI in the first period of gestation. These data suggest that exposition to high THI during in utero development can alter the expression of fundamental proteins related to normal development of preovulatory follicles. In this sense changes during early development can influence the fertility of the cows through changes in the balance in proliferation / apoptosis mechanisms in the dominant follicle.

697. (410) **MATERNAL TREATMENT OF BUTYRATE DURING GESTATION AND LACTATION IMPACTS ON MATERNAL METABOLISM AND IMPROVES METABOLIC PROGRAMMING OF THE OFFSPRING**

Florencia Heinecke[1], Marina Labiano[1], María Agustina Meneghini[1], Jeremías Pablo Flores Quiroga[1], Alicia Graciela Faletti[1] and Verónica White[1]

[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET-UBA), Buenos Aires, Argentina

Maternal obesity programs metabolic abnormalities in fetuses that precede a high susceptibility to the development of fatty liver later in life. Butyrate (B), a product of fiber metabolism from intestinal microbiota, improves lipid metabolism and prevents inflammation. We previously observed that maternal oral administration of B prevented maternal hypertriglyceridemia, fetal overgrowth and liver lipid overaccumulation in a maternal model of overweight in rats. Our aim was to evaluate whether B was able to ameliorate the negative program induced by maternal obesity in the offspring. Methods: Female Wistar rats were fed standard diet (CT rats) or saturated fat-rich-diet (FD rats) for 8 weeks and mated with control males. Vehicle or B (3%) was orally delivered daily during gestation and 3 days per week during lactation (FDB rats). The mothers were euthanized after weaning and the offspring at 140 days of life. Maternal liver levels of triglycerides (TG) and cholesterol esters (CE) were assessed by TLC and lipoperoxidation by TBARS assay. Results: We found a decrease in maternal liver weight only in FD group (12% $p<0.05$ vs CT) and an increase in adipose tissue weight in FD group (120% $p<0.1$ vs CT), that was not observed in FDB group. The increase in TG and EC content that we had observed at birth persisted in maternal liver from FD (TG: 225%, ChE: 109%) and FDB group $p<0.001$ vs CT. Maternal liver from FD group presented an increase in lipoperoxidation (228% $p<0.05$ vs CT), that was not observed in FDB group. In the adult offspring we observed a decrease in body weight in males from the FDB group (16% $p<0.01$ vs CT). B also prevented the increase in adipose mass in males and females from the FDB group (32% and 10% respectively $p<0.05$ vs FD). Conclusions: B was able to attenuate the increase in adipose tissue weight and lipoperoxidation in mothers. Also, the adult offspring from the FDB group presented a decrease in adipose tissue weight.

698. (484) **ENDOMETRIAL PHYSIOLOGY AND EARLY PREGNANCY ARE AFFECTED IN A MOUSE MODEL OF METABOLIC SYNDROME**

Noelia Carnovale [1], Candela Velazquez [2], Inés Stella [3], Mónica Frungieri [4], María Eugenia Matzkin [4], Analy Fritzler [5], Jorge Palazzi [6], Dalhia Abramovich [2], Mariela Bilotas [7], Gabriela Meresman [1].

[1] Laboratorio de Fisiopatología Endometrial, IBYME-CONICET, [2] Laboratorio de Estudios Fisiopatológicos del Ovario, IBYME-CONICET, [3] Servicio de Patología del Hospital Rivadavia y CEMIC, Argentina, [4] Laboratorio de Neuro-Inmuno-Endocrinología Testicular, IBYME-CONICET, [5] Sector de Endocrinología del Hospital Interzonal General de Agudos Pedro Fiorito, [6] Laboratorio IICT Labs, Rosario, Santa Fe, [7] Laboratorio de Inmunología de la Reproducción, IBYME-CONICET.

The prevalence of metabolic syndrome (MS) is rising globally. There is growing evidence which links MS to poor reproductive health. The aim of this study was to investigate the effect of MS on endometrial physiology and early pregnancy in mice treated or not with metformin (Met). Sixty 21-day-old C57BL/6 female mice were used: 20 were fed with a control diet (C) and 40 with a high-fat diet (HFD). At week 12, 20 HFD animals started treatment with Met 300 mg/kg in drinking water (HFDMet). At week 16, 10 mice for each group were sacrificed. The rest of the animals were mated with proven fertility male mice and sacrificed on day 7 of pregnancy. This mouse model of MS was previously characterized showing augmented levels of cholesterol, triglycerides, HDL, glycemia and insulin, and increased visceral and gonadal adipose tissue. Non-pregnant HFD mouse endometrium showed an increase in: epithelial apoptosis evaluated by TUNEL ($p<0,05$ vs. C); the number of glands evaluated by histologic analysis ($p<0,01$ vs. C); the levels of TBARS as an index of lipid peroxidation ($p<0,01$ vs. C) and catalase activity ($p<0,05$ vs. C). In addition, elevated levels of serum progesterone were found in HFD ($p<0,05$ vs. C and HFDMet), and shorter estrous cycles were observed in HFD and HFDMet ($p<0,05$ vs. C). Endometrial epithelial cell proliferation, evaluated by Ki-67 IHQ, decreased in HFDMet ($p<0,05$ vs. HFD). Pregnant HFD mice showed a delay in appearance of vaginal plug ($p<0,05$ vs. C and HFDMet) and an increase in the corpora lutea/implantation sites ratio ($p<0,05$ vs. C and HFDMet). Metabolic imbalance caused by HFD feeding results in alterations in endometrial apoptosis, oxidative stress and glandular hyperplasia, and also modified progesterone levels and estrous cycle length in mice. Met could control endometrial glandular hy-

perplasia. Also, HFD affects early pregnancy parameters. Further studies are needed in order to clarify the mechanisms involved in female reproductive dysfunction in MS.

699. (592) **INTERGENERATIONAL TRANSMISSION OF METABOLIC ALTERATIONS IN THE LEFT VENTRICLE OF TYPE 2 DIABETIC MALE RATS**

Irune Pirrone, Cintia Romina Gatti, María Laura Leonardi, Alicia Jawerbaum, Evangelina Capobianco.
Laboratory of Reproduction and Metabolism, CEFYBO-CONICET, School of Medicine, University of Buenos Aires, Argentina.

The prevalence of type 2 diabetes (T2D) can be related to the intergenerational transmission of metabolic anomalies that affect the offspring's health. We previously observed development of T2D and cardiac alterations in the offspring of female diabetic rats. Diabetic cardiomyopathy (DCM) is described by anomalies in the left ventricle and is associated with oxidative stress, inflammation, endoplasmic reticulum stress (ERS) and other pathologic conditions. Peroxisome proliferator activated receptors (PPARs) are transcription factors related to metabolism and anti-inflammatory processes in the heart. The aim was to evaluate the expression of PPARs, inducible nitric oxide synthase (*iNos)*, the antioxidant enzyme *MnSod* and genes involved in the ERS, in the left ventricle of male rats that develop diabetes by intrauterine programming. Methods: The male offspring of female Control (C) and diabetic rats (D: diabetic males obtained by intrauterine programming) were euthanized at 8 months of age (n=8). Triglycerides (TG) and HDL-cholesterol (HDL-c) were measured in plasma by colorimetric assays. The TG/HDL-c ratio was calculated as a measure of insulin resistance. The left ventricle was stored at -80°C for the evaluation of mRNA (by qRT-PCR) of *Pparα*, *Pparγ, Pparδ*, *iNos*, *MnSod* and ERS genes (*Chop*, *sXbp1*, *Atf4*). Results: The TG/HDL-c ratio was increased in D males (73% p< 0.05). The expression of *Pparα* and *Pparγ* was decreased in the left ventricle of D males (52% and 70%, respectively, p<0.05), but no differences were observed in the expression of *Pparδ* between groups. The mRNA expression of *iNos, MnSod, Chop, sXbp1* and *Atf4* was similar in both groups. Conclusion: The male offspring of female diabetic rats developed insulin resistance and showed impaired expression of *Ppars* in the left ventricle, but genes related to oxidative and ERS were unchanged. Further studies are needed to study the effects of PPARs alterations and the possible activation of proteins of the ERS.

700. (610) **UTILITY OF THE APPLICATION OF THE PROBABILITY RATIO OF THE EVOLUTION OF URIC ACID LEVELS DURING PREGNANCY IN THE ASSIGNMENT OF RISK OF GESTATIONAL HYPERTENSIVE DISEASE**

Ana Corominas[1], Silvia Balconi[1], Julieta Ruiz, [1]Alberto Ferreiros[1], Roberto Casale[1], Damiano Alicia E[2,3].
[1]Hospital Nacional Prof. A Posadas, Buenos Aires, Argentina. [2]Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO)- CONICET-Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. [3]Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Buenos Aires, Argentina. ana_corominas@yahoo.com.ar

Clinical decision-making involves the appropriate application of evidence-based medicine and involves a numerical method for calculating risks. This method includes pretest risks (obstetric and anthropometric history) and biochemical analyzes (or any other test in which performance parameters are known). Identification of pregnant women at risk of preeclampsia is still a challenge. Biomarkers available involve highly complex equipment and, in most cases, they have a short-term predictive role. In contrast, uric acid levels show an interesting diagnostic performance for preeclampsia being its dosage simple and accessible. Objective: to analyze the uric acid diagnostic attributes in pregnant women with different pretest risks, to estimate the risk of suffering gestational hypertensive disorders. Methods: Retrospective study. We analyzed the Positive and Negative Likelihood ratios (LR+, LR–) for the uricemia ratio (uricemia after and before the 20th week of gestation) and the incidence of gestational disorders. The post-test risks were calculated by interpolating in the Fagan diagram based on the pretest risk risk established in the guide for the diagnosis and treatment of hypertension in pregnancy (national ministry of health). Results: Using the pretest risk values based on the obstetric history, and the performance parameters of the uricemia ratio with a cut-off value of 1.5,the post-test values (when the uricemia ratio was≥1.5) obtained were 0.54 for preeclampsia in a previous pregnancy; 0.34 for Diabetes type 1 or 2; 0.03 for multiple pregnancies; 0.29 for the previous history of preeclampsia; 0.20 for maternal age > 40 years; 0.29 for nulliparity and 0.18 for BMI > 3; and around 0.01 in general when the uricemia ratio was <1.5. Conclusion: In this scenario, uricemia ratio contributes to restructuring the positive risk, and reduces the negative risk to almost zero, becoming a very good predictor of normotensive pregnancy and and thus contributing to better pregnancy care

REPRODUCTION II *Friday, November 18, 9-10:30 hr*
Chairs: Julieta Aisemberg - Romina Higa - Gabriela Jaita

701. (41) **ANALYSIS OF AUTOPHAGY AND APOPTOSIS IN THE CORPORA LUTEA OF THE VIZCACHA DURING PREGNANCY**

Caram DA[1,2], Inserra PIF[1,2], Vitullo AD[1,2] and Leopardo NP[1,2]
[1]CEBBAD - Universidad Maimónides. [2]CONICET.

Activity and regression of corpora lutea (CL) during mammal's gestation are finely regulated. Autophagy would play a dual role in defining the survival or death of CLs. The vizcacha, *Lagostomus maximus* (Lm), presents a gestation of 5 ½ months with a pseudo-ovulation process at mid-gestation that allows the formation of new CLs. We investigated autophagic and apoptotic protein expression during Lm pregnancy aimed to discern a possible role of autophagy in luteal cell survival or death. Ovaries of female Lm at early, mid and late pregnancy (N=6/group) were used. BECLIN1, LC3BI-II, LAMP1, P62 and C3A expression in CLs was analyzed by immunohistochemistry and western blot (WB), whereas electron microscopy (TEM) was used to analyze the presence of autophagic vesicles. Results were expressed as mean ± SD. ANOVA and Bonferroni post-test (p<0.05) were used. The majority of CLs (90%) were positive for autophagic markers, while relative optical density (DOR) and immunoreactive area (AIR) for all markers increased significantly as gestation progressed. C3A expression showed the lowest DOR (< 10%) with no significant differences among groups. Protein levels of BECLIN1, LC3B I-II, LAMP1, and P62 detected by WB increased significantly throughout pregnancy, whereas C3A expression was scarce. Finally, the number of autophagosomes significantly increased in regressing corpora lutea. Present results pinpoint autophagy as a survival mechanism induced at early pregnancy that contributes to the homeostasis and activity of healthy CLs. On the other hand, autophagy contributes to luteolysis of CLs during the late luteal phase at late pregnancy. This provides a new insight into the mechanism modulating luteogenesis and luteolysis in pregnant mammals.

702. (46) **PRENATAL ANDROGENIZATION AFFECTS OVARIAN LOCAL FACTORS THAT NEGATIVELY IMPACT ON FOLLICULAR DEVELOPMENT**

María José Ferrer[1], Giselle Adriana Abruzzese[1], Alicia Beatriz Motta[1].
[1]Laboratorio de Fisiopatología Ovárica, Centro de Estudios Farmacológicos y Botánicos (CEFYBO), UBA, CONICET. Buenos Aires, Argentina. majoferrer60@gmail.com

Prenatal androgen excess contributes to Polycystic Ovary Syndrome (PCOS) development. PCOS is the main cause of oligo-anovulation at reproductive age. Ovarian factors influence folliculogenesis, but it is still unknown how androgen excess affects them, impacting follicular growth and fertility. Thus, we aimed to study the effect of prenatal androgenization on local ovarian factors involved in folliculogenesis. Pregnant Sprague Dawley rats were injected with

testosterone (Prenatal androgenized group, PA) or vehicle (Control group) from days 16 to 19 of pregnancy. The female offspring were characterized according to the estrous cycle, and we obtained: regular ovulatory Control animals, and irregular ovulatory (PAiov) and anovulatory (PAanov) animals in the PA group. At 90 days of age, we analyzed the hormonal profile and ovarian parameters by qPCR and Western Blot. The analyzed proliferation markers were altered in the PA group. We found decreased Estradiol2/Testosterone ratio and *Sf1* mRNA levels in the PA group (p<0.05). The PAanov animals showed decreased AMH and IGF1r protein levels (p<0.05). The serum IGF1 levels were also decreased in the PA group (p<0.05). The pro/anti-apoptotic balance was deregulated in the PA group. The PAiov animals showed lower mRNA levels of *Bax* and *Bcl2*, while the PAanov animals showed higher *Bax* mRNA levels compared to the Control group (p<0.05). The ovarian inflammatory pathways and oxidative stress markers were altered in the PA group. We found decreased mRNA expression of *Nfkb*, *Cox2* and *IL6* in the PA group and a decreased expression of *TNFa* in the PAanov animals (p<0.05). We also found increased mRNA levels of the antioxidant enzyme *CuSOD* and decreased levels of GSH and mRNA expression of *GPX4* and *eNOS* in the PA group (p<0.05). To conclude, prenatal androgenization alters the ovarian proliferation/apoptosis balance, the inflammatory state, and oxidative stress, contributing to deregulating folliculogenesis in a phenotype-specific manner.

703. (47) **CHANGES IN THE EXPRESSION OF GENES INVOLVED IN THE OVARIAN FUNCTION BY DIET-INDUCED MATERNAL OBESITY**

María Agustina Meneghini, Jeremías Pablo Flores Quiroga, María Florencia Heinecke, Alicia Graciela Faletti
Centro de Estudios farmacológicos y botánicos (CEFYBO-CONICET-UBA), Buenos Aires, Argentina

Previously, we showed that female offspring from overweight rats showed higher body weight, early puberty, altered estrous cycle, lower number of primordial and primary follicles, higher ovulation rate, and lower number of healthy oocytes, than control offspring.
Thus, to identify which genes could be modified by maternal overweight, induced by a high-fat diet, this study aimed to assess the expression of some genes involved in the ovarian function. To this end, female rats were fed standard or cafeteria diet from 22 days of age until weaning of their offspring, including pregnancy and lactation. Female offspring were euthanized on the estrous day of the second estrous cycle. To evaluate the results according to the maternal overweight achieved, rats were divided into three groups: offspring from rats with 18%, 25% and 35% overweight (OCD18, OCD25 and OCD35, respectively). Total mRNA from ovaries was extracted and processed by real-time RT-PCR with specific primers. Compared with controls and expressed as relative units, we found increased expression of some genes involved in steroidogenesis, including: i) *Cyp17* in OCD35 rats (7±2; p<0.01); ii) *Cyp19* (29±7; p<0.001) and Foxo1 (1.4±0.2; p<0.01) in OCD25 rats; iii) *Cyp11* in OCD35 (67±11; p<0.001) and OCD25 rats (43±9; p<0.01); and iv) *17βHsd* in OCD18 (36±23) and OCD25 rats (33±5; p<0.05). However, the expression of Akt1, involved in folliculogenesis, showed a decrease compared with controls (0.8±0.1; p<0.05), which ranged between 0.4±0.1 and 1.4±0.2 (p<0.05) in OCD rats. Consistent with these results, OCD rats showed an increase in genital fat, which was around 5±0.3, p<0.01 compared with controls (4±0.2). These results indicate that the negative changes found in the reproductive system of female offspring induced by maternal overweight may be, at least in part, due to epigenetic changes in the genes involved in steroidogenesis and folliculogenesis.

704. (48) **CHANGES IN GENE EXPRESSION AT TESTICULAR LEVEL CAUSED BY DIET-INDUCED MATERNAL OBESITY**

María Agustina Meneghini, Jeremías Pablo Flores Quiroga, María Florencia Heinecke, Verónica White, Alicia Graciela Faletti
Centro de Estudios farmacológicos y botánicos (CEFYBO-CONICET-UBA), Buenos Aires, Argentina

Previously, we showed that male offspring from overweight rats showed higher body weight, lower testis and epididymal indices, early puberty, lower number of germ cells and motility, deficiency in the sperm mitochondrial function and acrosome reaction, and abnormal metaphases, as compared to control offspring. The present study aimed to evaluate the expression of some selected genes involved in the reproductive function. To this end, maternal overweight in rats was induced by a cafeteria diet, which was supplied continuously until weaning of their offspring, including pregnancy and lactation.
To evaluate the results according to the maternal overweight achieved, rats were divided into three groups: offspring from rats with 18%, 25% and 35% overweight (OCD18, OCD25, OCD35, respectively). Total mRNA was extracted from testes and processed using a real-time RT-PCR with specific primers. Compared with controls and expressed as relative units, *mTor* expression (involved in spermatogenesis) increased in all groups (between 2.0±0.2 and 2.2±0.4 vs 0.7±0.2; p<0.05), *Ahr* expression (involved in obesity) increased in OCD18 (9±4 vs 0.7±0.1; p<0.05), and *Nlrp3* expression (involved in inflammation) increased in OCD35 (2.8±0.6 vs 1.1±0.2; p<0.05), respectively. Regarding steroidogenesis, *Cyp17* expression increased in OCD35 (9±2 vs 1.0±0.3; p<0.01), *Cyp19* expression increased in OCD25 and OCD35 (342±33 and 374±77, vs 1.1±0.2; p<0.001), but no differences were found in *Cyp11* expression. Consistent with these results, OCD35 rats showed higher content of genital fat (5.4±0.3 vs 3.8±0.1; p<0.001) and greater presence of reactive oxygen species (11±3; p<0.001), by fluorescence probe, and TUNEL-positive cells in spermatozoa (6.4±0.7; p<0.001), all compared with controls (2.3±0.3; 1.9±0.6; respectively).
These results suggest that maternal overweight programs the offspring to develop changes in essential genes involved in different functions likely leading to a reproductive imbalance.

705. (116) **CCR2 SIGNALING PATHWAY IS DOWNSTREAM EGFR WITHIN THE CUMULUS-OOCYTE COMPLEX IN THE PERIOVULATORY PROCESSES**

Conte Julia Gaetana[1,2], Ballerini María Gabriela[1], Jaita Gabriela Alejandra[2], Peluffo Marina Cinthia[1]
[1]*Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) -CONICET–FEI–División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez"*
[2]*Instituto de Investigaciones Biomédicas (INBIOMED) -UBA-CONICET, Facultad de Medicina UBA*
e-mail: mpeluffo@cedie.org.ar

Recently, we have proposed the chemokine CCR2 receptor and its main ligand (monocyte chemoattractant protein-1, MCP1) as novel mediators of the ovulatory cascade. Our previous results also showed their interaction with the epidermal growth factor receptor (EGFR, a key signaling pathway in the ovulatory cascade) regarding the expression of periovulatory genes stimulated by the gonadotropins (GNT). The aim of the present study was to further evaluate the interaction between EGFR and CCR2 signaling in cumulus-oocyte expansion (C-OE) and oocyte maturation. To evaluate this, ovaries from adult female cats (*Felis catus*, n=32) at different stages of the estrous cycle during the breeding season were used. Cumulus oocyte complexes (COCs, n=301) were cultured either for 24 h (in C-OE media) or 28 h (in oocyte maturation media) under different treatments: Control: Media alone, GNT: FSH+LH, GNT/INH: GNT+EGFR inhibitor (INH), GNT/INH/MCP1: GNT+INH+MCP1, INH control: INH, INH/MCP1: INH+MCP1, MCP1: MCP1. At the end of the culture, the COCs were fixed in 4% PFA to assess hyaluronic acid (HA) by IF, media stored to measure estradiol (E2), progesterone (P4), and testosterone (T), and COCs were treated with hyaluronidase to evaluate oocyte maturation. Confocal analysis showed that HA stimulation by GNT within the expanded matrix in the COC was reduced in the presence of the INH. Whereas MCP1 could revert this effect observing HA through the whole COC. Almost all the samples showed E2 and P4 levels with the highest concentrations observed within the GNT and GNT/INH/MCP1 groups. T was not detected in any of the samples. Regarding oocyte maturation, interestingly the highest percentage of MII oocytes was observed in the GNT/INH/MCP1 group (p<0.05; compared to the control). In conclusion, our results confirm the interaction between CCR2 and

EGFR pathways in the C-OE process and oocyte maturation, with the MCP1/CCR2 pathway being downstream of the EGFR in the ovulatory cascade.

706. (202) **URINE CELL-FREE DNA AS A POTENTIAL BIOMARKER FOR OBSTETRICS COMPLICATIONS. AN ALTERNATIVE DETECTION METHOD**

María E. Racca[1,2], Paula J. Cepeda[1,2], María F. Rossetti[1,2], Joge G. Ramos[1,2], María A. Cardozo[1,2,5], Enrique H. Luque[1,4], Mónica Muñoz-de-Toro[1,3], María M. Milesi [1,4], Jorgelina Varayoud [1,4]

*[1] Instituto de Salud y Ambiente del Litoral (ISAL), CONICET-UNL, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2] Departamento de Bioquímica Clínica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [3] Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [4] Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [5] BLUT Laboratorios, Santa Fe, Argentina. *These authors contributed equally to this work.*

Urine cell-free DNA (cfDNA) could serve as a biomarker for different diseases (e.g., obstetric complications). Noninvasive sampling and the possibility to analyze large volume samples are advantages of urine-based protocols. Urine cell-free DNA assessment usually includes commercial kits. We aimed to determine the efficiency of an alternative purification method to extract and quantify urine cfDNA, comparing cfDNA levels on pregnant (P) and non-pregnant (NP) women. cfDNA was isolated from urine samples of P (first, second and third trimester, n=47) and NP (n=23) women. After urine pH neutralization and high-revolution centrifugation, cfDNA was purified from 800 μL supernatant applying an organic extraction method. First, urine samples were lysed with 5M NaCl and 20% sodium dodecyl sulfate solutions, then cfDNA was extracted with chloroform followed by ethanol precipitation. cfDNA was resuspended in 50 μL Tris-EDTA buffer and resulting concentrations were measured by UV spectrophotometry. Purified cfDNA integrity was analyzed by agarose gel electrophoresis. Finally, cfDNA levels were quantified by real time PCR amplification of Actin-β gene (*ACTB).* cfDNA was detected in 69% urine samples and yielded specific *ACTB* amplification with low standard deviation between PCR duplicates (Ct difference<0.5). A wide variability in cfDNA level was detected with values ranging from 10380 to 1217340 copies, respectively. There was no difference in cfDNA level between groups. A highly fragmented DNA was found in electrophoresis without a predominant fragment size. The present work proposes an alternative urine cfDNA detection method. Future studies will focus on evaluating urine cfDNA in a greater number of P women to analyze its utility in different obstetric outcomes prediction.

707. (220) **SARS-CoV-2, VACCINES AND FEMALE REPRODUCTION: EFFECTS ON OVARIAN FUNCTION**

Yamila Herrero 1, Candela Velázquez 1, Pomponio Valeria 1, Vanesa Hauk 2, Ignacio de Zúñiga 3, Gustavo Martínez 4, Mariano Lavolpe 5, Florencia Veiga 6, Leopoldina Scotti 1,7, Dalhia Abramovich 1, and Fernanda Parborell 1.

1 Laboratorio de Estudios de Fisiopatología Ovárica, Instituto de Biología y Medicina Experimental (IByME) – CONICET, Buenos Aires, Argentina, 2 Laboratorio de Inmunofarmacología, Instituto de Química Biológica (IQUIBICEN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires-CONICET, Buenos Aires, Argentina, 3 Pregna Medicina Reproductiva, Buenos Aires, Argentina, 4 Medicina Reproductiva Fertilis, Buenos Aires, Argentina, 5 In Vitro Buenos Aires, Buenos Aires, Argentina, 6 WeFIV, Buenos Aires, Argentina, 7 Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – CONICET - UNNOBA – UNSAdA, San Antonio de Areco, Argentina.

We have previously shown that alterations in ovarian function can be observed up to 9 months after SARS-CoV-2 infection. Until now, no study has evaluated the effect of SARS-CoV-2 infection over longer periods and the effect of vaccines on the female gonad. In the present study, we propose 2 objectives: a) Evaluate ovarian function in patients who have suffered from COVID-19 (mild symptoms) up to 18 months post-infection and b) Evaluate the effect of vaccines against COVID-19 (inactivated virus and adenovirus) on reproductive performance. A total of 125 women (21–43 years old) under ART were recruited between November 2020 and May 2022 from 4 reproductive medicine institutions. For objective a), the patients were classified into: control group (n=30) (no positive test for COVID-19) and post-COVID-19 group (n=55) (at least one positive test by PCR). For objective b), patients are classified into: unvaccinated group (n=70) and group vaccinated for COVID-19 (n=55). The results showed that in patients (without vaccination) the number of retrieved, mature and 2PN oocytes decreased up to 12 months after COVID-19 infection compared to the control regardless of age ($p<0.01$). When patients with and without vaccine are considered together, the number of retrieved and mature oocytes decreases even up to 18 months post-infection in those patients older than 35 years. Reduced levels of IL-1β in FF were observed in patients up to 18 months post-infection compared to the control group ($p<0.001$) without changes in IL-10 levels. Additionally, the number of retrieved and mature oocytes did not change in vaccinated patients compared to unvaccinated patients regardless of the type of vaccine and infection. In conclusion, our results show that ovarian function is altered up to 18 months after infection. In addition, the vaccines do not affect female fertility.

708. (261) **ROLE OF HYPOXIA INDUCIBLE FACTOR-1 ALPHA (HIF-1α) IN MOUSE OVARIAN LUTEINIZATION AND ANGIOGENESIS**

Rocío Marinoni, María José España de Marco, Candela Velazquez, Marta Tesone, Dalhia Abramovich.

Instituto de Biología y Medicina Experimental (IByME- CONICET).

Introduction: Angiogenesis is an essential process in the ovary that allows follicles to grow and develop a competent oocyte. It also allows correct ovulation and adequate corpora lutea formation. Hypoxia inducible factor-1 alpha (HIF-1α) is the main angiogenesis inductor capable of sensing hypoxia and, in response to low oxygen concentration, it promotes the formation of new blood vessels. Objective: To elucidate the role of HIF-1α in the ovary during corpus luteum formation using a superovulated mouse model. Methods: Prepubertal (25do) female F1 mice (BALB/c x C57) were used. The animals received 5 IU of eCG i.p followed by 5 IU of hCG i.p 48h later. One group of animals received i.p 5 mg/kg of the HIF-1α inhibitor Acriflavine (ACR) the same day of hCG injection (ACR group). The other group received saline (control group). 48h after hCG, mice were sacrificed and the ovaries recovered. WB and histological techniques were performed. Statistical analysis was carried out using unpaired t-test. Results: Ovarian VEGF levels were decreased and PDGFB was increased after ACR administration. The levels of ANGPT2 did not change between groups. The number of corpora lutea and follicular structures and the endothelial cell area were similar in both the control and the ACR groups. Periendothelial cell area was significantly decreased within the corpora lutea of ACR group. Hemorrhagic cysts were found in some ovaries only from ACR group. Conclusions: HIF-1α inhibition decreased VEGF and periendothelial cell area in the recently formed corpora lutea. PDGFB was increased, probably due to an attempt to stabilize the new vessels. Therefore, HIF-1α inhibition modifies angiogenic factor secretion by ovarian cells leading to the formation of unstable vessels and an alteration in blood supply to the newly formed corpora lutea. More studies are needed to assess the effect of these alterations in corpus luteum function.

709. (329) **LONG-TERM EFFECTS OF POLYCYSTIC OVARY SYNDROME ON THE RAT UTERUS**

Gisela Soledad. Bracho, María Virginia. Acosta, Inri. Iñiguez, Enrique Hugo. Luque, Verónica Lis. Bosquiazzo

Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. It is known that in PCOS, the hormonal imbalance could increase the risk of developing uterine alterations such as endometrial hyperplasia or even cancer. The aim of this study was to investigate the long-term effects of PCOS on the rat uterus. For this, we evaluated a) serum steroid hormone profiles and b) incidence and multiplicity of uterine abnormalities. To induce PCOS, Wistar female rats were injected subcutaneously with dehydroepiandrosterone (6mg/100g body weight, PCOS group) from 21 to 40 days of age. The CONTROL group received oil sesame injections. After the treatment, the animals were allowed to grow until adulthood (18-24 months approximately) and were euthanized in vaginal diestrous. Blood samples were collected to evaluate the serum levels of sex steroids and the uterine horns were dissected and processed for histological studies. All PCOS rats presented detectable levels of 17β-estradiol (E2) compared to 77.8% of CONTROL rats. In addition, the PCOS group showed a higher serum E2 levels (CONTROL: 10,7 ± 2,7 vs. PCOS: 17,6 ±1,9 pg/mL, $p<0.05$). Progesterone and testosterone serum levels did not show differences between the experimental groups. Alterations in the luminal epithelium and glandular compartment were observed in all experimental groups. However, in PCOS rats the uterine histopathology analysis showed a higher incidence of intraepithelial lumens, intraepithelial glands and stratification of the luminal epithelium. Also, the multiplicity of squamous metaplasia and conglomerates of glands increased in PCOS rats ($p<0.05$). These results demonstrated long-term effects of PCOS, evidenced by high serum E2 levels in association with an increased in the incidence and multiplicity of uterine lesions. These uterine lesions may be responsible for low fertility and high risk of endometrial cancer in PCOS rats.

710. (373) **INCREASED RECRUITMENT OF GRANULOCYTES IN THE OVARIES OF DAIRY COWS WITH CYSTIC OVARIAN DISEASE (COD)**

Antonela Stassi[1,2], Lucas Etchevers[1,2], Verónica Acosta[1], Lucía Villalba[1], Sofía Cainelli[1], Natalia Salvetti[1,2], Florencia Rey[1,2], Hugo Ortega[1,2].

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET) Universidad Nacional del Litoral, Argentina. [2]Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina.

COD is among the most relevant cause of subfertility in dairy cattle and is the result of ovulation failure. Ovulation has been characterized as an inflammatory process in which granulocytes are involved. Therefore, the aim of this study was to evaluate the distribution of granulocytes (CH138+) in ovaries of animals with spontaneous COD and control (C) animals. Ovariectomy was performed in animals with COD to obtain ovaries (n = 5), also control cows were ovariectomized in proestrus (n = 5). CH138+ cells were evaluated through immunohistochemistry in total ovary (TO), in ovarian cortex (OC), medulla (OM), theca interna (TI) and externa (TE) of cysts and dominant follicles. In addition, CH138+ cells were evaluated by immunocytochemistry performed on samples of follicular fluid from (FF) cysts and dominant follicles. The specificity of the antibody was corroborated by western blot (WB). The data was analyzed using ANOVA with Duncan's post-test and T-student test. WB analysis detected a strong positive band of 17 KDa for CH138. Comparison between groups showed that, the number of CH138+ cells was higher in TO, OM and TE of COD group than in C group ($p<0.05$). The analysis within group showed that, CH138+ cells in the C group were lower in TI and TE than OC and OM ($p<0.05$). CH138+ cells in the COD group were lower in TI than TE, OC and OM ($p<0.05$). Finally, CH138+ cells in FF of the COD group were similar to the C group ($p>0.05$). This is the first description about the evaluation of granulocytes in FF in cattle with COD. Growing evidence indicates that infiltrated leukocytes, such as granulocytes, produce additional cytokines and chemokines, acting as *in situ* modulators of ovarian function. These results demonstrate that the immune system and inflammatory pathways are strongly involved in ovarian events related to this disease. Therefore, we propose that this high proportion of these cells could be related to the anovulation observed in COD.

711. (397) **EVALUATION OF STEROIDOGENIC ENZYMES IN A MODEL OF FOLLICULAR PERSISTENCE IN DAIRY COWS**

Cattaneo Moreyra ML[1,2], Gareis NC[1,2], Hein GJ[1,3], Angeli E[1,2], Ortega HH[1,2], Salvetti NR[1,2], Rodríguez FM[1,2], Rey F [1,2].

[1]Laboratorio de Biología Celular y Molecular Aplicada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe.[2]Facultad de Ciencias Veterinarias-UNL, Esperanza, Santa Fe. [3]Centro Universitario Gálvez (CUG-UNL), Gálvez, Santa Fe.

In dairy cows with cystic ovarian disease, steroid hormones and protein levels of steroidogenic enzymes have been reported to be altered. Furthermore, in cows with ovarian follicular persistence, altered concentrations of estradiol in serum and follicular fluid were observed. Therefore, we aimed to evaluate the protein expression of enzymes involved in the synthesis of steroid hormones (StAR, CYP19, CYP17 and 3β-HSD) in ovarian tissue of cows with follicular persistence. Holando Argentino cows with healthy reproductive tract (n=25) were subjected to estrous cycles synchronization with a G6G-Ovsynch protocol. While cows of the control group (CG, n= 5) were only synchronized, cows of the experimental groups with 0 (P0, n=5), 5 (P5, n=5), 10 (P10; n=5) and 15 days of persistence (P15; n=5), were also treated with progesterone intravaginally (P4). Finally, the ovaries were obtained by bilateral ovariectomy. Histological ovarian sections were used for the indirect immunohistochemistry to localize and quantify *in situ* protein expression of StAR, CYP19, CYP17 and 3β-HSD in persistent and control follicles. Protein expression of CYP17 in theca cells and StAR in granulosa and theca cells was similar in the analysed groups ($p>0.05$). Lower CYP19 expression was detected in the P5 and P15 groups compared to the control group ($p<0.05$). The expression of 3β-HSD was greater in granulosa and theca cells in the persistent follicles of P15 group compared to follicles of P0 group ($p<0.05$). In addition, in granulosa cells, a greater expression was detected in the P10 group compared to the P0 group ($p<0.05$). These results show a local alteration of the enzymes involved in the steroidogenic pathway during persistence. Considering the results registered in cows with cysts, we could infer that the alterations in CYP19 and 3β-HSD occur before the cysts development.

712. (445) **EXTRACELLULAR VESICLES FROM THE PERIODONTAL BACTERIA *PORPHYROMONAS GINGIVALIS* REDUCE GLUCOSE METABOLISM IN TROPHOBLAST CELLS MODIFYING THEIR ANTIINFLAMMATORY AND TOLEROGENIC EFFECTS**

Brenda Lara[1], Iñaki Loureiro[1], Laura Gliosca[2], Guillermina Calo[1], Soledad Gori[1], Ana Schafir[1], Florencia Sabbione[3], Analía Trevani[3], Rosanna Ramhorst[1], Daiana Vota[1], Claudia Pérez Leirós[1], Vanesa Hauk[1]

[1] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inmunofarmacología. Buenos Aires, Argentina. CONICET, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

[2] Universidad de Buenos Aires, Facultad de Odontología. Laboratorio de Diagnóstico Microbiológico, Cátedra de Microbiología. Buenos Aires, Argentina.

[3] Laboratorio de Inmunidad Innata, Instituto de Medicina Experimental (IMEX), Academia Nacional de Medicina, CONICET, Buenos Aires, Argentina.

Adverse pregnancy outcome and deep placentation disorders are associated with periodontal disease. *Porphyromonas gingivalis (Pg),* one of the main pathogens causing periodontitis, releases extracellular vesicles known as outer membrane vesicles (PgOMV) that play an important role in the host-pathogen interaction. During

placentation, trophoblast cells (Tb) secrete soluble factors in a close interaction with maternal leukocytes to maintain immune homeostasis. An impaired trophoblast-immune interaction is associated with pregnancy complications. Previously we reported that PgOMV diminished the migration and invasion capacity of Tb. Our aim was to study the effect of PgOMV on Tb cell metabolism and trophoblast-immune interaction. Human first trimester trophoblast cell line HTR-8 was stimulated with 1 μg/ml PgOMV for 4-24h. Lipid droplets, glucose and fatty acid uptake were analyzed by flow cytometry using Bodipy 493/503, 2-NBDG and FLC12 probes, respectively. Lactate production was measured by Accutrend Plus system and mRNA expression of inflammatory mediators by qPCR. Peripheral blood monocytes and neutrophils were purified from healthy donors and cultured with conditioned media from Tb (CM) pre-treated or not with Pg-OMV (PgOMV-CM). Reactive oxygen species (ROS) production was evaluated by flow cytometry. PgOMV treatment reduced glucose uptake and the release of lactate by trophoblast cells (Basal 8.3±0.8 mM; Pg-OMV 7.2±0.4 mM; X±SEM n=4 $p<0.05$), whereas it increased fatty acid uptake. PgOMV also altered IL-6, CCL-2, TGF-b1 and IL-8 mRNA expression with >50% decrease in IL-8 ($p<0.05$). PgOMV-CM lost the capacity to reduce PMA-induced ROS production in neutrophils and of inducing tolerogenic HLA-G+ dendritic cells. Our results indicate that Pg-OMV reprograms the metabolism of trophoblast cells affecting their regulatory effect on dendritic cells and neutrophils. This mechanism might contribute to the pathogenic effect of Pg on pregnancy outcome.

713. (478) **PERIODONTITIS AND PREGNANCY COMPLICATIONS: VIRULENCE FACTORS OF PERIODONTAL BACTERIA INDUCE THE EXPRESSION OF INFLAMMATORY FACTORS IN HUMAN PLACENTAL EXPLANTS**

Iñaki Loureiro[1], Brenda Lara[1], Luciana Doga[2], Sofía Novoa[3], Luciana D`Eramo[2], Ariana Santillán[3], Guillermina Calo[1], Laura Gliosca[4], Pablo Fabiano[3], Aldo Squassi[2], Claudia Pérez Leirós[1], Vanesa Hauk[1].

1 Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inmunofarmacología. Buenos Aires, Argentina. CONICET, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina.

2 Universidad de Buenos Aires - Facultad de Odontología. Instituto de Investigaciones en Salud Pública.Cátedra de Odontología Preventiva y Comunitaria. Buenos Aires, Argentina.

3 Hospital General de Agúdos Dr. Cosme Argerich, Servicio de Obstetricia, Ciudad Autónoma de Buenos Aires, Argentina.

4 Universidad de Buenos Aires – Facultad de Odontología. Instituto de Investigaciones en Salud Pública. Cátedra de Microbiología. Buenos Aires, Argentina.

Placental inflammation is an important cause of placental dysfunction and pregnancy complications. Several studies have linked periodontal disease to adverse pregnancy outcomes although the pathogenic mechanisms are still unclear. It is proposed that periodontal pathogens could directly induce a mild inflammation of the placenta, compromising placentation and pregnancy outcome. The aim of this work is to characterize placental inflammation associated with the presence of periodontopathic bacteria and their virulence factors. We used a model of human third-trimester term placental explants to study the effects of periodontal bacteria virulence factors (LPS from *P. gingivalis* (PgLPS) and extracellular membrane vesicles) or gingival crevicular fluid (GCF) from pregnant women at 38-40 weeks of gestation (n=15). The effects of PgLPS were compared to *E. coli* lipopolysaccharide (EcLPS) or GCF from non-pregnant women, respectively. Gene and protein expression of pro and antiinflammatory cytokines were analyzed by qPCR and ELISA. PgLPS treatment induced the expression of proinflammatory mediators (TNF-α, IL-6 and IL-8; $p<0.05$) however the effect was weaker than EcLPS. On the other hand, PgLPS did not induce the secretion of IL1β compared to EcLPS (basal 126,4±54,8 pg/ml; PgLPS 171±63,73 pg/ml; EcLPS 1314±44,98 pg/ml basal/PgLPS vs EcLPS $p<0.05$). Upon conditioning of placental explants with GCF, only the GCF from pregnant women stimulated TNF-α expression. Results point to PgLPS as one of the virulence factors that could be present in the GCF and induce a mild inflammation of the placenta, a characteristic of pregnancy in patients with periodontal disease.

714. (588) **ALTERED GALECTIN EXPRESSION IN THE OVARY OF COWS WITH INDUCED FOLLICULAR PERSISTENCE**

Juan Alberto Chiaraviglio[1], Lucas Etchevers[1,2], Nadia Estefanía Ormaechea[1,2], María Lucia Cattaneo Moreyra[1,2], Pablo Uriel Díaz[1,2], Hugo Héctor Ortega[1,2], Florencia Rey[1,2], Natalia Raquel Salvetti[1,2].

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET) Universidad Nacional del Litoral (UNL), Argentina. [2]Facultad de Ciencias Veterinarias, UNL, Esperanza, Santa Fe, Argentina.

In dairy cattle, reproductive alterations cause important economic losses. Cystic ovarian disease is one of the most important reproductive alterations characterized by follicles larger than 20 mm in diameter that persist for at least 10 days in the absence of corpus luteum and uterine tone and interrupting estral cycles. Galectins are proteins with affinity for polylactosamines residues that have different functions in the regulation of apoptosis and proliferation. The objective of this study was to characterize galectins expression in ovarian follicular structures in cows with follicular persistence induced model. Galectins (Gal) 1, 3, 4 and 7 were detected by immunohistochemistry in dominant (DF) and persistent follicles (PF). The model was performed by using an intravaginal progesterone device and obtaining ovaries with DF in the expected day of ovulation (n=5; P0) and PF at 5 (n=5; P5), 10 (n=5; P10) or 15 days (n=5; P15) relative to the expected day of ovulation. Controls cows were ovariectomized in proestrus (n=5). Gal 1, Gal3, Gal4 and Gal7 were detected and evaluated in the nucleus and cytoplasm in granulosa and theca cells of DF and PF. The expression of Gal1 in the nucleus of granulosa of PF of P5 was lower than in DF from control group ($p<0,05$). The expression of Gal3 was lower in the cytoplasm of theca cells of PF of P15 group than DF of control group ($p<0,05$). The expression of Gal4 in the cytoplasm of both granulosa and theca cells was higher in PF of P10 and P15 than DF of control group ($p<0,05$). The expression of Gal7 was lower in the nucleus of granulosa cells of PF of P10 group than DF of control group ($p<0,05$). This altered expression of different galectins could participated in the development and persistence of dominant follicles leading to develop and maintain cystic follicles.

715. (592) **INTERGENERATIONAL TRANSMISSION OF METABOLIC ALTERATIONS IN THE LEFT VENTRICLE OF TYPE 2 DIABETIC MALE RATS**

Irune Pirrone, Cintia Romina Gatti, María Laura Leonardi, Alicia Jawerbaum, Evangelina Capobianco.

Laboratory of Reproduction and Metabolism, CEFYBO-CONICET, School of Medicine, University of Buenos Aires, Argentina.

The prevalence of type 2 diabetes (T2D) can be related to the intergenerational transmission of metabolic anomalies that affect the offspring's health. We previously observed development of T2D and cardiac alterations in the offspring of female diabetic rats. Diabetic cardiomyopathy (DCM) is described by anomalies in the left ventricle and is associated with oxidative stress, inflammation, endoplasmic reticulum stress (ERS) and other pathologic conditions. Peroxisome proliferator activated receptors (PPARs) are transcription factors related to metabolism and anti-inflammatory processes in the heart. The aim was to evaluate the expression of PPARs, inducible nitric oxide synthase (*iNos)*, the antioxidant enzyme *MnSod* and genes involved in the ERS, in the left ventricle of male rats that develop diabetes by intrauterine programming. Methods: The male offspring of female Control (C) and diabetic rats (D: diabetic males obtained by intrauterine programming) were euthanized at 8 months of age (n=8). Triglycerides (TG) and HDL-cholesterol (HDL-c) were mea-

sured in plasma by colorimetric assays. The TG/HDL-c ratio was calculated as a measure of insulin resistance. The left ventricle was stored at -80°C for the evaluation of mRNA (by qRT-PCR) of *Pparα*, *Pparγ*, *Pparδ*, *iNos*, *MnSod* and ERS genes (*Chop*, *sXbp1*, *Atf4*). Results: The TG/HDL-c ratio was increased in D males (73% p< 0.05). The expression of *Pparα* and *Pparγ* was decreased in the left ventricle of D males (52% and 70%, respectively, p<0.05), but no differences were observed in the expression of *Pparδ* between groups. The mRNA expression of *iNos, MnSod, Chop, sXbp1* and *Atf4* was similar in both groups. Conclusion: The male offspring of female diabetic rats developed insulin resistance and showed impaired expression of *Ppars* in the left ventricle, but genes related to oxidative and ERS were unchanged. Further studies are needed to study the effects of PPARs alterations and the possible activation of proteins of the ERS.

716. (621) **ESTRÉS OSMOTICO Y EXPRESIÓN DE CAVEOLINA 1 EN CÉLULAS AMNIÓTICAS**

Magali Berton, Nazarena Fernandez, Matias Sierra, Ana Corominas, Alicia E Damiano, Mauricio Castro Parodi.
Catedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Osmotic stress and Caveolin-1 expression in amniotic cells
Amniotic fluid is essential for normal fetal growth and development. Emerging evidence shows that the primary mechanism that regulates the intramembranous flow is an active unidirectional vesicular transport of water and solutes across the amnion cell It has been proposed that this transport process is mediated by transcytosis of caveolae-like vesicles. Oligohydramnios is a pregnancy pathology in which amniotic fluid volume decreases, increasing the osmolarity. This pathology is usually associated with preeclampsia in which we previously observed a decrease in caveolae in trophoblast cells. We hypothesize that hyperosmolarity alters the number of caveolae affecting the expression of caveolin-1 (Cav-1). Our objective was to evaluate the expression of caveolin 1 in amniotic and trophoblast cells exposed to hyperosmolarity. This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. Alejandro Posadas. Human amnion and trophoblast explants were cultured in complete DMEM-F12 and sucrose hyperosmolar solutions were added to generate the hyperosmolar conditions. Cav-1 expression was analyzed by Western Blot analysis and its localization by immunohistochemistry. Results: In villus explants cultured in hyperosmolarity, we observed a 2.2-fold increase in caveolin 1 expression (p=0.0124, n=3), while no significant changes were observed in amniotic explants (p=0.08, n=3). The labeling of Cav-1 was found in the plasma membrane. Conclusions: Our results suggest that vesicular transport in the amnion would not be mediated by caveolae-like vesicles in hyperosmolarity. More studies are necessary to evaluate the increase of Cav-1 in trophoblast cells in this osmotic condition.

717. (739) **SYNCYTIOTROPHOBLAST STRESS PROMOTES THE RELEASE OF AQP-POSITIVE EXTRACELLULAR VESICLES**

Sierra, Matías Nicolás[1,2]; Fernandez, Nazarena[1]; Berton, Magalí[1]; Castro Parodi, Mauricio[1]; Ferreiros, Alberto[3]; Corominas, Ana[3]; Casale, Roberto [3]; Szpilbarg, Natalia[2]; Damiano, Alicia E[1,2]
[1] Cátedra de Biología Celular y Molecular, Departamento de Cs. Biológicas. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (FFYB-UBA). Buenos Aires, Argentina.
[2] Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay). UBA-CONICET. Universidad de Buenos Aires. Buenos Aires, Argentina
[3] Hospital Nacional Profesor Alejandro Posadas. El Palomar, Provincia de Buenos Aires, Argentina.

Introduction: Preeclampsia is a gestational disorder unique to human pregnancy. Although its etiology remains unclear, this syndrome is associated with placental insufficiency, which increases syncytiotrophoblast stress and the release of extracellular vesicles (EVs). This cellular stress may affect placental transport functions altering the expression of different proteins as aquaporins (AQPs). We previously reported that placental AQPs may have a key role during placentation, and we found an abnormal expression of AQP3, AQP4, and AQP9 in preeclamptic placentas. Recent evidence shows that EVs not only may reflect the state of the tissue from which they come but also, can modulate cell communication. However, the significance of placenta AQP-positive EVs was not studied yet. We hypothesized that syncytiotrophoblast stress promotes the release of AQP-positive EVs that are involved in the etiology of preeclampsia. Objective: To study AQP3, AQP4, and AQP9 expressions in EVs isolated from placenta explants exposed to stress. Methods: Normal placental explants were cultured with 100 μM of $CoCl_2$ to induce syncytiotrophoblast stress. After incubations, a histological examination of the explants was performed to evaluate tissue integrity and quantify syncytial nuclear aggregates (SNA). EVs from the culture medium were isolated by differential centrifugation and analyzed in Zetasizer Nano-ZSP equipment. AQP3, AQP4, AQP9, and PLAP expression were assessed by Western blot. Results: $CoCl_2$ treatment enhanced the number of SNA (p<0.05; n=6). According to their expression in placental explants, AQP4 and AQP9 significantly increased in EVs after the stress induction (p<0.01; n=4, p<0.05; n=3, respectively), while AQP3 decreased (p<0.01; n=4).
Conclusion: Syncytiotrophoblast stress promotes the release of AQP-positive EVs, reflecting the status of the tissue. Further studies are required to investigate the clinical utility and the role of these AQP-positive EVs in preeclampsia.

718. (748) **REGULATION OF THE HYPOXIA INDUCIBLE FACTOR HIF-1α IN THE HTR8/SVneo CELL LINE**

Sofía Nardi[1], María Sol Pessino[1], Lucille T. Kourdova[1], María Laura Rojas[1], Jesica Flores-Martín[1], Susana Genti-Raimondi[1], Graciela M. Panzetta-Dutari[1], Ana C. Racca[1].
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

During pregnancy oxygen concentration is tightly regulated and alterations in its physiological levels are associated to pregnancy disorders such as preeclampsia. HIF-1α protein is rapidly degraded in the presence of oxygen and stabilized when oxygen levels decrease, regulating more than 50 genes in mammals thus influencing cell behavior. In this work, we identified a negative regulator of HIF-1α under hypoxia in the extravillous trophoblast-derived HTR8/SVneo cell line. We have previously determined that the tumor suppressor KLF6 is transiently increased in hypoxia in a HIF-1α dependent manner. Interestingly, here we established that KLF6 downregulation with specific siRNAs further increases HIF-1α transcript and protein levels under hypoxia, as evaluated through qPCR and western blot respectively, suggesting a regulatory loop between these two transcription factors. The scavenge of reactive oxygen species (ROS) with N-acetyl cysteine and the expression of negative dominant variants of the NFκB pathway revealed that both signals are involved in the induction of HIF-1α in response to KLF6 silencing. Also, we determined that KLF6 downregulation under hypoxia induces an active HIF-1α protein since in that context, the vascular endothelial growth factor (VEGF) mRNA, a classical HIF-1α target, is increased. Concomitant downregulation of KLF6 and HIF-1α using specific siRNAs restored VEGF transcript levels. On the other hand, the overexpression of KLF6 under hypoxia led to a decrease in HIF-1α protein but its mRNA level remained unmodified. Moreover, treatment of KLF6-overexpressing HTR8/Svneo cells with 250 μM of cobalt chloride (an inhibitor of HIF-1α degradation) prevents HIF-1α protein decrease indicating that KLF6 also regulates HIF-1α protein stability. These results suggest a complex regulatory loop between HIF-1α and KLF6 that may be involved in the pathophysiology of pregnancy diseases and other disorders in which oxygen plays an essential role.

719. (875) **INTRAFOLLICULAR APPLICATION OF ACTH IN DAIRY COWS DELAY OVULATION WITHOUT CHANGES IN FOLLICULAR BLOOD FLOW**

E. Matías Belotti[1,2], Lucas Etchevers[1,2], Elias Filippa[1,2], Ayelén

Amweg[1,2], Nadia Ormaechea[1], Juan A. Chiaraviglio[1], Ulises Notaro[1], Hugo H. Ortega[1,2], Natalia R. Salvetti[1,2].
[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET) Universidad Nacional del Litoral (UNL), Argentina. [2]Facultad de Ciencias Veterinarias, UNL, Esperanza, Santa Fe, Argentina.

Dairy cattle are subjected to stressful situations some of which can be associated to reproductive disruptions. In the ovary, follicles receive blood supply through a complex network of blood vessels. Functional and structural changes occur mainly in microvasculature of the follicular-luteal complex and have important roles in folliculogenesis and ovulation. Under stressful conditions, the microenvironment of the ovary is modified, affecting normal follicular dynamic. Previous studies have determined that adrenocorticotropic hormone (ACTH) is able to stimulate cortisol secretion in follicular wall *in vitro* and, additionally follicular cells express MC2R receptors. The aim of this study was to evaluate time to ovulation and hemodynamic changes in the ovulatory follicles after intrafollicular ACTH administration. Multiparous Holstein x Jersey cows without reproductive disease checked by US were included on the study. Estrous cycles were synchronized by G6G protocol. Then, ACTH (10IU in 0.1mL) was administered two days before expected day of ovulation, by US-guided intrafollicular injection (AG; N=5). Control group (CG; N=4) received 0.1 mL of physiological solution. Power Doppler US was performed once a day during proestrus until ovulation. Follicular blood flow was quantified by Image J Software and a repeated-measures analysis was performed using the Generalized Linear Model approach to evaluate follicular blood flow. No differences were observed on follicular blood flow, although a delay on ovulation time (days) was observed on treated cows (CG=19.75 +/- 0.95 vs AG=22.5 +/- 3.1). Considering ACTH receptors on follicular cells, it is likely that the delay in ovulation could be related to effects of this hormone at local level. More studies for confirmation of this delayed ovulation and changes at molecular level must be carried out, however it can be proposed that these results are consistent with possible local effects of ACTH on mechanisms involved in ovulation.

REPRODUCTION III

Saturday, November 19, 14-15:30 hr
Chairs: Rosanna Ramhorst – Evangelina Capobianco - Fernanda Parborell

720. (19) **COCAINE-INDUCED EPIGENETIC REPROGRAMMING IN MALE GERM CELLS AND THE ROLE OF DRD1: CHANGES IN EPIGENETIC ENZYMES PROFILES AND TARGET HISTONE MARKS WITH FUNCTIONAL IMPLICATIONS**

Gastón Barbero[1], Sahira Janeir Garazatua[1], Maximiliano de Sousa Ferro[2], Camila Perez Lujan[2], Alfredo D. Vitullo[1], Candela R. González[1*], Betina González[2*]
[1]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y de Diagnóstico (CEBBAD), Universidad Maimónides, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina. [2]Instituto de Investigaciones Farmacológicas (Universidad de Buenos Aires–Consejo Nacional de Investigaciones Científicas y Técnicas), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.
**These authors have contributed equally to this work*

Spermatogenesis presents windows of vulnerability for epigenetic reprogramming by environmental stressors that transmit developmental, metabolic, and behavioral traits to offspring. We previously demonstrated that cocaine (coc) administration downregulated dopamine receptors DRD1/2 and increased acetylated histone (H) protein levels in male germ cells (GC). Here, we analyzed specific post-translational modifications (PTMs) of H and epigenetic enzymes in isolated mouse GC treated with coc (10mg/kg) or vehicle (veh) in an intermittent binge protocol (3i.p. injections, 1h apart, one day on/off for 13 days). To evaluate the involvement of DRD1 in the deleterious action of coc, DRD1 antagonist SCH23390 (SCH 0.05mg/kg) was injected 15 min before each coc/veh injection. Additionally, we curated transcriptomic data from spermatogonia to mature sperm populations and analyzed the expression profile of *writers* and *erasers* of H lysine (K) acetylation and methylation, which act as mediators of epigenetic reprogramming events. GC from coc-treated mice showed increased H3K9me3/H3K27me3 silenced chromatin marks and decreased H3K27ac/H3K4me3 active promoter/enhancer marks. Coc also increased levels of H3K9ac/H4K16ac, involved in the replacement of H by protamines. Coc altered protein levels of LSD1 demethylase, G9A methyltransferase, MOF acetylase and HDAC1/2 and SIRT1 deacetylases. Pretreatment with SCH reversed the effects of coc on H3K4me3/H3K27me3/H4K16ac and restored HDAC1/2, SIRT1 and MOF levels. We also identified epigenetic enzymes with known functions during spermatogenesis and by the rule of "guilty by association", many others not even described yet in GC development. We provide evidence that coc alters specific H PTMs and that DRD1 is able to mediate epigenetic changes. Moreover, some of the enzymes' mRNAs were detected in mature sperm, suggesting a paternal contribution to the zygote. Our study contributes to the mechanistic aspects behind transgenerational epigenetics.

721. (27) **EFFECT OF LACTATE DEHYDROGENASE (LDH) ACTIVITY SUPPRESSION ON THE REGULATION OF SERTOLI CELL (SC) PROLIFERATION BY FSH**

Cecilia Lucía Centola, Marina Ercilia Dasso, Daniel Soria, María Fernanda Riera, Silvina Beatriz Meroni, María Noel Galardo.
Centro de Investigaciones Endocrinológicas "César Bergadá" (CEDIE)-CONICET/FEI/División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez". Ciudad de Buenos Aires, Argentina.

The final number of SC reached during the proliferative periods determines sperm production capacity in adulthood. It is well known that FSH is the major SC mitogen exerting its action by activation, at least in part, of mTORC1/p70S6K pathway. mTORC1 regulates a wide range of cellular functions, including glycolytic metabolism and cell proliferation. Moreover, new evidence evinces that glycolytic flux is involved in the regulation of mTORC1 activity. Proliferating cells tend to allocate substantial fraction of glucose to glycolytic ATP production followed by lactate generation accompanied by an increase in LDH activity. Although the role of LDH activity in mature SC was thoroughly documented, the role of LDH in immature SC remains obscure. The aim of this study was to analyze whether LDH activity is necessary to modulate mTORC1/p70S6K pathway and to regulate SC proliferation by FSH. SC obtained from 8-day old rats were maintained under basal conditions (B) or stimulated with FSH in the absence or presence of oxamate (OXA) -inhibitor of LDH activity. Lactate (Lac) production, phosphorylated (P)-mTORC1 and P-p70S6K levels by Western blot, and bromodeoxyuridine (BrdU) incorporation by immunocytochemistry were evaluated. Results are expressed as mean±SD of three independent experiments (different letters indicate statistically significant differences, $P<0.05$). It was observed that OXA inhibited the FSH-stimulated Lac production (B:2.95±0.41^a, FSH:4.13±0.15^b, OXA:3.23±0.28^a, FSH+OXA:3.27±0.19^a µg Lac/µg DNA) verifying, in this way, that OXA is a useful tool to block LDH activity. In addition, OXA was able to prevent FSH effect on P-mTORC1 and P-p70S6K levels; and finally, FSH-stimulated BrdU incorporation was inhibited by OXA (B:8.3±0.9^a, FSH:13.8±1.7^b, OXA:6.5±0.9^a, FSH+OXA:9.56±0.6^a % BrdU positive cells). These results suggest that LDH activity is involved in the regulation by FSH of both mTORC1/p70S6K pathway and SC proliferation. PICT2018 N°1291.

722. (75) **MORPHOLOGICAL CORRELATION BETWEEN THE MAIN SEGMENTS OF THE SEMINIFEROUS TUBULE, DETERMINED BY TRANSILLUMINATION, STRUCTURE AND CELLULAR ISOLATION. PRELIMINARY RESULTS**

Virginia Avena 1, Abi Karenina Funes1, Rocío Agüero 1, María Monclus 1,2, Inés Conte1, Paola Boarelli 3, Estefanía Saez Lancellotti 1,2, Miguel Fornés 1,2.

1 Laboratorio de Investigaciones Andrológicas de Mendoza (LIAM), IHEM, Universidad Nacional de Cuyo, CONICET. 2 Centro de Investigaciones de la Universidad del Aconcagua (CIUDA), Universidad del Aconcagua. 3 Laboratorio de Enfermedades Metabólicas (LEM), Universidad Maza.

The seminiferous epithelium (ES), of the seminiferous tubule (TS), goes through various epithelial stages (ES) forming a cycle of the germinal epithelium, before delivering a finished sperm. These stages have their own rhythm in each species, temporally and histologically. In turn, in the TS - isolated and intact - 3 main zones are observed by transillumination: clear (Z1), intermediate (Z2) and dark (Z3) that are associated with the ES cycle. Each cycle of the ES results from a still unknown regulation. The study of cycles involves their correct isolation and characterization. In the rabbit, the EE (VIII Correlative Cycles) have been described, but the corresponding zones have not been isolated or characterized, and even less their regulation. The objective of the work was to relate zones and EE by fine-tuning the isolation of the main zones of the ST, verifying the success by isolating specific germ cells of the stage. New Zealand adult rabbit testes were used. One half was fixed in Bouin and the other was decapsulated, releasing the TS in a Petri dish. They were incubated 10 minutes/37°C with collagenase. Under magnification and transillumination, the loose TS were separated into Z1, Z2 and Z3. Some were fixed in Bouin and others were subjected to "squash" detaching the cells from the ES by fixing them with p-F 4%. Testis, isolated TS and cells were processed for light and electron microscopy. The morphological correlation between TS zones and ES cycles indicates that stages III and IV correspond to Z1, V to VII to Z2 and I, II and VIII to Z3. The proportion of germ cells obtained by squash from each zone corresponds to the cell types observed in the histological analyses, a higher proportion of round and elongated spermatids in Z2, more spermatocytes and no sperm in Z1, inversely to what was seen in Z3 ($p < 0.05$). These observations allow us to use transillumination to isolate EE and advance in the study of their regulation, within the areas of the rabbit TS.

723. (130) **DYNAMIC CHANGES IN SPERM FLAGELLUM DURING FERTILIZATION**

Martina Jabloñski[1], Guillermina María Luque[1], Matías Daniel Gómez-Elías[1], Adán Guerrero[2], Mariano Gabriel Buffone[1]

1 Laboratorio Biología Celular y Molecular de la Reproducción, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina;

2 Laboratorio Nacional de Microscopía Avanzada, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca, México.

Mammalian sperm undergo a series of biochemical and cellular changes in the female reproductive tract to acquire fertilizing ability. These changes are collectively known as capacitation. As a result, sperm lose the acrosome in a process called acrosomal exocytosis (AE). Most of the studies are conducted in vitro, using a mixture of acrosome-intact and reacted sperm without discriminating the acrosomal status. Moreover, little is known about acrosome-reacted sperm which are the ones that are able to fertilize the egg. In this work, we investigated the cellular and molecular changes that occur in the sperm flagellum after the initiation of AE. In single-cell live imaging experiments and using super-resolution algorithms, a decrease in the midpiece diameter was seen upon AE. This contraction is associated to changes in intracellular calcium and a dramatic reorganization of the actin cytoskeleton. By 3D-STORM microscopy, changes in the double-helix structure of actin in the midpiece were observed in acrosome-reacted sperm. This observation led us to investigate the motility of sperm after AE. A gradual decrease in flagellar beat frequency occurs in coincidence with the midpiece contraction associated with AE. The dynamic changes in the midpiece and acrosomal status during in vitro fertilization were imaged live, where it was observed that the midpiece contraction may occur after ZP penetration. In addition, by performing sperm-egg fusion live imaging experiments, it was determined that after several minutes of the gamete fusion being initiated, the midpiece folds on itself and decrease the midpiece diameter. At this point, the sperm ceased its motility. Altogether, these results demonstrate that the sperm midpiece encounters structural changes after fusion with the egg, driven by calcium and actin dynamics.

724. (152) **SPERM LACKING SLO3 CHANNELS BEND THEIR FLAGELLUM AFTER INCUBATION UNDER CAPACITATING CONDITIONS**

Lucila Gomez Olivieri[1], Paula A. Balestrini[1], Celia Santi[2], Clara I. Marin Briggiler[1], Guillermina M. Luque[1], Mariano G. Buffone[1].

[1]Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

[2] Department of obstetrics and Gynecology, Washington University, St Louis, MO. USA.

During capacitation, where sperm acquire the ability to fertilize the egg, the gametes undergo a hyperpolarization of the plasma membrane potential (Em). This change in their Em is essential for the occurrence of acrosomal exocytosis and in mice, is mainly driven by the sperm-specific K^+ channel SLO3. In this work, by using SLO3 KO sperm, we found that once cells are incubated under capacitating conditions, a fraction of them (~50%, vs WT, $p < 0.05$) display a bending of the flagellum called hairpin. In time course experiments, we observed that this increase occurs very rapidly (within the first 15 min of incubation). Moreover, the hairpin rise in SLO3 KO mice was also observed in sperm obtained from the female uterus after mating. To evaluate how Em could be altering sperm morphology, SLO3 KO sperm were incubated in conditions that pharmacologically promote Em hyperpolarization: valinomycin (K^+ ionophore), amiloride (Na^+ channel inhibitor), ouabain (active Na,K-ATPase inhibitor), and genistein (CFTR voltage-dependent channel inhibitor). Only the addition of valinomycin significantly decreased the percentage of SLO3 KO sperm with hairpin ($p < 0.05$). In addition, SLO3 WT sperm were incubated in a high K^+ concentration medium to keep the Em depolarized. However, in this condition, sperm did not develop this change in morphology. In conclusion, sperm from SLO3 KO mice *in vitro* and *in vivo* develop flagellar abnormalities during capacitation that are not fully explained by the alteration on Em.

725. (156) **RELEVANCE OF CRISP PROTEINS FOR MALE FERTILITY AND EMBRYO DEVELOPMENT**

Sulzyk Valeria, Rebagliatti Abril, Weigel Muñoz Mariana, Cuasnicú Patricia.

Instituto de Biología y Medicina Experimental (IBYME-CONICET)

(sulzykvaleria@gmail.com)

Cysteine-Rich Secretory Proteins (CRISP1-4) are mainly expressed in the male reproductive tract and play key roles in mammalian fertilization. In spite of this, knockout (KO) mice for individual proteins are fertile, suggesting compensation among CRISP family members. Based on this, the aim of this work was to generate and characterize the reproductive phenotype of a triple KO lacking CRISP1/3/4 (TKO). For this purpose, DKO1/3 and CRISP4 KO colonies were crossbred, and fertility of TKO males analysed by mating them with control females. Results showed that TKO males exhibited fertility rates significantly lower than controls (4,13±2,43 vs 8,53±1,08; $p<0,01$). To investigate the mechanisms underlying this subfertility, superovulated females were mated with TKO males and the percentage of fertilized eggs in the ampulla was analysed the following day and 4 hr after mating. In both cases, no significant differences were found between groups However, when the recovered fertilized oocytes were incubated *in vitro* for additional 5 days to analyse their ability to develop into embryos, the percentage of eggs reaching the blastocyst stage was significantly ($p<0,001$) lower for mutant than for control males, indicating that the lack of CRISP was affecting early embryo development. To investigate potential functional deficiencies in mutant cells, TKO and control epididymal sperm were co-incubated *in vitro* with control cumulus oocyte complexes and the percentage of fertilized eggs determined. Under these conditions, significantly lower fertilization rates were found for mutant

than for control sperm (3,8±3,8 vs 49±7,1; p<0,001), supporting the existence of deficiencies already present in epididymal sperm that could be responsible for subsequent embryo development defects. Together, these findings support the relevance of CRISP for both fertility and fertilization and contribute to a better understanding of the impact of male factors on embryo development.

726. (227) **CYCLIC EXPRESSION AND LOCATION OF DELETED IN MALIGNANT BRAIN TUMOR 1 (DMBT1) IN PORCINE OVIDUCT AND EFFECT ON SPERMATOZOA**

Agustín Vanzetti[1,2], Melina Faggi[1], Camila Palacios[1], Patricia Marini[1,3], Juan Manuel Teijeiro [1,2].
[1]Laboratorio de Medicina Reproductiva. Facultad de Ciencias Bioquímicas y Farmacéuticas. [2]CONICET, [3]CIUNR.
E-mail: jteijeiro@fbioyf.unr.edu.ar

The oviductal protein Deleted in Malignant Brain Tumor 1(DMBT1) is involved in the sperm-oviduct interaction, through the negative selection of sperm. The objective of this work was to evaluate the transcriptional expression of DMBT1, the presence in oviductal fractions through the porcine estrous cycle and to analyze the effect on sperm in its molecular and physiological context. Oviducts classified in follicular phase (FP) or luteal phase (LP) were perfused with PBS to collect oviductal fluid (OF) and oviductosomes (OVS). Epithelial cells were obtained by scrapping and then proteins from different subcellular fractions were obtained. Transcriptional and translational regulation of DMBT1 were assayed by RT-qPCR and Western blot, respectively. Sperm were incubated in capacitating medium (control), supplemented with (mOVS) or with (mOF) corresponding to FP or LP using 0.1, 0.2 or 0.5 µg/µL of protein. The specificity of the effect of DMBT1 was evaluated by inhibition using anti-DMBT1 antibody. Vitality, acrosome alterations and motility were analyzed. The results show that DMBT1 mRNA expression levels are higher in the FP than in the LP ($p < 0.05$), while protein levels are higher in the LP than in the FP ($p < 0.05$). Higher sperm mortality was found in mOVS from the LP compared to the control ($p < 0.05$) for the treatment with 0.2 µg/µL of protein. No significant differences were found for acrosomal alteration in any condition. A decrease in the curvilinear velocity (VCL) of the spermatozoa was observed in mOF from LP ($p < 0.05$) and mOVS from FP ($p < 0.05$) for 0.1 µg/µL of protein compared to the control. In addition, higher VCL was found in the mOF from FP compared to mOF from LP ($p < 0.05$), in the treatment with 0.5 µg/µL. The results demonstrate that DMBT1 expression is regulated at the transcriptional and translational levels during the sexual cycle and that the context may modify its action, but also, this study reveals a relevant and separated role for OVS and OF

727. (248) **EFFECT OF ARTIFICIAL OOCYTE ACTIVATION ON EARLY EMBRYONIC DEVELOPMENT**

Lucas Nicolás González, Patricia Sara Cuasnicú, Débora Juana Cohen.
Instituto de Medicina y Biología Experimental (IBYME-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

The fertilizing sperm triggers egg activation which is required for embryo development. In the clinic, artificial oocyte activation (AOA) emerged as an experimental alternative in cases of fertilization failure after ICSI (intracytoplasmic sperm injection). The most widely used activating agent for human eggs is the Ca2+ ionophore A23187 which, differently from sperm, induces a single Ca2+ peak. Using the mouse as a model and by applying different AOA strategies: 1 or 2 stimuli of A23187 in a medium with or without extracellular Ca2+ and SrCl2 as control, we have previously demonstrated that the activation with A23187 causes a delay in the progression of meiosis and a decrease in the development to blastocyst. The present work is aimed to deepen the mechanisms by which A23187-induced activation impacts blastocyst development. First, we analyzed the kinetics of embryonic development which did not change with the different activation protocols ($p>0.05$), indicating that the decrease in development could be due to the delay in meiosis progression. To evaluate this, eggs were treated with cycloheximide (CHX) which induces meiosis resumption independently of Ca2+ dynamics. We observed that eggs activated with 1 or 2 stimuli of A23187 in the presence of CHX showed an improvement in the meiosis progression ($p<0.001$) and an increase in the percentage of 2 cell embryos obtained ($p<0.05$). Even more, development to the blastocyst stage was significantly improved in eggs activated with 2 stimuli of A23187 in presence of CHX ($p<0.05$). Considering the relevance of mitochondrial activity for both meiosis and embryonic development, we next evaluated the production of ROS and ATP levels which did not change with the different activation strategies employed ($p<0.05$). Taken together, these results indicate that egg activation with A23187 induces a delay in meiosis progression, presumably due to the Ca2+ levels achieved, which negatively impacts embryo development.

728. (271) **HIGH-THROUGHPUT SCREENING METHOD FOR DISCOVERING CATSPER INHIBITORS USING MEMBRANE DEPOLARIZATION CAUSED BY EXTERNAL CALCIUM CHELATION AND FLUORESCENT CELL BARCODING.**

Guillermina M. Luque[1]; Liza J. Schiavi-Ehrenhaus[1]; Martina Jabloñski[1]; Paula A. Balestrini[1]; Analia G. Novero[2]; Nicolás I. Torres[1]; Claudia E. Osycka-Salut[3]; Alberto Darszon[4]; Dario Krapf[2]; Mariano G. Buffone[1].
[1]Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.
[2]Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario, Argentina.
[3]Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM-CONICET), Buenos Aires,Argentina.
[4]Instituto de Biotecnología, UNAM, Cuernavaca, México.

Sperm hyperactivation requires a rise in intracellular Ca^{2+} brought in by sperm-specific CatSper channels present in the flagellum. The exclusive expression of CatSper in sperm and its critical role in sperm function makes this channel an attractive target for contraception. The strategy of blocking CatSper as a male, non-hormonal contraceptive has not been achieved due to the lack of good screening methods. We aimed to develop a high-throughput method to screen drugs with the capacity to block CatSper based on the combination of two remarkable tools: 1) the Fluorescent Cell Barcoding (FCB); 2) the membrane potential (Em) assay. For this purpose, we assigned unique fluorescent signatures by combining two fluorescent dyes, CellTrace CFSE and CellTrace Violet, to different sperm samples as a way to enable higher throughput flow cytometry. These differentially labelled samples incubated in distinct experimental conditions (presence or absence of the CatSper inhibitor HC-056456) were mixed into one tube for simultaneous acquisition. We employed FCB flow cytometry in combination with propidium iodide (PI) to assess viability, and a reporter of Em $DiSC_3(5)$ to determine CatSper function, only in live sperm. Removing external divalent cations by adding EGTA, allows CatSper to efficiently conduct monovalent cations, where an influx of Na^+ depolarizes the cell. The magnitude of this depolarization mainly depends on the extent of CatSper opening. The Em depolarization after EGTA addition was diminished in CatSper1 KO sperm, validating a simple strategy for assessing CatSper function. Altogether, these results suggest that we have developed an efficient and simple method to screen drugs with the capacity to block CatSper.

729. (278) **LIRAGLUTIDE REGULATES FATTY ACID STORAGE IN SERTOLI CELLS**

Marina Ercilia Dasso, Cecilia Lucia Centola, Daniel Soria, María Noel Galardo, Silvina Beatriz Meroni, María Fernanda Riera.
Centro de Investigaciones Endocrinológicas "César Bergadá" (CEDIE)-CONICET/FEI/División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez". Ciudad de Buenos Aires, Argentina.

Sertoli cells (SC) are necessary to provide the structural and nutri-

tional support for germ cell development. SC convert glucose to lactate, the main energy substrate for spermatocytes and spermatids. Consequently, SC use fatty acids (FA) -stored as triacyglycerides (TAG) in lipid droplets (LD)- as its own energy source, being LD essentials to keep ATP levels. Obesity prevalence has risen dramatically and liraglutide (Lira), a glucagon-like peptide-1 analogue, has emerged as a useful drug for treatment of obesity and type 2 diabetes mellitus. It has been observed that Lira regulates cell metabolism in different cell types however, little is known about its possible effects on SC function. Considering that the regulation of FA storage may be relevant to seminiferous tubule physiology, the aim of this work was to study the effect of Lira on lipid storage in SC. For this purpose, SC cultures obtained from 20-day old rats were used. First, we determined that SC express GLP-1 receptor by RT-PCR. Then SC cultures were maintained under basal conditions (B) or incubated with Lira (100nM) for different periods of time. We observed that Lira promotes an increase in LD number (B: 0.37±0.1; Lira:0.70 ±0.1*. means ± SD of number of LD per SC in one representative experiment out of three. *P < 0.05 vs B) that is accompanied by an increase in TAG content. Next, we analyzed the effects of Lira on the expression of proteins involved in FA storage such as FAT/CD36 -a FA transporter- and glycerol-phosphate-acyl-transferases 1 and 4-GPAT1 and 4 enzymes involved in TAGs synthesis- by RT-qPCR. We observed that Lira increases FAT/CD36 mRNA levels after 6h of treatment (2.0±0.3* fold variation vs. B). Taken together, these results suggest that Lira can modulate lipid storage, which is essential to sustain SC energetic metabolism. (PICT2020-0642; PIP2021-162).

730. (430) **EVALUATION OF SEMEN PARAMETERS AFTER SARS-COV2 INFECTION**

María Eugenia Matzkin[1,2], Ignacio De Zuñiga[3], Celina Beguerie[4], Gustavo Martinez[4], Mónica Beatriz Frungieri[1]

[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET), CABA, Argentina, [2]Cátedra 1, Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, CABA, Argentina, [3]PREGNA Medicina Reproductiva, CABA, Argentina, [4]Fertilis Medicina Reproductiva, San Isidro, Buenos Aires, Argentina

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is known to affect multiple tissues, including male reproductive organs, raising the possibility to affect male fertility. This study aimed to examine several semen parameters in coronavirus disease (COVID19) non-infected and infected men (recruited 1 to 12 months after COVID19 diagnosis). Sperm concentration, motility, vitality, and morphology were significantly higher in the COVID19-negative group compared to COVID19-positive 1 to 3 months-post infection patients (p<0.05). Western blot evaluation of protein expression levels of cyclooxygenase 2 (COX2, inducible form of the key enzyme in prostaglandins synthesis) in semen cell populations exhibited a five-fold increase in COVID19-positive patients compared to the COVID-19-negative group (p<0.05). Co-immunofluorescence analysis in semen cell populations revealed COX2 expression in CD68-immunopositive leukocytes. Assessment of seminal plasma levels of prostaglandins (by colorimetric immunoassays) showed that, while PGF2α levels remained unchanged, a statistically significant increase for PGD2 levels in the COVID-positive group could be detected (p< 0.05). Because SARS-CoV2 can rely on polyamines for varied stages in its replication, we measured seminal plasma levels of the polyamines putrescine, spermidine, and spermine by thin layer chromatography. Although not significant, our data show a tendency for reduction of these polyamines levels in COVID19-infected patients compared to the COVID-19-negative group. Altogether, our results suggest that COVID19 may have a negative effect on male fertility.

731. (448) **ASSOCIATION OF METABOLIC SYNDROME WITH MALE REPRODUCTIVE STATUS IN THE SECOND GENERATION**

María Milagros Giaccagli, Jael Dafne Herzfeld, Mariana Weigel Muñoz, Patricia Sara Cuasnicú, Vanina Gabriela Da Ros, Débora Juana Cohen

Instituto de Biología y Medicina Experimental (IByME-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

The prevalence of metabolic syndrome (MS) has increased lately in alarming proportions, now affecting men in reproductive age. Previous studies from our laboratory have shown that MS has minor effects on male fertility, leading us to hypothesize that diverse factors could contribute to an MS-induced reproductive effect. As males with MS remain competent to father children, the aim of this study was to evaluate whether the metabolic status of the father affects the reproductive performance of their offspring. To this end, male offspring of C57BL/6J fathers that had been fed a control diet (CD, 6% fat) or a high-fat diet (HFD, 34% fat), were randomly assigned into groups to receive either: CD (CD-CD, HFD-CD) or HFD (CD-HFD, HFD-HFD). Starting from the 10th week of treatment, both HFD-fed groups showed statistically significant metabolic disturbances compared to CD-fed mice. Testicles of HFD-CD and HFD-HFD were smaller than those of CD-CD fed mice (p<0.05), while there were no differences in the epididymal weights. Interestingly, all groups had a higher amount of gonadal fat than CD-CD fed mice. *In vitro* studies showed no difference among groups in sperm parameters (sperm count, viability, motility and acrosome reaction) as well as in fertilization and embryo development rates. Consistently, when HFD-fed males were able to mate with females, evidenced by the presence of vaginal plug, no differences in fertility parameters were observed compared to control. However, HFD-fed animals presented a lower plug index (an indicator of successful mating) over time (p<0.01). In agreement, observational studies during a short period after caging with a female revealed a variety of impairments in their copulatory behavior. In summary, metabolic alterations in the second generation generate a disminished sexual behavior, exacerbated by the metabolic condition of the father that could lead to subfertility.

732. (582) **EXPRESSION OF SERINE PROTEASE AR2 IN MOUSE SPERM AND ASSESSMENT OF ITS PARTICIPATION IN FERTILIZATION**

Ania Antonella Manjon[1], Gustavo Luis Verón[1], Paula Duek[2], Lydie Lane[2], Mónica Hebe Vazquez-Levin[1]

[1]Instituto de Biología y Medicina Experimental (IBYME; CONICET-FIBYME), Buenos Aires, Argentina; [2]Swiss Institute of Bioinformatics (SIB), Ginebra, Suiza. aniaamanjon@gmail.com

Proteases play important roles in biological processes, among them in mammalian fertilization. Human AR2 is an inactive testis-specific serine protease identified using an algorithm developed by our group and members of Swiss Institute of Bioinformatics. We have previously reported AR2 expression in human sperm and its role in sperm-oocyte interaction. This study aimed to begin AR2 characterization in the mouse model. Mouse AR2 is localized in chromosome 8; the gene contains 6 exons and 5 introns, and encodes 5 transcripts and 3 protein isoforms (>95% identity) that lack the catalytic triad residues and depict an overall high homology with the human isoforms (83%). To characterize its expression and biological role in mouse sperm, two commercial polyclonal anti-human AR2 antibodies were used (Ab1 towards N-term, Ab2 towards C-term). Protein alignment confirmed >85% homology of antibodies antigen sequences and the mouse isoforms. AR2 was immunolocalized in fixed acrosome-intact non-capacitated (NC) and capacitated (C) sperm, and in A23187 (10 μM) acrosome-reacted (AR) sperm. Specific immunolocalization patterns were: Ab1=Acrosomal region + Flagellum (A+F) and Flagellum (F) (NC=100% A+F; C= 16% A+F and 84% F; AR=100% F). Ab2= Head (H), head depicting a highly intense signal in the Acrosomal region (H+A), patchy head (P) and no signal (N) (NC and C=21% H, 79% H+A; AR= 21% H, 50% P, 29% N). AR2 was also immunodetected in NC live sperm: Ab1= H and F; Ab2= H. *In vitro* fertilization (IVF) of cumulus-oocyte complex was impaired when sperm were pre-incubated with Ab1 (46% inhibition; p<0.01; n=109) and Ab2 (64% inhibition; p<0.01; n=108). In conclusion, AR2 is a mouse sperm inactive serine protease localized in the head and flagellum; IVF inhibition by specific AR2 antibodies supports its involvement in fertilization, in agreement with findings in the human model. Presence of AR2 alterations (expression/localiza-

tion) in sperm from infertile men is currently under study.

733. (596) **GAMETE RECOGNITION PROTEINS IN THE LIGHT OF COMPARATIVE GENOMICS**

María Clara Campos*[1], Francisco Pisciottano*[1], David Bruque[2] and Patricia Saragüeta[1]
[1] Instituto de Biología y Medicina Experimental - CABA, Provincia de Buenos Aires, Argentina
[2] Unidad de Conocimiento Traslacional Hospitalaria Patagónica, Hospital de Alta Complejidad SAMIC - El Calafate, Provincia de Santa Cruz, Argentina
**equal contribution*

The mammalian fertilization process has been extensively described by both physiology and molecular biology. Gamete recognition proteins (GRP) such as members of the Zona Pellucida protein family (ZP) and IZUMO1-JUNO pair play a key role in this process, facilitating the interaction and fusion between the oocyte and the sperm, respectively. The interactions between oocyte and sperm counterparts may constitute a prezygotic reproductive isolation mechanism. The story of protein evolution has greatly changed with the large number of genomes reported as their sequencing advanced. We have improved the phylogeny of gamete interaction proteins using chromosome-resolved genome assembled by Hi-C long-range sequencing. As we reported before, ZP3 and ZP2, gamete interaction ZP proteins, display a similar pattern of evolution along their phylogenies showing adaptive changes in the canids subtree but not among felid species. On the other hand, structural cross-linker ZP1 and ZP4 display a different evolutionary history, showing signatures of positive selection inside felids subtree and, the fusion protein IZUMO1R did not show positive selection among all studied phylogenetic groups. Moreover, we showed that conservation of all carnivora gamete interaction proteins was significantly higher in felids compared to canids (p-value < 0.05), except for the case of ZP4. Here we extend our analysis to sperm surface proteins involved in gamete intraction. In order to reveal the evolutionary history of sperm GRP in carnivores and bring light to the process of coevolution with their oocyte conterparts we analysed IZUMO1, ZP3r, PKDREJ, SLLP1. The results will be useful to undestand the molecular basis of the lack of precygotic reproductive isolation mechanisms in felids and compare them to canids, its sister group. In addition, they will allow us delve into the coevolution process between sperm and oocyte GRP.

734. (613) **THE ONSET OF THE WOMAN'S FERTILITY WINDOW: IN SEARCH OF ACCURATE BIOMARKERS.**

Guillermo I. V. Kerz[1], Ingrid Paul[1], Elsa Zerbini[1], María B. Peralta[1,2], Melisa M. L. Velázquez[1,2].
[1]Instituto de Ecología Humana y Desarrollo Sustentable, Universidad Católica de Santa Fe, Santa Fe, Argentina. [2]CONICET.

The identification of precise biomarkers and cut-off values to determine the onset of the woman's fertility window has been subject of several research in reproductive physiology. However, little is known about precise parameters that contribute to an upgrade in women's reproductive health. The aim of this study was to evaluate some markers during the menstrual cycle in women fertile age, at the beginning of the fertility window. The number of volunteer women that met the inclusion criteria was 44. The measurement of endometrial thickness and ovarian follicular diameter was evaluated by ultrasound from each volunteer. At the same time, blood samples to the evaluation of serum estradiol concentration by biochemistry and cervical mucus by microscopic analysis, were obtained. The ROC curves were used to analyze the results. The onset of the fertile phase was identified when the cervical mucus turned from infertile into fertile by microscopic observations. The serum estradiol concentration at the beginning of the fertile phase was 113 pg/ml, whereas the ovarian follicle diameter and the endometrial thickness were 15 mm and 7.5 mm, respectively, all of them chosen through Youden's index. On the other hand, the area under the curve for serum estradiol was 0.772, for the follicular diameter was 0.794, whereas that for the endometrial thickness was 0.744 (CI 95%). These findings suggest the importance of identifying accurate biomarkers of the fertile period in women, which could be useful and good predictors for certain purposes. Furthermore, these results add information to a histoarchitecture of biomarkers aimed at improving accuracy in fertility management.

735. (639) **SPERM DNA FRAGMENTATION AND ITS ASSOCIATION WITH ROUTINE SEMEN PARAMETERS**

Verón GL[1], Errecalde T[2], Marchioni N[2], Catalano D[2], Blanco E[2], Maggi L[2], Vazquez-Levin MH[1,2]
(1) Instituto de Biología y Medicina Experimental (CONICET-FIBYME, (2) CDRossi. Buenos Aires, Argentina.

Routine male reproductive workup involves basic semen analysis, although with limited value. Considering the relevance of the sperm nuclear integrity in sperm fertilizing potential, Sperm DNA Fragmentation (SDF) analysis has been included in the 6th WHO Manual (2021). Herein, we aim to assess the association between routine semen parameters and SDF. Semen samples (n=318) obtained from men consulting for infertility at CD Rossi (40.5 ± 6.7 years; mean ± SDM; 2019-2022), were subjected to semen analysis (WHO guidelines) and SDF (TUNEL assay using fluorescent microscopy). As a result, SDF was negatively correlated with sperm count, vitality, and motility (P<0.01), and positively correlated with male age, mid-piece abnormalities and cytoplasmic droplets presence (P<0.01). Population dichotomization considering the 20% SDF cut-off showed lower values for sperm count, vitality, and motility in the TUNEL-positive group, and higher age and mid-piece abnormality rates (P<0.05). While TUNEL-positive samples (≥20%) represented 19.8% of the population, abnormal SDF was determined in 23.1% oligozoospermic, 39.5% asthenozoospermic (P<0.005 vs total population) and 18.3% teratozoospermic men samples. Interestingly, 19.2% in normozoospermic samples also had abnormal SDF. Considering the wide range for cut-off values found in the literature, a new cut-off value was determined based on seminal abnormality rates (Lower Reference Limits) using a selection algorithm based on Youden index. As a result, a cut-off of 15.3 ± 5.8% (4-19%) was determined. TUNEL-positive samples (SDF ≥15.3%) depicted similar results to those found using the 20% cut-off, both in the 318 total and in the 156 normozoospermic samples. Based on these findings, SDF assessment should be included in the routine male fertility workup, since normo, astheno and oligozoospermia diagnosis may be concealing an underlying nuclear pathology affecting male reproductive potential.

736. (670) **EVALUATION OF PROLIFERATION AND MATURATION MARKERS OF SERTOLI CELLS IN THE TESTES OF INFERTILE PATIENTS**

Leilane Glienke[12], María Belén Maio[1], Thaisy Munduruca Pires[12], Ignacio Ezequiel Rojas Campión[1], Hernán Oxilia[23], Mariana Barberis[3], María Florencia Fulco[4], Esperanza Berensztein[25], Livia Lustig[12], Roberta Sciurano[26], Cristian Marcelo Sobarzo[12], María Susana Theas[12]
1 Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Ciencias Médicas, Universidad de Buenos Aires, Argentina
2 II Unidad Académica de Histología, Facultad de Ciencias Médicas, Universidad de Buenos Aires, Argentina
3 Hospital General de Agudos Parmenio T. Piñero, División Anatomía Patológica, CABA, Argentina
4 Hospital de Clínicas General San Martín, División Urología, CABA, Argentina
5 Laboratorio de Cultivo Celular, Servicio de Endocrinología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, CABA, Argentina
6 CONICET

Testis immunopathology is similar in humans and rats undergoing chronic testicular inflammation and infertility. We showed that the number of Sertoli cells (SCs) per seminiferous tubules (STs) increases in testicular biopsies of patients, who underwent assisted fertilization procedure, displaying severe hypospermatogenesis (HypoS) and Sertoli cell only syndrome (SCOS); as well as in rats with autoimmune orchitis (EAO). EAO is a well-established rodent

model of organ-specific autoimmunity associated with infertility. To verify if the increased number of SCs is associated with their maturity degree we analyzed by immunohistochemistry the expression of anti-Müllerian hormone (AMH), Ki67, and serum FSH (RIA). STs wall thickness was also evaluated. In severe HypoS (n=3-5) and SCOS (n=3-5) patients the percentage of STs with AMH+ SCs significantly increased compare to patients with obstructive azoospermia and complete spermatogenesis (control group, C, n=3-6) (mean±SEM, C: 0,00±0,00; severe HypoS: 86,78±5,18; SCOS: 94,23±5,28; p<0,01, p<0,01, Dunn's Test). AMH expression was highest in STs of SCOS (pixel intensity, arbitrary units, mean±SEM, C: 0,05±0,02; severe HypoS: 0,08±0,02; SCOS: 0,14±0,02; p<0,05 vs. C, Tukey's Test). The number of Ki67+ SCs increased significantly in patients with SCOS (mean±SEM, C: 0,32±0,10; severe HypoS: 3,08±1,13; SCOS: 5,61±0,88; p<0,01, Tukey's Test). In patients with severe HypoS and SCOS serum FSH was increased vs. C (ng/ml, mean±SEM, C: 5,07±1,28; severe HypoS: 22,92±5,69; SCOS: 20,70±2,79; p<0,01, Dunn's Test). STs wall thickness significantly increased in HypoS and SCOS patients (μm, mean±SEM, C: 5,31±0,72; severe HypoS: 10,20±1,09; SCOS: 16,40±4,83; p<0,05, Dunn's Test). Our results showed that SCs from infertile patients with severely damaged STs displayed an immature-like phenotype correlative with their proliferative capability. These facts might explain the abnormal function of SCs in the analyzed infertile patients.

737. (899) **EVALUATION OF SMALL NON-CODING RNA LEVELS IN SPERMATOZOA FROM PATIENTS WITH NEGATIVE OUTCOMES IN ASSISTED REPRODUCTIVE TECHNOLOGIES**

Julieta B. Grosso1, Clara Claus1, Karina Calvo2, Claudia Brignardello2, Carlos Morente2, Silvana V. Spinelli1
1instituto de Inmunología Clínica y Experimental de Rosario (IDICER), 2Centro Médico Proar

Fertility problems affect 15% of couples worldwide and the causes can be attributed equally to the male or female partner. Since conventional semen analysis provides a limited prediction of male fertility, in our laboratory we evaluated the potential use of sRNAs as markers of semen quality in assisted reproduction treatments (ART). Based on previous research, we focused on tRNA-derived fragments produced by the ribonuclease angiogenin (ANG), called tiRNAs. These molecules are key regulators of physiological and pathological processes with a great impact on reproductive function. In this work, we analyze sperm cell (SPZ) to assess their quality and possible associations with ART results. We conducted a prospective study with couples who underwent ART with donated oocytes at the PROAR Medical Center 2018-2019. Samples were collected from normozoospermic men and different transcripts were quantified by RT-qPCR (N=17, 10 ART+ and 7 ART-). Data was statistically evaluated using the Mann Whitney test. As endogenous controls we employed U6 for sRNA and PPIA for mRNA. Even though reactions were prepared using the same amount of total RNA, levels of both transcripts showed strong dispersion, with differences in Ct of more than 10 cycles for U6. These results indicate that RNAs stability in mature SPZ is variable between samples. Interestingly, levels of U6 in SPZ tend to be lower in patients with failed ART cycles, showing a discrete group with the lowest levels that could potentially account to patients with unexplained infertility. Levels of tiRNAGlu, tiRNA-Gly and tiRNALys showed no differences between groups, but their absolute levels correlate with U6 expression only in samples form successful ART (p:0.01, p:0.03, p:0.03, Spearman r). We also tried to measure the ANG transcript but it was not detected in SPZ, suggesting that sperm tiRNA may be incorporated from seminal plasma. The SPZ maturation marker SLFNL1, GAPDHS and ZBPB1 were also quantified. No significant differences were observed between the groups but GAPDHS and ZBPB1 showed higher detection in samples from patients whose couples reach pregnancy. In sum, these results provides preliminary data showing the potential role of RNA stability and tiRNAs in male infertility

SIGNAL TRANSDUCTION
Thursday, November 17, 14-15:30 hr

Chairs: Leonardo Romorini - Fernanda Riera - Fernando Dominici

738. (70) **THE ROLE OF TNF AND NF-*K*B IN LIPID METABOLISM IN 3T3-L1 ADIPOCYTES**

Francisco Damián Rosa, María Cecilia Lira, Iara Castellanos, Juliana Lourdes Bernacchia, Alejandra Graciela Palma, Mónica Alejandra Costas, María Fernanda Rubio.
Laboratorio de Biología Molecular y Apoptosis, Instituto de Investigaciones Médicas A. Lanari, IDIM UBA-CONICET.

The study of the tumor microenvironment is important to understand the biology of cancer. It has been known that the adipose tissue of breast cancer obese patient has higher TNF expression than the lean adipose tissue of breast cancer patient. Therefore, we have studied the effect of TNF on the lipid metabolism of murine mammary adipose tissue. In this model, TNF decreased adipocyte size and total lipid content, and increased glycerol secretion. With the aim of getting insights into the molecular pathways by which TNF is involved in the lipid metabolism of adipocytes, we used an *in vitro* model of 3T3-L1 cells differentiated into adipocytes. Hence, 3T3-L1 cells were stimulated with a differentiation cocktail and, after one week, the medium was changed for medium with or without TNF 20 ng/ml for 72 h. TNF treatment decreased the adipocytes size (0.89 ± 0.03 respect to Basal, p<0.01). In addition, we have also seen that TNF decreased total lipids content (0.31 ± 0.06 respect to Basal, p<0.01). In order to determine if the effects observed so far could be mediated by NF-*k*B, we stimulated the cells with 200 nM sulfazalazine (ssz), NF-*k*B pathway inhibitor. The co-treatment of ssz with TNF (S+T) failed to reverse the effect of TNF on adipocyte size (0.89 ± 0.03 TNF; 0.87 ± 0.04 S+T respect to Basal, p<0.01); and the effect of TNF on the total lipids content (0.31 ± 0.06 TNF; 0.61 ± 0.05 S+T respect to Basal, p<0.01). In conclusion, as seen in the *ex vivo* model, TNF decreased the adipocyte size and the total lipid content in adipocyte differentiated 3T3-L1 cells. However, the effects of TNF observed in this work on the lipid metabolism do not appear to be NF-*k*B-dependent.

739. (78) **AKAP350 PARTICIPATES IN LFA-1 CLUSTERING AT THE IMMUNE SYNAPSE IN *EXVIVO* NATURAL KILLER CELLS**

Pariani AP[1], Almada E[2], Rivabella Maknis T[1] , Favre C[1], Larocca MC[1]
1. Instituto de Fisiología Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Argentina.
2. Instituto de Inmunología clínica y experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario, Argentina.

Natural Killer Cells (NK) are cytotoxic cells from the innate immune system. They form a specialized junction with their target cells, the immune synapse (IS), which is highly dependent on NK receptors such as the integrin LFA-1. AKAP350 is an scaffold protein central for microtubule nucleation at the Golgi apparatus that, in NK derived YTS cells, participates in LFA-1 recruitment to the IS, thus conditioning NK cytolytic activity. The aim of this work was to analyze the participation of AKAP350 in LFA-1 clustering in *ex vivo* NK cultures. Methods: *ex vivo* NK (eNK) from volunteer blood donors were purified by negative selection and cultured in IL-2 supplemented medium. eNK with reduced expression of AKAP350 (AKAP350KD) were prepared by transduction with shRNA expression lentiviral particles. eNK were exposed to erythroleukemia KT-86 cells (2:1 ratio) for 30 minutes. LFA-1 distribution was analyzed by immunofluorescence confocal microscopy. Relative distance to the IS (RD) was estimated as the difference between the distance from each LFA-1 vesicle to the IS and the distance from the cell centroid to the IS, related to the latter. Results are expressed as media±standard error. Results and Conclusion: Our results revealed the presence of an intracellular pool of LFA-1 which partially colocalized with the Golgi apparatus in eNK cells. LFA-1 vesicles polarization to the IS was im-

paired in AKAP350KD cells (CONTROL: -0.14±0.01, AKAP350KD: 0.05±0.02, n=30, p<0.05). Concomitantly, LFA-1 clustering at the IS was decreased in AKAP350KD cells (Control: 60±15%, AKAP-350KD:16±6%, n=20, p<0.05). Those results are in agreement with our previous findings in YTS cells, confirming that AKAP350 participates in LFA-1 polarization and clustering at the IS during NK interaction with susceptible target cells and revealing a novel model for regulation of LFA-1 reorganization during NK activation.

740. (91) **PTTG POST-TRANSLATIONAL MODIFICATIONS CONTROL ITS PROTEIN STABILITY AND TRANSCRIPTIONAL ACTIVITY**

Manuel Fiz[1]*, Mariana Fuertes[1]*, Sergio Senin[1], Eduardo Arzt[1,2]

[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society, Buenos Aires, Argentina, [2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

** These authors contributed equally to this work*

Oncogene activation is a widely known tumorigenesis mechanism. The proto-oncogene pituitary tumor transforming gene (PTTG) arises as a key factor due to its frequent overexpression in tumors and by its regulation of cell cycle. Post-translational modifications control PTTG protein: PTTG is modified by ubiquitination, phosphorylation and in particular the SUMOylation enhancer RSUME increases the stability of the PTTG protein, its tumor abundance and prolongs its half-life. In this work we studied the molecular modulation of PTTG protein stability at post-translational level and the role of RSUME.
In COS-7 cells we studied the stability of mutated PTTG variants in their possible conjugation sites to SUMO (K25R and K168R) or in the most important site of conjugation to ubiquitin (ΔD-box) by western blot (WB) from cellular extracts previously transfected with PTTG wt or mutated. We found that the mutant K25R presents the same protein stability as PTTG wt, and RSUME increases its stability. The K168R mutant has lower stability than PTTG wt and RSUME increases its stability. The ΔD-box is much more stable than PTTG wt and RSUME does not control its stability. By 100µg/mL cycloheximide COS-7 treatment we observed that the K25R and K168R mutants have a shorter half-life than PTTG wt. By co-transfecting cells with RSUME expression vector, we found that RSUME prolongs the half-life of the K25R mutant but has no effect on the half-life of the K168R mutant. In AtT-20 cells (ACTH-secreting murine pituitary line) by quantitative RT-PCR experiments we found that PTTG wt induces the expression of c-Myc, while K25R and K168R mutants showed less induction of c-Myc transcription. We conclude that the ubiquitination ΔD-box site is critical for PTTG stability and does not involve RSUME action, while the SUMO attachment sites are important for the SUMOylation action of RSUME on stability and activity.
Supported by ANPCyT and FOCEM (COF 03/11).

741. (177) **PHOSPHOLIPASE D 1 AND 2 INHIBITION PREVENTS OXIDATIVE STRESS IN RETINAL PIGMENT EPITHELIUM CELLS EXPOSED TO HIGH GLUCOSE LEVELS**

María Sol Echevarría, Paula Estefanía Tenconi, Melina Valeria Mateos.

Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Departamento de Biología, Bioquímica y Farmacia (DBByF), Universidad Nacional del Sur (UNS), 8000 Bahía Blanca, Argentina.

Objective: Oxidative stress (OE) and inflammation are involved in the pathogenesis of several retinal diseases. We previously demonstrated that classical phospholipase D isoforms (PLD1 and 2) mediate the inflammatory response of retinal pigment epithelium (RPE) cells induced by high glucose (HG) levels. Furthermore, a significant increase in reactive oxygen species (ROS) was observed in RPE cells exposed to HG. The aim of the present work was to study the relationship between OE and PLD activation observed in HG- treated RPE cells. Methods: RPE cells (ARPE-19) were exposed to HG (33 mM) or to normal glucose levels (NG, 5.5 mM) for 24 h. To inhibit PLD1, PLD2 and cyclooxygenase-2 (COX-2) VU0359595 (PLD1i, 0.5 µM), VU0285655-1 (PLD2i, 0.5 µM) or celecoxib (10 µM) were used, respectively. ROS production was assessed using the probe DCDCDHF. Immunocytochemistry assays (ICC) and western blots were performed to evaluate nuclear factor erythroid 2–related factor2 (Nrf-2) pathway. Results: HG-exposure for 24 h increased ROS levels (148%) in ARPE-19 cells with respect to NG. The incubation with PLD1i and PLD2i prevented HG-induced ROS generation in RPE cells. On the contrary, the inhibition of COX-2 was not able to prevent OE induced by HG. ICC showed Nrf-2 nuclear translocation in cells exposed to HG and this effect was not observed when cells were pre-treated with PLD1i and PLD2i. Nrf-2 activation correlated with and increased heme oxygenase-1 (HO-1) expression (42%) in HG-exposed cells but no differences were observed in cells treated with PLD1i or PLD2i with respect to NG. Conclusions: Our results demonstrate that PLD1 and PLD2 inhibition not only prevents the inflammatory response of RPE cells, but also decreases OE generated in RPE cells exposed to HG in a Nrf-2 and COX-2 independent manner. Further experiments are needed to fully elucidate the mechanisms by which the PLD pathway mediates OE in RPE cells exposed to inflammatory injury.

742. (180) **TARGETING PHOSPHOLIPASE D PHARMACOLOGICALLY PREVENTS PHAGOCYTIC FUNCTION LOSS OF RETINAL PIGMENT EPITHELIUM CELLS EXPOSED TO HIGH GLUCOSE LEVELS**

Paula Estefanía Tenconi1, Vicente Bermúdez1, María Sol Echevarría1, Aram Asatryan2, Jorgelina Calandria2, Norma María Giusto1, Nicolas G. Bazan2, Melina Valeria Mateos1

1. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Departamento de Biología, Bioquímica y Farmacia (DBByF), Universidad Nacional del Sur (UNS), 8000 Bahía Blanca, Argentina.

2. Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health New Orleans, New Orleans, LA 70112, USA.

Objective: Inflammation and oxidative stress are a key factor in retinal and ocular diseases. We previously described the participation of classical phospholipase D isoforms (PLD1 and PLD2) in the inflammatory response of human retinal pigment epithelium (RPE) cells exposed to high glucose concentrations (HG). The aim of this work was to study the role of the PLD pathway in RPE phagocytic function. Materials and Methods: Two human RPE cell lines were used, ARPE-19 and ABC cells. ARPE-19 cells were exposed to HG (33 mM) or to normal glucose concentration (NG, 5.5 mM, control condition) for 24, 48 or 72 h and then non-specific phagocytosis was measured using pHrodo™ green bioparticles®. Also, photoreceptor outer segments (POS) phagocytosis was measured. To inhibit PLD1 and PLD2 cells were treated with 0.5 or 5 µM pharmacological PLD1 (VU0359595 or PLD1i) or PLD2 (VU0285655-1 or PLD2i) selective inhibitors. Also, PLD1 and PLD2 siRNA were used. Cell viability was measured by flow cytometry using propidium iodide (PI). Results: HG exposure for 48 and 72 h reduced phagocytic function of ARPE-19 cells. The loss of function induced by HG was prevented when cells were treated with 5 µM PLD1i or PLD2i. Furthermore, PLD1i and PLD2i did not affect RPE phagocytic function under physiological conditions and were able to prevent the increase in reactive oxygen species (ROS) induced by HG in RPE cells. In addition, RNAseq analyses showed for the first time PLD1 and PLD2 expression in hRPE49 and ABC cells, a novel human RPE cell line. Under physiological conditions, PLD1i and PLD2i did not affect ABC cells viability and partial silencing of both PLDs did not affect ABC cells POS phagocytosis. Conclusion: Our findings demonstrate that PLD1i and PLD2i prevent the loss of phagocytic function of RPE cells exposed to HG, without affecting RPE function or viability under non-inflammatory conditions, pointing to their potential use as therapeutic agents for retinal inflammatory diseases.

743. (196) **SECURIN PTTG CHANGES ITS STABILITY AND INTERACTION WITH RSUME DURING THE CELL CYCLE**

Lara Falcucci[1], Alejandra Attorresi[1], Mariana Fuertes[1]*, Eduardo Arzt[1,2]*
[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society, Buenos Aires, Argentina, [2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
** These authors contributed equally to this work*

Altered expression of cell cycle regulators, resulting in aberrant control of mitosis, is common in tumors. Among these, the securin pituitary tumor transforming gene (PTTG), isolated by differential display from rat pituitary tumor cells, is a widely distributed protein in eukaryotic organisms that control metaphase-anaphase transition, inhibiting premature separation of sister chromatids in mitosis. PTTG also controls the transcription of genes regulators of cell cycle as c-Myc, p21, cyclins D3, A2 and B2. RWD-containing SUMOylation enhancer (RSUME), a protein identified also from pituitary tumor cells, increases PTTG protein stability and tumor abundance resulting in increased proliferation, aneuploidy, and tumor development. In this work we intend to know the mechanism of regulation of PTTG stability throughout the cell cycle and the role of RSUME. In COS-7 cells we studied the stability of PTTG in the different phases of the cell cycle by western blot (WB) of asynchronous or synchronized cell extracts in the G1, S, G2 and M phases. We verified the synchronization by flow cytometry with propidium iodide staining and by WB against specific markers of each phase. We found that RSUME increases PTTG protein stability only in G1 and M phases, but the effect is lost when PTTG levels are reduced in S and G2. We then studied whether the protein interaction of RSUME with PTTG in asynchronous cells, is maintained or lost in cells synchronized in each of the cell cycle phases. By immunoprecipitation with HA antibody in COS-7 cells transfected with expression vectors for HA-PTTG and V5-RSUME, and WB with V5 antibody, we observed that PTTG interacts with RSUME in G1 and M phases, but this interaction is lost in S and G2 phases where the protein stability of PTTG is reduced and RSUME does not act on PTTG stability. We conclude that PTTG protein stability changes during cell cycle in accordance with the interaction with RSUME. Supported by ANPCyT and FOCEM (COF 03/11).

744. (285) **CRHR1-MEDIATED Akt AND ERK1/2 ACTIVATION FROM ENDOCYTIC COMPARTMENTS INVOLVES SPECIFIC SIGNALING PLATFORMS**

Karen E Lindl[1], Paula A dos Santos Claro[1], Luciana Ant[2], Ignacio Dellavalle[1], Micaela Silbermins[1,3], Patricia Saragüeta[2], Susana Silberstein[1,3]
[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET-Partner Institute of the Max Planck Society, Buenos Aires, Argentina.
[2] Instituto de Biología y Medicina Experimental (IBYME)-CONICET.
[3] Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

The main goal of our work is the identification and characterization of cellular mechanisms and molecular components involved in Corticotropin-releasing hormone (CRH) signaling downstream of its type 1 receptor (CRHR1), which will be instrumental for understanding the physiological function of CRH in the central nervous system. We have shown that the neuronal hippocampal cell line HT22 stably expressing CRHR1 (HT22-CRHR1 cells) recapitulates essential features of the CRH responses seen in primary cell cultures, making this an in vitro model useful to perform molecular and cellular experiments. We previously demonstrated that a sustained ERK1/2 activation downstream CRHR1 depends on endocytosis and have recently determined that Akt kinase is also phosphorylated by activated CRHR1 from endocytic compartments. Internalized receptors enter a complex endocytic network composed by a variety of compartments with heterogeneous membrane composition. The protein repertoire available in each conditions receptors responses and final fate within the network. By fluorescent immunocytochemical analysis in HT22-CRHR1 cells, we characterized the cell compartments that CRH-activated CRHR1 transits after internalization. Activated CRHR1 colocalizes with early and recycling endosomal markers and Rab5 effectors APPL1 and EEA1. Silencing of these Rab5 effectors disclosed that APPL1 is required for CRHR1-mediated Akt activation whereas EEA1 is needed for ERK1/2 activation. Thus, different protein complexes are involved in CRHR1 signaling from inside the cell. Akt signaling controls a wide variety of neuronal functions, being crucial in pathophysiological processes and it is functionally linked to stress-related disorders. To determine the impact of Akt activation downstream CRHR1 in regulation of gene expression, we performed transcriptional profiling analysis in CRH-stimulated HT22-CRHR1 cells. Initial results are presented and discussed. Supported by ANPCyT and FOCEM (COF 03/11).

745. (305) **LONG NON-CODING RNAs TRANSCRIBED FROM TELOMERES ARE INDUCED DURING THE PROCESS OF ADIPOGENESIS AND DEPENDENTS ON ROS AND PKA SIGNALING**

Marina C. Ruiz[1], Natalia M. Galigniana[1], Romina Uranga[2], Graciela Piwien-Pilipuk[1]
1-Instituto de Biología y Medicina Experimental – CONICET; 2-Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur- CONICET

TERRAs (*telomeric repeat containing RNAs*), are long non-coding RNAs associated with telomere and genome stability. Telomeres are particularly prone to be damaged by oxidative stress and, thus their protection is relevant to avoid their dysfunction. We showed that the level of TERRAs are increased in response to oxidative stress as a mechanism of telomere protection. However, ROS have proven to be signaling molecules, and we found that physiologic increase of ROS in brown adipose tissue of mice exposed to cold triggered the induction of TERRAs. It is also well known that ROS increase during adipocyte differentiation. We found that TERRAs increased their level of expression during the differentiation of 3T3-L1 preadipocytes. Their increase was prevented in the presence of the antioxidant N-acetyl-L-cysteine. Next, we tested which component of the adipogenic cocktail (MDI: IBMX, DEXA, Insulin, Rosiglitazone) was responsible for TERRAs induction, and TERRAs increased upon IBMX or rosiglitazone treatment of 3T3-L1 preadipocytes. Further, PKA inhibitors H89 and PKI blocked the increase of TERRA induced by MDI as well as the increased mediated by IBMX or rosiglitazone treatments, suggesting that TERRA induction is under PKA control. We analyzed the formation of telomere-associated DNA damage foci (TAFs) in 3T3-L1 preadipocytes induced to differentiate by IIF labelling TRF1, a component of the sheltering complex in telomeres and gH2AX, the phosphorylated histone variant found at sites of DNA damage. No colocalization was observed between TRF1 and gH2AX in images obtained by confocal microscopy indicating no TAF formation, thus the telome integrity was preserved during adipogenesis. In summary, we showed for the first time that TERRAs are induced during the process of adipocyte differentiation dependent on ROS and PKA signaling in order to protect telomers and possibly having other potential functions that need to be unveiled.

746. (317) **THE MITOCHONDRIAL-NUCLEAR SHUTTLING OF THE IMMUNOPHILIN FKBP51 IS DEPENDENT ON THE ACTIVATION OF HSF1**

Camila Rubino[1], Valentina Hansen[1], Sol Ciucci[1,2], Alejandra Erlejman[1,2], Mario Galigniana[1,3], Gisela Mazaira[1,2]
[1] Departamento de Química Biológica, FCEN, UBA, [2]IQUIBICEN-CONICET, [3] IBYME-CONICET.

In previous works we reported that the immunophilin FKBP51 is a mitochondrial factor that migrates to the nucleus when cells are exposed to oxidative stress. In this work we analyzed the regulation of this phenomenon. Confocal microscopy studies show that the mitochondrial-nuclear shuttling of FKBP51 is accompanied by Hsp70, a chaperone induced under stress conditions and interacts with the TPR domains of the immunophilin. To evaluate whether FKBP51 trafficking occurs with other types of stress, 3T3-L1 cells were exposed to heat-shock (43ºC for 30'), UV light (30'), osmot-

ic stress (425 mOsm), hypoxia (250 μM DFO), Ca-ATPase inhibition (0.5 μM tapsigargina), metabolic stress (0.5% serum for 16 h), glutathione depletion (0.5 mM BSO for 16 h), etc. FKBP51 always abandons mitochondria and accumulates in the nuclei. The common factor for such heterogeneous stimuli could be the activation of the Heat-Shock Factor-1 (HSF1). To assess whether HSF1 affects FKBP51 trafficking, HSF1-KO MEF cells were exposed to 0.25 mM H_2O_2 for 1 h. FKBP51 concentrated in the nucleus (and nucleoli) in control wild-type cells only, suggesting that HSF1 is necessary for its FKBP51 trafficking. The mere overexpression of GFP-HSF1 already shows FKBP51 in the nucleus without any stimulation. Accordingly, since cancer cells have high metabolism and production of ROS, we show that the expression of FKBP51 is high, and its location is nuclear. Hence, cell viability was evaluated under normal and oxidative stress conditions (0.25 mM H_2O_2) by MTT assay in HEK cells overexpressing FKBP51 and/or HSF1. While H_2O_2 has harmful action on cell proliferation, FKBP51 and/or HSF1 overexpression show protective action. It was confirmed in MEF-KO cells for HSF1, whose growth is reduced, and the inhibitory effect of peroxide is enhanced. It is concluded that FKBP51 and HSF1 cooperate in the cell proliferation mechanism and the resistance to cell death.

747. (345) **EVALUATION OF THE LEUKOTRIENE B4 (LTB4) SIGNALLING PATHWAY LTA4H/LTB4/BLT2 ON DIFFERENT STAGES OF LIVER CANCER DEVELOPMENT**

Lucía Oviedo Bustos[1]; María P. Ceballos[1]; Carla G. Comanzo [1,2,3]; Anabela C. Ferretti [2]; Magalí Frattini [2]; Nicolás F. Palma [1,2]; Marina C. Vera[1], Ariel D. Quiroga[1,2,3]; María de Luján Álvarez[1,2,3].
1. Instituto de Fisiología Experimental (IFISE–CONICET). Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR).
2. Área Morfología. Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR).
3. CAECIHS, Universidad Abierta Interamericana (UAI).

LTB4 is generated from arachidonic acid through the sequential action of several enzymes, including leukotriene A4 hydrolase (LTA4H). LTB4 is overexpressed in different types of cancers and plays a key role in cancerous cell proliferation. Additionally, recent studies indicate that the activation of BLT2 (a cell surface receptor for LTB4 and 12-hydroxyeicosatetraenoic acid) is necessary for cell proliferation, survival and metastasis; however, its role in hepatocellular carcinoma (HCC) remains largely unknown. Aim: to assess the status of LTA4H/LTB4/BLT2 signalling pathway on different stages of hepatocarcinogenesis, using animal models. Methods: Early-stage liver cancer (preneoplasia) was evaluated using a two-stage chemical carcinogenic model in adult male Wistar rats (initiator: diethynitrosamine (DEN); promotor: 2-acetylaminofluorene). Preneoplastic lesions developed after 6 weeks of treatment. A late stage of hepatocarcinogenesis was evaluated using a single dose of DEN in 14-day-old C57/BL6 mice. HCC developed after 10 months. LTA4H and BLT2 expressions were tested by western blot assays in rat preneoplastic livers, mouse HCC tissues and healthy livers. Results: Western blot studies showed a significant increase in the expression of LTA4H and BLT2 proteins between healthy and preneoplastic livers [LTA4H (arbitrary units, AU): Healthy: 2080± 163, preneoplastic: 3258±177*; BLT2 (AU) Healthy: 1664±264, preneoplastic: 3476± 427*]. In addition, the expressions of LTA4H and BLT2 were significantly different between healthy and HCC livers [LTA4H (AU): Healthy: 2531±524 HCC: 3812±123*; BLT2 (AU) Healthy: 672±104, HCC: 2196±366]. *p<0.05 vs healthy tissue of each corresponding model. Conclusion: The present results, despite being preliminary, indicate that LTA4H/LTB4/BLT2 signalling pathway might be involved in the different stages of liver cancer development. Targeting this signalling pathway could establish future strategies for the treatment of liver cancer.

748. (348) **A NOVEL ROLE OF MAGE-C2 ONCOPROTEIN IN THE ACTIVATION OF PI3K/AKT PATHWAY**

Franco Andrés Pascucci 1, Micaela Escalada 1, Melisa Suberbordes 1, Martín Monte 1 2.
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. 2 Instituto IQUIBICEN, UBA-CONICET.

MAGE-C2 belongs to the type I MAGE protein family. Type I *MAGE* genes are expressed in cancer and silenced in normal tissues with the exception of testis. Recently, we reported that the oncogenic cellular context affects MAGE-C2 pro-oncogenic activity. In particular, the RASV12 oncogene stabilizes MAGE-C2 turning it into a potent inhibitor of p53. Then, we decided to study the interactome of MAGE-C2 in the presence of RASV12 to discover new functions in these conditions. For this purpose, we expressed MYC-MAGE-C2 and RASV12 in HEK293T cells, immunopurified (IP) MAGE-C2 and identified MAGE-C2-interacting proteins by mass spectrometry (LC-MS/MS). Among the interactions identified; we focused on IRS4, a PI3K activator highly expressed in cancer cells. We first studied the effect of MAGE-C2 on the PI3K pathway using these models: A375-MAGE-C2 WT and -KO cells; and overexpression of MAGE-C2 in HEK293T (both high endogenous IRS4-expressing cells). We observed a positive correlation between MAGE-C2 expression and pAKT (Thr-360) levels, determined as readout for PI3K activation. To find out if MAGE-C2 increases the activity of IRS4 as a PI3K activator, we expressed MAGE-C2 with or without IRS4 in non-T HEK293 cells (no endogenous IRS4-expressing cells) and observed that MAGE-C2 was able to activate the PI3K/AKT pathway only when exogenous IRS4 is present. Since the PI3K/AKT signaling pathway is a prototypic survival pathway, we measured the survival rate of 72h starved A375-MAGE-C2 WT and -KO cells using trypan blue exclusion and found that A375-MAGE-C2 WT cells were significantly more resistant to starvation-induced cell death (1.8 % viability decrease) than the A375-MAGE-C2 KO cells (13.4 % viability decrease). Here we propose a novel pro-oncogenic function of MAGE-C2 protein by stimulating the PI3K/AKT pathway through IRS4.

749. (486) **MITOCHONDRIAL RESPIRATORY PARAMETERS AND BIOGENESIS REGULATORY FACTORS ARE INCREASED BY ANGIOTENSIN II IN H295R HUMAN ADRENOCORTICAL CELL**

Matías Belluno, Katia Helfenberger, Ariana Balcázar Martínez, Alejandro Bozzano, Ana Fernanda Castillo, Yanina Benzo, Cecilia Poderoso.
INBIOMED (UBA-CONICET). Departamento de Bioquímica Humana. Facultad de Medicina. Universidad de Buenos Aires. Paraguay 2155 5to piso. C1121ABG. CABA, Argentina.

Mitochondrial homeostasis is preserved by the fine coordination between the generation of new mitochondria by mitochondrial biogenesis; and the removal of damaged mitochondria. Biogenesis increases oxidative phosphorylation capacity, diminishes pathologic oxidative stress and repair mitochondrial-associated dysfunctions. This process is driven by transcription factors such as PCG-1α, TFAM, NRF1 and usually is accompanied by an increase in mitochondrial DNA (mtDNA). Mitochondrial normal function is essential for hormones signal transduction pathway, such as angiotensin II (Ang II). We have previously reported that Ang II favors mitochondrial fusion in H295R human adrenocortical cells. The effect of Ang II on mitochondrial regulators genes and metabolic parameters is not known up to date. The aim of this work is to determine the effect of Ang II on several transcription factors involved in the regulation of mitochondrial parameters in H295R cells. We observed that 100 nM Ang II promoted a significant increase in NRF1 mRNA levels in a time-dependent manner, as assessed by qPCR ($p<0.001$). Furthermore, after Ang II stimulation, a significant increase on TFAM protein levels was detected by western blot ($p<0.05$). In agreement, mitochondrial mass increased in response to Ang II treatment, determined by the relationship between nuclear DNA and mtDNA by qPCR. mRNA expression of NRF2 and UCP2, genes involved in mitochondrial function, is regulated by Ang II in a biphasic manner. Then, we analyzed mitochondrial bioenergetics in Ang II-stimulated and control cells by using the SeaHorse XF Cell Mito Stress Test and observed that mitochondrial basal and maximal respiration and ATP production are significantly increased by this hormone ($p<0.01$; $p<0.001$). Together, our results indicate that Ang II favors mitochondrial biogenesis and thus increases mitochondrial bioenergetics and function.

750. (534) **REGULATION OF THE ANDROGEN RECEPTOR FUNCTION BY TPR-DOMAIN PROTEINS**

Valentina Hansen[1], Camila Rubino[1], Sol Ciucci[1,2], Alejandra Erlejman[1,2], Mario Galigniana[1,3], Gisela Mazaira[1].
[1] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, [2] IQUIBICEN - CONICET, Buenos Aires, Argentina, [3] IBYME - CONICET, Buenos Aires, Argentina.

Tetratricopeptide repeats (TPR) sequences of 34 amino acids arranged in tandems of antiparallel α-helices. They are responsible of protein-protein interactions. The best characterized TPR proteins are those found associated to Hsp90 in steroid receptor complexes. In previous works, our laboratory demonstrated that the main TPR-domain immunophilins FKBP51 and FKBP52 regulate the activity of the glucocorticoid receptor (GR) in a competitive fashion with other coexisting TPR proteins, such as PP5, SGT1α and 14-3-3σ. In this work, the study was extended to the androgen receptor (AR). The nuclear import of AR was evidenced by fluorescence microscopy, and the transcriptional activity was assessed by a luciferase reporter gene. Like for the case GR, the results demonstrate that both FKBP52 and PP5 enhance AR transcriptional activity, whereas SGT1α only shows a slight inhibitory effect on AR rather than the strong stimulation measured for GR. Regarding 14-3-3σ, an expression-dependent inhibitory effect was measured for AR, unlike the biphasic regulation previously observed for GR (i.e., stimulation at for low expression levels and inhibitory action for high levels). Microscopy studies showed that, in contrast to our expectations, the subcellular localization of AR was unaffected by the proteins tested here. In conclusion, TPR domain proteins regulate the transcriptional activity of AR showing patterns that differ from those of GR. This indicates receptor specificity of action. It is also implied that the expression balance between all these TPR factors should affect the final biological response. This may be relevant for cases like prostate cancer, where the functional balance between AR and GR affects the development and progression of the pathology.

751. (585) **THE REGULATORY ACTION OF HSP90-BINDING IMMUNOPHILINS ON NF-κB BIOLOGICAL ACTIVITY IS IMPAIRED BY β-CATENIN**

Sol Ciucci[1,2], Alejandra Erlejman [1,2], Gisela Mazaira[2], Mario Galigniana[2,3].
[1] IQUIBICEN – CONICET; [2] Departamento de Química Biológica, FCEN, UBA; [3] IByME – CONICET.

Immunophilins (IMMs) belong to a subfamily of chaperones that show rotamase activity. Two of the best characterized IMMs, FKBP51 and FKBP52, were first described in the steroid receptor-Hsp90 complex. Recently we showed that they regulate the biological activity of NF-κB, a factor that plays a dual role in apoptosis since it is required for p53-dependent cell death but impairs TNF-induced apoptosis. In turn, β-catenin is a protein originally related to the E-cadherin cell-cell adhesion system, but it is also a key canonical factor of the Wnt signalling cascade. Importantly, aberrant expression of β-catenin induces malignant transformation of normal cells, and its abnormal activity has been reported in many cancer types. Since β-catenin is an Hsp90-client protein and both IMMs bind to Hsp90, we hypothesized a potential functional involvement of β-catenin and the IMMs in the regulation of NF-κB biological activity. The respective inhibitory and stimulatory action of FKBP51 and FKBP52 on NF-κB transcriptional activity is evidenced here in HEK cells stimulated with PMA. Importantly, this treatment as well as that with TNFα, recruits FKBP52 to the NF-κB. Thereafter, cells overexpressing increasing amounts of β-catenin and stimulated with PMA show that both, the basal activity of NF-κB and the stimulatory action of FKBP52, are supressed in a β-catenin concentration-dependent fashion. Similarly, β-catenin enhances the inhibitory effect of FKBP51 on NF-κB response. To test the eventual functional competition of β-catenin and FKBP52, HEK cells were transfected with constant amounts of β-catenin and increasing amounts of FKBP52. As a result, the IMM reverses the inhibitory action of β-catenin. It is concluded that β-catenin abrogates the regulatory actions of both IMMs on NF-κB. Inasmuch as β-catenin is constitutively active in most cancer cells, these findings may justify the limited response seen for NF-κB in these cells, even though FKBP52 is highly expressed.

752. (608) **EFFECT OF NEW HSP90 INHIBITORS IN TWO CANCER MODELS: PROSTATE AND BREAST CANCER**

Iara Santa Cruz[1], Agostina Aramburu[1, 2], Alejandra Erlejman[1,3], Mario Galigniana[1,4], Gisela Mazaira[1], Sol Ciucci[1,3].
[1]Departamento de Química Biológica, FCEN, UBA. Buenos Aires, Argentina, [2]Departamento de Industrias-ITAPROQ FCEN, UBA. Buenos Aires, Argentina, [3]IQUIBICEN/CONICET. Buenos Aires, Argentina, [4]IBYME/CONICET. Buenos Aires, Argentina

Hsp90 stabilizes the active conformation of cognate client proteins already exhibiting a stable tertiary structure, among them, steroid receptors and several oncoproteins. Cancer cells are dependent on chaperones given their elevated proteotoxic stress. Hsp90 inhibitors are to date the only known chemotherapeutic agent that shows strong effects in all hallmarks of cancer. Therefore, it is considered a promising molecular target for cancer treatment. Various Hsp90 inhibitors are being tested in clinical and preclinical studies with different results, but proven nephro- and hepatotoxic effects. The aim of this work was to analyze the biological actions of synthetic compounds predesigned by computational modeling according to the potential inhibitory effect on the intrinsic ATPase activity, which is regarded as essential for Hsp90 biological function. We assessed the effects of the drugs on the Hsp90 ATPase activity *in vitro*, cell viability and migration in two cancer cell models (prostate and breast), as well as their putative inhibitory action on the glucocorticoid receptor (GR) nuclear translocation. Geldanamycin (GA), a known Hsp90 inhibitor, was used as a positive control in all experiments. Pyrazoline-derivative compounds (C3 and C6) confirmed *in silico* predictions regarding their ability to inhibit Hsp90 ATPase activity. As expected, cell treatment with GA prevented nuclear import of the steroid receptor and decreased cell viability and cell migration capacity in both cell lines, PC3 (prostate cancer) and MDA-MB-231 (breast cancer). Both synthetic drugs also showed equivalent inhibitory action on cell viability and migration. Interestingly, none of the novel drugs showed any effect on the GR nuclear import. These properties could have pharmacological relevance, given the lack of side effects such as steroid receptor inhibition, which is a desirable characteristic for pharmacological applications. Moreover, the study provides novel insights that could contribute to the design of more active and less toxic drugs.

753. (773) **CHARACTERIZATION OF NUCLEOLAR-LOCALIZED MAGE PROTEINS**

Micaela Escalada, Candela Vidal, Franco Andrés Pascucci, Melisa Suberbordes, Fátima Ladelfa, Martin Monte
Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. Instituto IQUIBICEN, UBA-CONICET.

Our group is dedicated to the characterization of proteins of the MAGE-A, -B and -C groups. Studies form the last years have positioned them as nuclear oncogenic drivers, mainly targeting the p53 onco-suppressor pathway. During our investigation, we observed that MageB2 failed to affect p53 activity and can localize to the nucleoli in unstressed cells, while relocalizes to the nucleoplasm upon nucleolar stress. These distinctive features prompted us to further study nucleoli-related MAGE proteins. First, with the aim to further study MageB2, we analyzed the kinetics of its re-localization to the nucleoli upon nucleolar stress removal. Cell treatment with 1uM BMH-21 (a specific polymerase I inhibitor) for 3 hours delocalized MageB2 from the nucleolus to the nucleus and 42 hours after BMH-21 washing, MageB2 localization resembles the one in normal cell conditions. Then, to understand whether other MAGE-B members could behave analogous to MageB2, we cloned MageB3, that similar to MageB2, possesses a potential nucleolar localization signal. In this context, we effectively observed that MageB3 accumulates in the nucleolus and relocalizes to the nucleoplasm upon treatment with BMH-21. Considering that our group has already reported heterodimeric interaction between members of MAGE-A group, we decided to investigate whether an interaction between MageB2 and

MageB3 could be detected. Data obtained from immunoprecipitation approach, showed that MageB2 co-immunoprecipitates with MageB3. Finally, to address whether this interaction could result in functional consequences, we performed a series of MageB2 and MageB3 co-expression studies. Protein analysis by western blot indicated that MageB2 is able to enhance MageB3 protein levels, suggesting a potential protein stabilization effect. In conclusion, this work extends our studies on the response of MageB2 to nucleolar stress and proposes a model of interaction between MageB2 and MageB3 that results in the stabilization of MageB3.

754. (788) **FUNCTIONAL AND STRUCTURAL ANALYSIS OF GLUCOCORTICOID AND PROGESTERONE RECEPTOR CROSSTALK**

Norma Roxana Carina Alves[1,2], María Virginia Dansey[1,3], Adali Pecci[1,2], Lautaro Damián Álvarez[1,3]
[1-] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires. [2-] Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad Nacional de Buenos Aires. CONICET. [3-] Unidad de Microanálisis y Métodos Físicos Aplicados a Química Orgánica (UMYMFOR). Universidad Nacional de Buenos Aires. CONICET.

Steroid Receptors (SR; Estrogen, Androgen, Progesterone, Glucocorticoid, and Mineralocorticoid Receptors) are ligand-activated transcription factors involved in cell signaling, survival, and proliferation. SRs share conserved sequences and structures arranged in an N-terminal domain, a highly conserved DNA-binding domain (DBD), and a conserved ligand-binding domain (LBD). Activated SRs dimerize and bind to hormone response elements (HRE). Since SRs are coexpressed and coactivated in many cell types, their integral study requires including their crosstalk mechanisms. Specifically, our group focused on PR/GR crosstalk and found an antagonistic effect of activated GR on PR-dependent features, showing GR-PR complexes formation and co-recruitment to shared sites; on that basis, we aimed to assess a potential GR/PR direct interaction. In reporter gene assays with different HREs in various cell lines in the presence of selective GR agonist dexamethasone, selective PR agonist R5020, or both, we obtained marked interferences within their activity with combined treatment. In particular, using a single-HRE (AGAACAgttTGTTCT) reporter, we observed that one SR activation inhibits the other's transcriptional activity. Picturing a possible heterodimerization scenario and based on GR/MR reports, we performed GR-DBD and PR-DBD structural analysis and molecular dynamics (MD) simulations of GR/PR homo and heterodimers bound to such HRE. MD analysis showed different dynamic behavior of the dimerization interface (D-loop) and adjacent residues (lever-arm) between homo and heterodimers. We identified unique interactions within each SR involved in D-loop stabilization; in particular, the orientation of a His in the lever-arm appears critical for GR proper dimer binding. Indeed, shifts in its orientation led to conformational D-loop rearrangements that destabilize heterodimers. In line with our experimental results, these observations could aid explain the interference we found *in vitro*.

755. (794) **FUNCTIONAL STUDY OF MAGE-A9 AND ITS EFFECT ON P53**

Melisa Suberbordes1, Franco Andrés Pascucci1, Micaela Escalada1, Martín Monte1,
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA e Instituto IQUIBICEN, UBA-CONICET.

MAGE-A9 is a tumor-expressed protein, within the group of MAGE type I proteins (Melanoma Antigen Genes). MAGE type I gene expression is silenced in healthy adult tissues, except in the germ cells, but are abnormally re-expressed in tumor tissues where they display pro-oncogenic functions. Little is known about MAGE-A9 protein and its mechanisms of action, most of the clinical observations show it as an indicator of poor prognosis, metastasis, poor survival and cell resistance to chemotherapy. To start a molecular characterization, we generated a MAGE-A9 expression vector containing a Flag tag. We observed for the first time that MAGE-A9 is able to inhibit the transactivation function of the tumor suppressor p53 in reporter-gene assay. Given that p53 promotes apoptosis upon different stresses, we studied whether MAGE-A9 expression could protect wt-p53 bearing cells (U2OS) form apoptosis. Cells transfected with MAGE-A9 and treated with staurosporine (an apoptosis inducer) showed a lower percentage (22,30%) of apoptotic nuclei compared to MAGE-A9 negative cells (39,1%). Since it has been proposed different mechanism of action for other MAGE proteins in regulating p53, we performed co-inmunoprecipitation assays of MAGE-A9 with some potential p53 regulators such as HDAC -2, -4 and -6, TRIM28 and MDM2. We observe that MAGE-A9 interacts with HDAC2 but not with the other tested proteins. By means of immunofluorescence we saw that its cellular location is nuclear and cytoplasmic and it is excluded from the nucleolus. This observation was confirmed by the absence of the NoLS sequence at its N-terminus. Also, co-expressing MAGE-A9 with p14ARF does not relocalizes it to the nucleolous as it does with MAGE-C2. Additionally, we found for the first time that MAGE-A9 protein is phosphorylated at serine. This work presents the initial molecular characterization of MAGE-A9 but further work has to be developed to better know MAGE-A9 with the aim of inhibit its pro-oncogenic activity.

756. (858) **GPRC5A REGULATION BY VITAMIN D3 AND CIGARETTE EXCTRAC SMOKE IN COLON CANCER CELL LINES**

Pablo Iglesias, Camila Dib, Nadia Núñez, Tatiana M. Limpias del Valle, Consuelo Mori, Ángel G. Valdivieso, Tomás A. Santa Coloma
Laboratory of Cell & Molecular Biology, BIOMED-UCA-CONICET, Buenos Aires, Argentina

GPRC5A is a G protein-coupled receptor 5A, also known as Retinoic acid-induced gene 3 (RAI3) and Retinoic acid-induced (RAIG1). In our laboratory, we named this gene PEIG-1 (phorbol ester-induced gene-1) because it was found to be a 12-O-tetradecanoyl phorbol 13-acetate (TPA)-inducible gene. Recently, we reported that TPA regulated *GPRC5A* mainly through the PKC pathway. Commonly, TPA is employed as a tumor-promoting agent. GPC5A dysregulation is associated with diverse types of human cancers and its role as a tumor suppressor or as an oncogene is unclear yet. This dual behavior makes GPRC5A an interesting gene to study. The aim of this work was to study the expression of this gene in colon (T84 and Caco-2) and lung (Calu-3) cells treated with cigarette extract smoke (CSE), a potential oncogenic inductor. Also, cells were treated with vitamin D3 because of its similar immunomodulatory action that retinoic acid (vitamin A), a known activator of the promoter region of *GPRC5A* through the RAR/RXR transcription factor. The transcriptional expression of *GPRC5A* in cells treated with CSE, vitamin A, vitamin D3, and TPA was measured by qRT-PCR. To test if ROS could be involved in the *GPRC5A* regulation, the cytoplasmic ROS (cROS) and mitochondrial ROS (mtROS) were measured with DCFH-DA and Mitosox, respectively. The mitochondrial membrane potential (mΨ) was measured by flow cytometry with the fluorescent probe TMRE. The treatment with CSE and vitamin D3 for 48 h induced an increased ($p<0.05$) expression of *GPRC5A*, like the controls with vitamin A. Preliminary results show that the cROS, mtROS and mΨ were increased ($p<0.05$) in T84 cells treated with TPA. In conclusion, CSE and D regulated the expression of *GPRC5A*. Further research is needed to determine the implication of these changes in the *GPRC5A* regulation by vitamin D and/or CSE Supported by CONICET PUE 22920160100129CO, PIP 11220150100227CO, and ANCPYT PICT-2018-4429 to TASC, and PICT-2015-1031 and PICT-2020 SERIE A-03854 to AGV.

757. (886) **A NOVEL ROLE OF MAGE-C2 ONCOPROTEIN IN THE ACTIVATION OF PI3K/AKT PATHWAY**

Franco Andrés Pascucci 1, Micaela Escalada 1, Melisa Suberbordes 1, Candela Vidal 1, Fatima Ladelfa 1 2, Martín Monte 1 2.
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. 2 Instituto IQUIBICEN, UBA-CONICET.

MAGE-C2 belongs to the type I MAGE protein family. Type I *MAGE*

genes are expressed in cancer and silenced in normal tissues with the exception of testis. Recently, we reported that the oncogenic cellular context affects MAGE-C2 pro-oncogenic activity. In particular, the RASV12 oncogene stabilizes MAGE-C2 turning it into a potent inhibitor of p53. Then, we decided to study the interactome of MAGE-C2 in the presence of RASV12 to discover new functions in these conditions. For this purpose, we expressed MYC-MAGE-C2 and RASV12 in HEK293T cells, immunopurified (IP) MAGE-C2 and identified MAGE-C2-interacting proteins by mass spectrometry (LC-MS/MS). Among the interactions identified; we focused on IRS4, a PI3K activator highly expressed in cancer cells. We first studied the effect of MAGE-C2 on the PI3K pathway using these models: A375-MAGE-C2 WT and -KO cells; and overexpression of MAGE-C2 in HEK293T (both high endogenous IRS4-expressing cells). We observed a positive correlation between MAGE-C2 expression and pAKT (Thr-360) levels, determined as readout for PI3K activation. To find out if MAGE-C2 increases the activity of IRS4 as a PI3K activator, we expressed MAGE-C2 with or without IRS4 in non-T HEK293 cells (no endogenous IRS4-expressing cells) and observed that MAGE-C2 was able to activate the PI3K/AKT pathway only when exogenous IRS4 is present. Since the PI3K/AKT signaling pathway is a prototypic survival pathway, we measured the survival rate of 72h starved A375-MAGE-C2 WT and -KO cells using trypan blue exclusion and found that A375-MAGE-C2 WT cells were significantly more resistant to starvation-induced cell death (1.8 % viability decrease) than the A375-MAGE-C2 KO cells (13.4 % viability decrease). Here we propose a novel pro-oncogenic function of MAGE-C2 protein by stimulating the PI3K/AKT pathway through IRS4.

TOXICOLOGY I *Wednesday, November 16, 14-15:30 hr*
Chairs: Laura Kass - Gabriela Altamirano

758. (458) **INHALATION AIR POLLUTANTS IMPAIR THE LUNG ANTIOXIDANT SYSTEM INVOLVED IN ALVEOLAR BARRIER INJURY REPAIR RESULTING IN OXIDATIVE DAMAGE**

Sofía Reynoso, Agustina Freire, Mariana Garcés, Lourdes Cáceres, Florencia Sarno, Timoteo Marchini, Pablo Evelson, Natalia Magnani
Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL), Facultad de Farmacia y Bioquímica.

It has been suggested that breathing polluted air leads to pulmonary oxidative stress and inflammation which might affect tissue damage repair mechanisms. The aim of our work was to study if exposure to Buenos Aires urban air impairs lung antioxidant system activity shifting the cell redox metabolism to a more oxidase status, which could be associated with a delayed injury repair. In order to address such mechanisms, BALB/c mice were exposed to filtered air (FA) or urban air (UA) from Buenos Aires City, in whole-body exposure chambers for up to 8 weeks. Then, moderate lung injury was induced by intratracheal instillation of 0.1 N hydrochloric acid (HCl). Samples were evaluated 5 days after HCl treatment. Regarding the antioxidant system, even though transcription factor Nrf2 expression was higher in the UA+HCl group (p<0.05), Glutathione peroxidase (GPx) expression levels (p<0.05) along with catalase activity (p<0.01) were decreased compared to their respective control group. UA -induced oxidative damage was evaluated by the expression of 4-HNE as a lipid peroxidation marker. Our results have shown that after UA exposure, HCl treatment leads to augmented expression of 4-HNE (p<0.05). Moreover, the superoxide produced by the NADPH oxidase (Nox) activity showed the same trend (p<0.05) in comparison to the control group. Taken together, these results showed that the present of urban pollutants in the lung increased Nox activity resulting in oxidative damage due to an impaired antioxidant enzymes activity. Therefore, the antioxidant system of mice breathing UA might was overwhelm and unable to modulate the redox metabolism involved in the alveolar damage repair mechanisms previously observed. Consequently, a deficient antioxidant response after UA exposure may delay the tissue healing mechanisms hindering the complete alveolar barrier architecture recovery that extends the diminished lung function over time.

759. (464) **NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ALTERS THE OVIDUCTAL DIFFERENTIATION OF PREPUBERTAL EWE LAMBS**

Ramiro Alarcón[1], Lovera Lourdes[1], Ana Laura Alegre[1], Paola Inés Ingaramo[1], Oscar Edgardo Rivera[2], Gisela Haydee Dioguardi[2], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1]
[1] Instituto de Salud y Ambiente del Litoral (ISAL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Instituto de Investigación Agropecuaria, Ambiente y Salud (IIPAAS), Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora.

Glyphosate-based herbicides (GBH) are extensively used in Argentina and abroad. In the beginning, GBHs were declared safe, but many publications in recent years have reported their harmful health effects. We reported the adverse effects of neonatal exposure of ewe lambs to GBH on ovarian and uterine development. Here, considering oviduct is essential for fertility, we aimed to evaluate the GBH effects on oviduct development of neonatally exposed ewe lambs. To this, crossbreed (Corriedale x Hampshire down) ewe lambs received orally, from postnatal day (PND) 1 to PND14, GBH (1 mg/Kg/day of glyphosate, n=8) or saline solution (vehicle, n=12). On PND45, we performed the salpingectomy, and oviductal weight and length were measured. Later, we collected and processed ampullar and isthmic sections for both RT-PCR and histology analysis. No changes in oviductal weight or length were found. Luminal epithelial height, number of glands, and myosalpinx thickness were evaluated using Picrosirius-Hematoxylin staining; all animals showed similar results. Proliferative activity was assessed by immunodetection of Ki67. In GBH-exposed animals, higher cell proliferation was observed in the luminal epithelium and the subepithelial stroma of the ampullar region, but no changes were observed in the different compartments of the isthmus. Expression of molecules involved in cell proliferation and oviductal development [steroid receptors (ESR1 and PR) and IGF molecules (IGF-1 and -2, IGF-1R, IGFBP5)] were determined by RT-PCR. No altered expression of evaluated genes was observed in the isthmus nor the ampulla. Since cell proliferation is controlled through different pathways, further studies are necessary to identify the molecules involved. Deregulation of proliferative status might affect tissue homeostasis and development, with consequences on oviduct functionality and reproductive health.

760. (501) **MECHANISMS OF NLRP3 INFLAMMASOME ACTIVATION IN MACROPHAGES BY AIR POLLUTION PARTICULATE MATTER ($PM_{2.5}$)**

Lourdes Caceres[1], Timoteo Marchini[1,2], Sheu-Tijani Olawale Abogunloko[2], Sara Malchow[2], Fabienne Ehret[2], Julian Merz[2], Larissa Fischer[3], Oliver Gorka[3], Peter Stachon[2], Olaf Groß[3], Pablo Evelson[1], Dennis Wolf[2]
[1]Universidad de Buenos Aires, CONICET, Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina. [2]Cardiology and Angiology, Medical Center, University of Freiburg, Freiburg im Breisgau, Germany. [3]Institute of Neuropathology, Medical Center, University of Freiburg, Freiburg im Breisgau, Germany.

Particulate matter ($PM_{2.5}$) exposure aggravates cardiorespiratory diseases by inflammatory cytokine secretion from alveolar macrophages. Inhalation of silica particles and asbestos triggers NLRP3 inflammasome activation and IL-1β release. However, the mechanisms involved in NLRP3 engagement by $PM_{2.5}$ remain unclear. To study this process, THP1-ASC-GFP cells were incubated for 6 or 24 h with 0, 1, 10, or 100 μg/mL of $PM_{2.5}$ surrogates, including ROFA, CAPs, SRM1648a, and SRM2975. NLRP3 priming and specks formation were detected by flow cytometry after incubation with ROFA (p<0.001) and SRM1648a (p<0.001). Increased IL-1β was detected by ELISA in cell culture supernatants of ROFA-exposed THP1-ASC-GFP cells (p<0.001) and BMDMs from wild type mice (p<0.001), but not from *Nlrp3*$^{-/-}$ or *Casp1*$^{-/-}$ mice, or after pre-incubation with the NLRP3-specific inhibitor MCC950. Upregulation of

Tnf gene expression (p<0.001) and increased TNF-α levels in cell culture supernatants (p<0.01) were also observed. Pre-incubation with anti-TNF-α antibody resulted in decreased IL-1β release from ROFA-exposed BMDMs (p<0.01). Increased mitochondrial superoxide anion ($O_2^{\bullet-}$) production by MitoSOX fluorescence was found in ROFA-exposed BMDMs (p<0.001), together with decreased maximal respiration by the Seahorse MitoStress Test. Accordingly, inhibition of mitochondrial complex I $O_2^{\bullet-}$ production with S1QEL resulted in decreased IL-1β levels (p<0.01). K^+ efflux contribution on NLRP3 activation was evident in ROFA-exposed BMDMs incubated with increasing concentrations of KCl (p<0.05). Lysosomal leakage in BMDMs was also observed after ROFA exposure (p<0.001). In conclusion, $PM_{2.5}$ induces NLRP3 inflammasome priming and activation in macrophages. TNF-α, mitochondrial $O_2^{\bullet-}$ production, K^+ efflux, and lysosomal disruption were identified as potential drivers of NLRP3 engagement after $PM_{2.5}$ exposure. These findings help to unravel the mechanisms by which $PM_{2.5}$ promotes cardiorespiratory inflammation and disease.

761. (512) **URBAN AIR INDUCES OXIDATIVE STRESS, INFLAMMATION AND DAMAGE OVER TIME IN MICE OLFACTORY BULB**

Freire, Agustina[1]; Garcés, Mariana[1]; Cáceres, Lourdes[1]; Sofía, Reynoso [1]; Marchini, Timoteo[1]; Octavio, Diana [1]; Martinefski, Manuela[2]; Tripodi, Valeria[2]; Alvarez, Silvia[1]; Calabró, Valeria; Magnani, Natalia[1]; Evelson, Pablo[1].

[1] Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica. Ciudad de Buenos Aires, Argentina.

[2] Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Tecnología Farmacéutica.

Growing evidence support the notion that the central nervous system (CNS) is a highly relevant target of air particulate matter (PM). Oxidative stress and neuroinflammation are suggested to play a major role in the neurotoxicity after PM inhalation. In order to address such mechanisms, the aim of this work was to study the chronic effects of urban air on mice olfactory bulb (OB), focusing on oxidative stress and inflammation markers over time. Balb/C 8-week-old mice were exposed to filtered air (FA, control group) or urban air (UA) in whole-body chambers for up to 4 weeks. Regarding OB redox status, UA exposed mice showed a decreased reduced glutathione level (GSH) (p<0.05), while glutathione reductase activity, and glutathione peroxidase expression, were increased 4 w after exposure compared to FA group. Total superoxide dismutase (SOD) activity, including a differential analysis of its cytosolic and the mitochondrial isoforms, SOD1 and SOD2 respectively, were determined. SOD1 activity showed an initial decrease after 1 w of UA exposure compared to FA, followed by an increase at week 4 (p<0.05), while no changes were observed for SOD2 activity. In addition, Heme oxygenase-1 expression was augmented in UA exposed group after 4 w (p<0.05). NADPH oxidases (NOX) expression, particularly NOX4 and NOX2 isoforms, which are predominant in the CNS, were augmented in UA exposed group at all the time points evaluated compared to FA-mice (p<0.05). The loss of redox homeostasis led to oxidative damage in the OB as the 4-hydroxynonenal expression was found increased in UA-exposed mice after 1 and 4 w (p<0.05), as well as nitrotyrosine levels 4 w after UA exposure. These findings suggest that the UA inhalation alters the OB redox metabolism even after as earlier as 1 w and the imbalance remained up to 4 w. Therefore, the oxidative and inflammatory responses might be, in part, responsible of the neurotoxicity associated to environmental PM.

762. (614) **HEXACHLOROBENZENE PESTICIDE EXPOSURE UPREGULATES THE EXPRESSION OF KYNURENINE PATHWAY ENZYMES IN VITRO, AND PROMOTES IMMUNOSUPPRESSION IN THE EXPERIMENTAL HER2-POSITIVE BREAST CANCER MODEL**

Zárate LV[1], Peña Agudelo JA[2], Miret NV[1], Nicola Candia AJ[2], Zappia CD[3], Chiappini FA[1], Pontillo CA[1], Monczor F[3], Candolfi M[2], Randi AS[1].

[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina;

[2]Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Biomédicas (UBA-CONICET), Laboratorio de Inmunoterapia Antitumoral, Buenos Aires, Argentina.

[3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Investigaciones Farmacológicas (UBA-CONICET), Laboratorio de Farmacología de Receptores, Buenos Aires, Argentina.

Breast cancer is the most common cancer in women worldwide. Among the proposed risk factors, environmental pollutants are increasingly involved in metastatic tumor cells emergence and progression. Hexachlorobenzene (HCB) pesticide is a weak ligand of the Aryl Hydrocarbon Receptor (AhR), a transcription factor related to tumor development. Previously, we have reported that HCB promotes cell proliferation, migration and invasion in breast cancer cells. Accumulating evidence confirms that IDO1/TDO2 overexpression in tumor leads to tryptophan depletion in the microenvironment, activating Kynurenine pathway, and suppressing the T-cell mediated immune response. In this study, we have examined the action of HCB exposure (0.03, 0.3 and 3 mg/kg body weight, bw) in a syngeneic mouse model of HER2+ breast cancer (ER-/PR-/HER2+) for 15 and 30 days. In addition, we have evaluated the effect of HCB (0.005; 0.05; 0.5 and 5 μM) on LM3 cells for 24 h. Results show that HCB exposure in LM3 enhanced AhR protein levels at all doses (p<0.001, Western blot). The pesticide also upregulated CYP1A1 mRNA levels at 0.005; 0.05 and 5 μM; TDO2 mRNA content at all doses, and IDO1 mRNA levels only at 5 μM (p<0.01; qPCR). In addition, HCB induced an increase in tumor volume at all doses tested, and a rise in tumor weight at 3 mg/kg bw (p<0.05). HCB promoted the development of spontaneous lung macrometastasis at 0.3 mg/kg bw and micrometastasis at 0.03 and 0.3 mg/kg bw (p<0.05). Furthermore, in the spleen, HCB (0.03 mg/kg bw) generated a change in the lymphocyte profile, causing a profound decrease in CD8+ (p<0.05) at 30 days. In mammary tumors, CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs) were reduced at 15 and 30 days, and 30 days respectively (p<0.05; HCB 0.03 mg/kg bw; flow cytometry). In conclusion, HCB exposure upregulates the expression of Kynurenine pathway enzymes in LM3 cells, which could inhibit the lymphocytic response against mammary tumors, facilitating tumor progression.

763. (641) **EFFECT OF ENDOCRINE DISRUPTING POLLUTANTS HEXACHLOROBENZENE AND IMIDACLOPRID ON THE REGULATION OF LIVER CELL GROWTH**

Giselle Romero Caimi[1], Zahira Deza[1], Lucia Coli[1], Ezequiel Ridruejo[1,2] and Laura Alvarez[1]

[1] Laboratory of Biological Effects of Environmental Contaminants. Department of Human Biochemistry, School of Medicine, Universidad de Buenos Aires. Ciudad Autónoma de Buenos Aires. Argentina. [2] Chief, Hepatology Section, Department of Medicine. Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC). Ciudad Autónoma de Buenos Aires. Argentina

Some compounds of anthropogenic origin cause serious and dangerous consequences for living beings. The pesticide Hexachlorobenzene (HCB) is a hepatic tumor promoter, its commercialization is currently prohibited, however it continues to be released into the environment. The insecticide Imidacloprid (IMI) is classified as moderately toxic and unlikely to be carcinogenic by the WHO. HCB is an endocrine disruptor (ED), alters thyroid hormones (TH), deregulates cell growth and develops liver tumors. Some research suggests that IMI could be ED, but its mechanism of action is unclear. Objective: To study the effect of HCB and IMI on proliferative and apoptotic processes, as well as on key molecules in tumor development in vitro. HCB (0.05, 0.5 and 5 uM), IMI (0.02, 0.2 and 2 uM) dose curves and (2, 6, 12 and 24h) time curves were performed in Hep-G2 and Huh-7 cells. Cells were pre-treated with exogenous T3 (10-9M, 10-7M and 10-5M), or with SB-431542 (TGF-B1 inhibitor) or conditioned medium (CM) from cells treated with HCB for 24hs. Statistic, ANOVA two way, * p 0.05. Evaluated: 1-proliferation: PCNA, Westernblot

(WB) and 5´BrDU incorporation (IH). 2-apoptosis: caspase-3 and cytochrome-c, (WB). 3-Growth regulators: TGF-β1 and p-21, RT-PCR. 4-COX-2 (WB). 5-pERK and pJNK, (WB). 6-Response to HT. Results: HCB: PCNA increased (134 and 169%) for (0.5 and 5 uM) from 12 hours; caspase-3 (28, 51,73%) and Cit-c (23, 46, 71%). TGF β1 and JNK increased dose-dependently (67, 178, 314%) and (37, 58, 64%). pERK increased (47 and 61%) and COX-2 (23 and 52%) at high doses, at 24 hours. T3 at 10^{-7}M normalized PCNA increases. MC on untreated cells increased PCNA 41%. Pre-treatment with SB-431542 prevented the increase in PCNA generated by HCB or MC. IMI: PCNA decreased (25 and 46%) for high doses, caspase-3 (20, 36, 67%), Cyt-c (31,46, 83% at 24h. TGF-β1 increased (23, 37, 41 %), pJUK (18, 30, 45%) and COX-2 (31, 53%) at high doses. Conclusion: HCB as well as IMI deregulate cell growth. TGF β1, JUNK and COX-2 could be involved in its mechanism of action.

764. (642) **THE PRESENCE OF CADMIUM ENHANCED CHLORPYRIFOS TOXICITY IN ENDOTHELIAL CELLS**

María del Carmen Martinez[1], Micaela Amespil[2], Eugenia Morini[2], Taiel Schwartz[2], Adriana Cochón[1]*, Silvina Gazzaniga[1]* (*equal contribution)

1. Departamento de Química Biológica- Facultad de Ciencias Exactas y Naurales- Universidad de Buenos Aires, 2. Escuela ORT

Chlorpyrifos (CPF) and cadmium (Cd) are coexistent pollutants dispensed to the environment due to industrial and agricultural activities and reach general population due to their presence in water and food. Nonetheless, their potential impact on vascular health has been scarcely described. We have previously demonstrated the *in vitro* effects of CPF on endothelial cholinesterase and non-cholinesterase targets, and the occurrence of oxidative stress due to the pesticide. The aim of this study was to evaluate whether Cd could enhance CPF toxicity. For this purpose, endothelial cell (EC) cultures were treated for 24 h with 50 μM CPF, two $CdCl_2$ concentrations (1 and 10 μM) or the combination of both toxicants. EC cultures treated with CPF presented less confluent well-adhered cells with normal morphology than control. Cd treatment did not affect cell morphology or adherence but the joint treatment yielded cultures with slightly adherent rounded cells with viable appearance under phase-contrast microscopy. Particularly, cell viability was ≈ 93% for cells treated with 50 μM CPF and remained unaffected for Cd treatment at any concentration. The treatment with the mixture diminished cell viability to ≈ 86% for 50 μM CPF + 1 μM Cd and reached significance when treated with 50 μM CPF + 10 μM Cd (64%, $p<0.05$). The main target of CPF, acetylcholinesterase (AChE), presented an inhibition of 40%. When CPF was combined with Cd, the inhibition of the enzyme was 46% for 1 μM Cd and 65% for 10 μM. Since AChE was not significantly inhibited by Cd ($p>0.05$), the higher enzyme inhibition with both toxicants could be attributable to a greater entry of CPF into the cell. Our results showed that, the pesticide in the coexistence with Cd can produce a higher damage to the endothelium than when it is alone, which could predispose to EC dysfunction.

765. (682) **CHLORPYRIFOS INDUCES LUNG METASTASIS AND MODULATION OF CANCER STEM CELL MARKERS IN *IN VIVO* MODEL**

Marianela Lasagna,[1-2] Daniel Zappia,[3] Mariana Mardirosian,[1-2] Noelia Miret,[2-4] Andrea Randi,[4] Lara Soledad Dahir,[5] Gabriel Cao,[5-6] Mariel Nuñez,[2] Claudia Cocca.[1-2]

[1]Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", UBA-CONICET, Buenos Aires, Argentina.

[2]Laboratorio de Radioisótopos, Cátedra de Física, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

[3]Instituto de Investigaciones Farmacológicas, UBA-CONICET, Buenos Aires, Argentina.

[4]Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

[5] Departamento de Patología, Hospital General de Niños Pedro de Elizalde, Buenos Aires, Argentina.

[6]Centro de Altos Estudios en Ciencias Humanas y de la Salud, UAI-CONICET, Buenos Aires, Argentina.

Breast cancer was the most frequently diagnosed malignant tumor in Argentina in 2020. In recent decades, exposure to different environmental pollutants, many of them endocrine disruptors (Eds) such as Chlorpyrifos (CPF), have been associated with an increased risk of breast cancer development and progression. To evaluate whether CPF was able to promote metastasis *in vivo*, we used an experimental model N: NIH athymic female mice in which tumors derived from the MDA-MB-231 cell line were xenotransplanted. They were randomly divided into 3 groups: control (C), CPF 0.001 mg/kg/day (CPF 0.001) or CPF 0.1 mg/kg/day (CPF 0.1) and treated for 22 days. The tumor doubling time was significantly shorter in CPF exposed animals (8.1 days for CPF 0.001 and 6.3 days for CPF 0.1) than C (9.4 days). Tumors from animals exposed to both doses of CPF showed greater cellular pleomorphism, anisokaryosis, apoptotic bodies and hyperchromatic nuclei, and appeared to have a lower degree of differentiation. We found a larger lung metastasis area in CPF 0.001 compared to C ($p<0.05$ vs C). We analyzed the expression of epithelial-mesenchymal transition (EMT) markers in the tumors. We observed a significant increase in mRNA expression of Slug in the CPF 0.1 group ($p<0.05$ vs C), but no differences were observed in Vimentin or β-catenin levels. Finally, we determined the expression of CSC markers and we found an increase in ALDH mRNA levels in CPF 0.001 ($p<0.001$ vs C) and CPF 0.1 ($p<0.001$ vs C) and an increase of CD44 and CD24 ($p<0.01$ vs C) in the CPF 0.1 group. Our results show that CPF promotes higher areas of pulmonary metastasis and modulates the expression of EMT and CSC molecular markers, which may have an impact on the breast cancer prognosis and evolution of women exposed to the toxicant.

766. (687) **PROLIFERATIVE EFFECTS OF CHLORPYRIFOS AND CYPERMETHRIN ON NORMAL AND TUMORAL HUMAN ENDOMETRIUM CELL LINES**

Giselle Fainberg[1], Marianela Lasagna[1,2], José Luis Rangel[1], Florencia Chiappini[3]; Clara Ventura[1,4], Mariana Mardirosian[1,2], Claudia Cocca[1,2], Mariel Núñez[1]

[1]Laboratorio de Radioisótopos, FFyB, UBA

[2]Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), UBA-CONICET

[3]Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Dto. de Bioquímica Humana, Fac. Medicina, UBA

[4]Instituto de Estudios Inmunológicos y Fisiopatológicos. CONICET-UNLP

Chlorpyrifos (CPF) and cypermethrin (CPM) are two pesticides widely used in Argentina. We previously demonstrated that the pesticide CPF acts as an endocrine disruptor in rats inducing alteration of the estrous cycle and proliferation of uterine endometrial cells. The aim of this work was to evaluate whether CPF or CPM affect the proliferation and/or the viability of THESCs and ECC-1, normal and tumoral human endometrial cells, respectively. We also studied the role of estrogen receptor alpha (ER) in the pesticides action. THESCs and ECC-1 cell lines were exposed to CPF or CPM concentrations from 0.05 to 50 μM. Clonogenic assays were performed in the presence or absence of the specific ERα inhibitor (ICI 182,780). Cell viability by MTT assay and ER isoforms expression by western blot were also assessed. CPF and CPM at 0.05 μM induced proliferation in both cell lines (THESCs: $p<0.001$ CPF 0.05 μM vs C; $p<0.001$ CPM 0.05 μM vs C; ECC-1: $p<0.001$ CPF 0.05 μM vs C; $p<0.01$ CPM 0.05 μM vs C) similarly to E2 ($p<0.001$ vs C). This effect was reversed when cells were pre-incubated with ICI 1 μM. We also observed that exposure to 50 μM of CPF and CPM decreased proliferation in THESCs and ECC-1 cells (THESCs: $p<0.001$ CPF 50 μM vs C; $p<0.001$ CPM 50 μM vs C; ECC-1: $p<0.01$ CPF 50 μM vs C; $p<0.$ 001 CPM 50 μM vs C) which was accompanied in CPF-treated ECC-1 by a decrease in viability at 24 h of exposure ($p<0.001$ vs C), whereas such effect was only seen at 48h when cells were exposed to CPM ($p<0.05$ vs C). The expression of ERα but not ERβ increased in THESCs cells exposed for 24 h to 0.05 μM CPF ($p<0.05$). These results demonstrate that concentrations of these pesticides found in the environment can induce proliferation of

human uterine endometrial cells, indicating that they could act as a risk factor in the development of gynecological pathologies, such as endometriosis and endometrial cancer.

767. (691) **CYTOTOXIC EFFECTS OF ANTHRACENE AND MAGNETITE NANOPARTICLES COATED WITH OLEIC ACID ON BREAST CANCER CELLS**

Mariana Mardirosian[1,2], Marianela Lasagna[1,2], Mariel Nuñez[2], Tamara Galarza[2], Nuria Espert[3], María Carolina Parra[3], Cecilia Lascano[3], Claudia Cocca[1,2], Andrés Venturino[3]

1. Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini". Buenos Aires, Argentina. 2. Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. 3. Centro de Investigaciones en Toxicología Ambiental y Agrobiotecnología del Comahue. Neuquén, Argentina.

The demand for crude oil hydrocarbons represents a growing concern worldwide due to pollution problems from drilling, production and transportation. Thus, it is important to perform toxicity studies to assess the risk associated with these incidents, not only for the environment but also for humans. Our aim was to evaluate the effects of anthracene, a polycyclic aromatic hydrocarbon (PAH), and magnetite nanoparticles coated with oleic acid (NP), developed and synthetized by our group for the remediation of water contaminated with PAH, on MCF-7 and MDA-MB-231 breast cancer cells. We studied anthracene and NP effects on survival and apoptosis. Furthermore, we performed Prussian blue staining to evaluate NP uptake and its cellular localization. MCF-7 and MDA-MB-231 cell lines were exposed for 72 h to either anthracene (0; 3,5; 7; 15 and 28 μM) or NP (0; 12,5; 25; 50; 100 and 200 mg/L). After the incubation, we performed MTT assay to assess cell viability and the Hoechst staining method to detect nuclear fragmentation during apoptosis. No significant differences were observed in the survival of anthracene treated cells compared to control. However, exposure to 100 and 200 mg NP/L significantly decreased the survival of both cell lines ($p< 0,05$). No significant differences were observed in the number of fragmented nuclei after anthracene or NP exposure. We observed that NP were internalized and located in the cytoplasm and around the nuclei in both cell lines. Surprisingly, our results suggest that the studied concentrations of anthracene do not affect viability or apoptosis. It will be necessary to further study other anthracene effects in order to understand the mechanism of action of the toxicity of this hydrocarbon. We also envisage the need of evaluating NP containing PAH after remediation to determine potential risks of the system. The effects derived from NP alone alert for secure uses avoiding nanomaterial release to the environment during remediation processes.

768. (800) **PERINATAL EXPOSURE TO DAILY DOSE OF THE UV FILTER BENZOPHENONE 3 (BP3) DECREASES FERTILITY IN C57BL/6 MICE**

Valentina Galliani[1], Julián Abud[1,2], María Laura Zenclussen[1,2], Horacio Adolfo Rodríguez[1,2]

[1] Instituto de Salud y Ambiente del Litoral (UNL-CONICET), Santa Fe, Argentina.

[2] Cátedra de Fisiología Humana (FBCB-UNL), Santa Fe, Argentina.

Benzophenone-3 (BP-3) is an ultraviolet (UV) blocking agent widely used in the composition of sunscreens. Previously, we have observed that prenatal exposure to BP3 provoked fetal growth restriction (FGR) and altered sex ratio in the mice progeny. Now, our aim was to identify the effects on reproductive physiology of the offspring caused by perinatal exposure to BP-3. To do this, C57BL/6 pregnant mice were dermally exposed to 50 mg BP-3/kg bw.day or olive oil (vehicle) from gestation day 9 (gd9) to postnatal day 21 (PND21). We recorded the weight of the dams during gestation and lactation, and of the offspring from PND0 to PND21, as well as the date of vaginal opening, gestation duration, pups per mother, estrous cycle duration and sex ratio. We observed no differences in mother's weights, duration of gestation, number of pups per mother, onset of puberty or sex ratio. The weights of the pups exposed to BP-3 were transiently lower than those of the control. Estrous cycle was not affected by perinatal exposure to BP-3. Besides, we performed a forced-breeding protocol: at 10 weeks of age, one F1 female and one F1 male mouse from each group was randomly chosen from each litter and housed together for a period of 6 months. The cumulative number of live pups delivered, time to first litter, number of deliveries (fertility) per dam, number of neonates per litter (fecundity), and number of mice having litters were recorded. We observed a decrease of pups/mother and deliveries/mother of dams perinatally exposed to BP-3. To see if this decreased fertility could be associated to an early onset of oocytes depletion, we estimated the ovarian reserve of germ cells. We found no differences in total number of oocytes between control and BP3. We conclude that perinatal exposure to BP3 leads to a decline in the reproductive capacity of female mice in a forced-breeding protocol not linked to oocyte depletion. This decline of fertility could be proposed as a lasting effect of perinatal exposure to BP-3.

769. (809) **IMPAIRED OVARIAN RESPONSE TO EXOGENOUS GONADOTROPINS IN FEMALE MICE OFFSPRING BORN TO MOTHERS PRENATALLY EXPOSED TO BISPHENOL A (BPA), BENZOPHENONE-3 (BP3), OR THEIR COMBINATION (BPA+BP3)**

Valentina Galliani[1], Julián Abud[1,2], Florence Fischer[3], Tobias Kretschmer[3], Ana Claudia Zenclussen [3], María Laura Zenclussen[1,2], Horacio Adolfo Rodríguez[1,2]

[1] Instituto de Salud y Ambiente del Litoral (UNL-CONICET), Santa Fe, Argentina.

[2] Cátedra de Fisiología Humana (FBCB-UNL), Santa Fe, Argentina.

[3] Helmholtz-Centre for Environmental Research - UFZ, Dept. of Environmental Immunology, Leipzig, Germany

BP3 belongs to organic sunscreens used in personal care products to help minimize the damaging effects of ultraviolet (UV) radiation. BPA is used in the production of polycarbonate plastics and epoxy resins. Several studies have evidenced that both chemicals can affect reproductive parameters by endocrine disruption, but there are no studies assessing the potential toxicity of their combined exposure. In this study we analyze if ovary´s ability to respond to stimulation with gonadotropins is affected by prenatal exposure to BP3, BPA or BP3+BPA. BP3 dissolved in olive oil was dermally applied whereas oral administration was used for BPA dissolved in ethanol-water (0.001%). Chemicals were administered to C57BL/6 pregnant females mated with BALB/c males according to the following scheme: a) BP3 (50 mg/kg/day); b) Control (olive oil and ethanol-water); c) BPA(4μg/kg/day); d) BPA+BP3. Dams were exposed from gestation day 0 (gd0) to gd10 in two successive pregnancies. In both pregnancies we recorded the weight of the dams and of the litters from postnatal day 0 (PND0) to PND21, gestations duration, pups per mother and sex ratio. Besides, female offspring were injected with PMSG at PND27 and hCG was injected 48 hours later. Oocyte count was performed 14hs after hCG injection. Duration of gestation, pups per mother, sex ratio and weights of offspring were similar between treated groups and control in both pregnancies. Interestingly, we observed a significant decrease in the number of ovulated oocytes of BPA-exposed female offspring of first pregnancy and of the three groups (BPA, BP-3, BP-3/BPA) in the offspring of the second pregnancy. Besides, we can conclude that animals prenatally exposed to BP3, BPA or their combination impaired the ability of the ovary to respond to gonadotropins stimulation.

770. (895) **BIOLOGICAL EFFECT OF RESPIRABLE DUST EMITTED FR.OM UNPAVED RURAL ROADS OF ARGENTINA ON LUNG AND SKIN CELLS**

Tatiana Ulchak1, Astort Francisco1, Fenoy Ignacio1; Palavecino Imanol1; Ramirez Haberkon Nancy B.2, Mendez Mariano2, Randi Andrea3, Panebianco Juan E. 2, Goldman Alejandra1, Orona Nadia S.1.

1Instituto de Tecnologías Emergentes y Ciencias Aplicadas (ITECA), UNSAM, CONICET, LIVA_CESyMA_ECyT, Colectora Avenida General Paz 5445 (B1650WAB) San Martín, Buenos Aires, Argentina.

2 Instituto de Ciencias de la Tierra y Ambientales de La Pampa (INCITAP) - CONICET - UNLPam. Mendoza 109 (L6302EPA) Santa Rosa, La Pampa, Argentina.
3 Universidad de Buenos Aires, Facultad de Medicina, Depto. de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina.

Air particulate matter (PM) is an environmental health problem affecting the world's population. Although several studies related the exposure to urban PM with the onset or exacerbation of allergies such as asthma and atopic dermatitis, there are few studies that link rural PM with the development of these allergic disorders. Rural PM comes mainly from agricultural activity, where agrochemical compounds are frequently used. Also it is known that unpaved roads exposed to wind erosion are an important source of air PM. Therefore, the aim of this study was to evaluate the effect of respirable dust emitted from unpaved roads from intensive cultivation areas where the use of agrochemicals prevails from the central Pampas region on pulmonary (A549) and skin (HaCaT) cells. A549 or HaCaT cells were exposed to PM (1-100µg/ml) emitted from unpaved roads from inside (high glyphosate/AMPA) or outside (low Gliphosate/AMPA) of a farm field. After 24h, metabolic activity (MTT), lactate dehydrogenase activity (LDH) and IL-8 cytokine release (ELISA) was assessed. We found that A549 cells exposed to PM showed a marked increase in IL-8 production for both particles ($P<0.001$) and an increase in LDH when cells were exposed to the highest concentration of PM from inside field roads. No changes were detected for MTT. Regarding HaCaT cells, we found that exposure to PM from unpaved roads inside of the farm provoked an increase in LDH at the highest dose employed ($P<0.01$), without changes in MTT. Both PMs increased significantly the levels of IL-8 (1-10 µg/ml) but decreased significantly for the highest dose. These results indicate that particles from roads of intensive culture fields induce inflammation and cytotoxicity in both lung and skin. The difference in cell cytotoxicity between the two particles (inside-outside roads) may be a consequence of the differential agrochemical content.

771. (905) **COULD SOY PROTEIN BE A PALLIATIVE ALTERNATIVE FOR THE CEREBELLUM UNDER CADMIUM CHRONIC INTOXICATION?**

Glenda Daniela Martin Molinero [1,2], Gabriel Boldrini [1,2], María Verónica Pérez Chaca [3] ; Mario Franco Moyano,[4] Samanta Armonelli Fiedler, [5] Nidia Noemí Gómez[3], Pablo Héctor Horacio López[5], Silvina Mónica Álvarez [1,2].
[1]Lab de Nutrición y Medio Ambiente, Universidad Nacional de San Luis, [2] IMIBIO-CONICET, San Luis-Argentina and [3] Laboratorio de Morfofisiología, Universidad Nacional de San Luis. [5]Departamento de Química Biológica "Dr Ranwel Caputto"-CIQUIBIC-CONICET; Facultad de Cs. Químicas; Universidad Nacional de Córdoba, Argentina.
E-mail: glendamartin@hotmail.com

Cadmium (Cd) is an environmental contaminant. Our aim was to study its toxicity in cerebellum and the potential palliative effect of a vegetarian diet. We determined Cd concentration in total blood and cerebellar tissue by ICP-MS. Oxidative stress and apoptosis markers were analyzed by PCR and immunohistochemistry. Female Wistar rats (12 animals/group) were fed with casein (Cas) and soybean (So) as protein source for 60 days. Simultaneously, half of the animals were administered either 15 ppm of Cd in water or water as control ad libitum. Our results showed that Cd concentration in total blood was incremented in CasCd vs. CasCo($p<0.01$) and in SoCd vs. SoCo ($p<0.05$). Cd level in tissue was increased in CasCd vs. CasCo and vs. SoCd ($p<0.0001$), with no significant differences between Soy groups. Total myelin area was quantified with Sudan black staining, and the intoxicated groups showed myelin alterations depicted by reduced staining in the paramedian section in the vermis, suggesting intramyelinic edema in the white (a less pronounced reduction was observed in the SoCd group). Proteolipid Protein (PLP) expression, an intrinsic myelin protein vital for the generation of the multilayered structure of myelin showed an increase in CasCo with no changes in the Cd groups. Nrf-2 mRNA expression measured by PCR, and immunofluorescence Nrf-2 (phosphorylated at ser 40) showed a significant reduction in the exposed groups, explaining previous results of antioxidant enzymes. As an indicator of oxidative stress, nitration of tyrosine (3-NT) residues showed immunoreactivity restricted to PC and cells located mainly in the white matter, significantly increased in CasCd. SoCd did not show significant differences, consistent with iNOS expression. Cd induces oxidative and nitrosative stress, known to contribute to neurodegeneration, which is attenuated by a soy based diet. These results suggest that soy would prevent Cd from entering the cerebellum, preventing most of its toxic effect.

772. (907) **EFFECT OF RESPIRABLE DUST EMITTED FROM UNPAVED RURAL ROADS ON SKIN AND INNATE IMMUNE RESPONSE**

Camila Azzi1; Fenoy Ignacio1; Tatiana Ulchak1; Orona Nadia S.1.; Valentina Martin1, Ramirez Haberkon Nancy B.2, Mendez Mariano2, Panebianco Juan E. 2, Goldman Alejandra1, Astort Francisco1
1Instituto de Tecnologías Emergentes y Ciencias Aplicadas (ITECA), UNSAM, CONICET, LIVA_CESyMA_ECyT, Colectora Avenida General Paz 5445 (B1650WAB) San Martín, Buenos Aires, Argentina.
2 Instituto de Ciencias de la Tierra y Ambientales de La Pampa (INCITAP) - CONICET - UNLPam. Mendoza 109 (L6302EPA) Santa Rosa, La Pampa, Argentina.

Environmental factors such as air particulate matter (PM) are involved in the development of allergic disorders like atopic dermatitis. The increase of allergic diseases observed in rural areas may be a consequence of respiratory and dermal exposure to air PM containing inorganic and organic soil elements as well as agrochemicals. The main source of rural PM is wind erosion of soils used for intensive cultivation. Also unpaved rural roads are an important source of PM emission. Although several studies related the exposure to urban PM with the onset or exacerbation of allergies such as atopic dermatitis, there are few studies that link rural PM with the development of disorders. Therefore, we aim to study the effect of PM emitted from unpaved roads from intensive cultivation areas where the use of agrochemicals prevails, on innate immune response. Adult BALB/c mice were exposed to PM emitted from unpaved roads from inside (PM in) or outside (PM out) of a farm field by simultaneous intranasal instillation (1mg/kg BW) and dermal exposure (16 $\mu g/cm^2$). After 24h, ILC2 population was assessed by flow cytometry and cytokine (IL-10, IL-5, IFN-ɣ and IL-6) levels were analyzed in supernatants from splenocytes and brachial draining lymph node cells (DLN) stimulated ex vivo with PMA/Ionomycin by ELISA. Mice exposed to PM in showed increased IL-6 ($p<0.01$) levels in splenocytes and decreased IFN-ɣ and IL-10 levels in DLN ($p<0.05$ and $p<0.01$) while mice exposed to PM out showed increased IL-5 and IL-10 ($p<0.05$ and $p<0.01$) levels in splenocytes with no changes in DLN. Interestingly, Mice exposed to either PMs showed a significant increase in ILC2 population in DLN ($p<0.05$) without changes in spleen. These results suggest that exposure to air particles emitted by unpaved roads of intensive cultivation fields may disrupt skin leading to increased ILC2 and systemic cytokine alterations, possibly involved in a higher susceptibility to developing allergic diseases.

TOXICOLOGY II *Thursday, November 17, 14-15:30 hr*
Chairs: Ana María Buzaleh – Noelia Miret

773. (37) **PROGESTERONE RECEPTOR SIGNALING IS IMPAIRED IN THE PUBERTAL MAMMARY GLAND AFTER THE INTRAUTERINE EXPOSURE TO THE UV-FILTER BENZOPHENONE-3**

Schierano-Marotti Gonzalo[1], Altamirano Gabriela Anahi[1,2], Gomez Ayelen Luciana[1,2], Sofia Lorena Oddi[1] Muñoz-De-Toro Mónica[1,2], Kass Laura[1,2]
[1]Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Mammary gland development is sensitive to endocrine disruption by environmental contaminants like the UV-filter benzophenone-3 (BP3), widely utilized in personal care products. Our aim was to evaluate whether intrauterine exposure to BP3 affects the progesterone receptor (RP) signaling in the mammary gland during puberty. Pregnant C57BL/6 mice were dermally exposed to vehicle (sesame oil; Control), 0.15 (0.15-BP3) or 50mg BP3/kg/day (50-BP3) from gestation day 8.5 to 18. Serum and mammary gland samples were taken from the female offspring on postnatal day 45 ± 3 at diestrus 1. Serum levels of estradiol (E2) and progesterone (P4) were assessed. In the mammary gland, the histoarchitecture and quantification of mast cells were performed in histological sections stained with hematoxylin-eosin and alcian-blue/safranin, respectively. The mRNA expression of estrogen receptor alpha (ESR1), PR, RANKL and WNT4 (both PR-induced genes) was assessed by real-time RT-PCR. Serum levels of E2 and P4, the histoarquitecture, and the number of mast cells were similar between all experimental groups ($p > 0.05$). The expression level of PR was below the limit of quantification in 3 of 6 and 4 of 7 animals in the 0.15-BP3 and 50-BP3 groups, respectively. Similarly, in those animals, WNT4 expression was also negligible. In contrast, RANKL and ESR1 expression of BP3-exposed groups was similar between groups ($p > 0.05$). Our results showed that intrauterine BP3-exposure could impair the PR signaling pathway, and therefore, affect the normal development of the mouse mammary gland during puberty.

774. (76) **STUDY OF SURVIVAL AND GROWTH IN THE SECOND-GENERATION OF RATS EXPOSED TO GLYPHOSATE OR GLYPHOSATE-BASED HERBICIDE**

Almirón Ailín[1], Lorenz Virginia[1,2], Doná Florencia[1], Di Paolo Sebastián[1], Varayoud Jorgelina[1,2], Durando Milena[1,2], Milesi María Mercedes[1,2]

[1] Instituto de Salud y Ambiente del Litoral (ISAL), UNL – CONICET, Santa Fe, Argentina. [2] Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas (FBCB) – UNL, Santa Fe, Argentina.

Glyphosate (Gly) is the active ingredient of herbicide formulations known as glyphosate-based herbicides (GBH), the most widely used pesticides worldwide. Previously, we found that fetuses (F2) whose mothers (F1) had been exposed *in utero* and during lactation to a low dose of a GBH or Gly exhibited intrauterine growth restriction (IUGR) in a rat model. The IUGR is the second cause of perinatal mortality, and it may induce cardiovascular (CV) remodeling, leading to short/long-term health dysfunctions. Here, we investigated whether GBH- or Gly-induced IUGR could affect the normal development and physiology of the F2 generation, from birth until peripubertal period. For this, pregnant rats (F0) were exposed to Gly or a GBH through food, in a dose of 2 mg of glyphosate/kg/day, from gestational day (GD) 9 until offspring (F1) weaning (postnatal day -PND-21). On PND90, F1 females became pregnant. One group was euthanized at GD19 to assess the length and the heart and body weight of F2 fetuses. Another group of dams was allowed to deliver and lactate their offspring (F2). In F2 generation, we determined the litter size and sex ratio, the survival rate, the weight gain from PND1 to PND21, and the onset of puberty in females. F2 fetuses from GBH and Gly groups exhibited reduced body weight and length, in association with higher relative heart weight ($p<0.05$), compared to Controls (C). At birth, no differences were observed in litter size or sex ratio. Interestingly, a lower survival rate in PND21 (C: 100%; GBH: 69%; Gly: 80%; $p<0.05$) was shown in F2 pups from GBH and Gly groups. At the peripubertal period, GBH and Gly F2 males were shorter ($p<0.01$) than C males; while females showed delayed vaginal opening (C: 45±0.5 day; GBH: 52±1.8 days; Gly: 49±0.9 days). Our results suggest that the IUGR induced in the F2 generation by GBH or Gly, decreases postnatal survival and causes developmental dysfunctions, which could be related to "a possible" impairment of fetal CV remodeling.

775. (84) **EFFECT OF LOW DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON BLOOD-TESTIS BARRIER (BTB) PERMEABILITY IN JUVENILE RATS**

Daniel Soria1, Marina Dasso1, Cecilia Centola1, Cristian Sobarzo2, María Noel Galardo1, María Fernanda Riera1, Silvina Meroni1

1Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergada" (CEDIE) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Fundación Endocrinológica Infantil (FEI) – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina 2Instituto de Investigaciones Biomédicas (INBIOMED), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Medicina, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina.

A progressive decline in male reproductive function has been observed over the past 50 years. Epidemiological and experimental studies suggest that one of the main causes is exposure to xenobiotics. 2,4-dichlorophenoxyacetic acid (2,4-D) was the first synthetic herbicide to be developed commercially and has been used agronomically and in nonagricultural applications for weed control for more than 60 years. In addition to its low cost, 2,4-D has environmentally friendly properties such as short environmental persistence and low toxicity to humans and wildlife. However, the possible effects on testicular function have been barely studied.
In the testis, the BTB isolates germ cells providing an adequate microenvironment for their development. Concordantly, defects in the BTB lead to harmful effects on spermatogenesis. No studies have been conducted to assess if early life exposure to 2,4-D represents any potential risk to BTB function. The objective of this work was to analyze the effects of low doses of 2,4-D on BTB integrity in vivo. Male rats were treated with oral doses of 2,4-D (Rfd: 0.005 mg/kg/day; NOAEL: 5 mg/kg/day) from postnatal day (pnd) 20 to 33, period in which BTB is being established. Controls (C) received vehicle (corn oil). The integrity of the BTB was evaluated on pnd 34 using a biotinylated tracer. Histological and meiosis progression analyses were also performed. It was observed that both doses of 2,4-D induced an increase in the permeability of the BTB that enables the passage of the tracer to the adluminal compartment of the seminiferous tubule (C: 5.8±1; 2,4-D 000.5: 9.9±2.1*; 2,4-D 5: 13.9±3.2*; % of permeated tubules, mean±SD, $P<0.05$). No changes in body weight, gonadosomatic relationship, testicular histology or meiosis progression were observed. In summary, the results suggest that rat juvenile treatment with low doses of 2,4-D could alter the integrity of the BTB. (PICT2020-1061, PIP 2020 0100162).

776. (119) **MYCOTOXINS IN BEER AND ASSOCIATED RISK**

Motta Estela[1], Sandra Medici[2,3], María Belén Berto Cuixart[1], Chisari Andrea[1,4]

[1] Departamento de Química y Bioquímica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata

[2] Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM, CONICET-CIC), FCEyN, UNMdP, Mar del Plata

[3] Centro de alimentos y medioambiente Fares Taie Instituto de Análisis. Magallanes 3019 Mar del Plata

[4] CONICET. Buenos Aires. Argentina

e-mail: estelaleonormotta@hotmail.com

The consumption of beer is widespread in the population. Beer is made from malting barley, often accompanied by other cereals such as hops, corn, wheat and its subsequent fermentation. **Objective:** was to know the occurrence of mycotoxins in industrial and craft beers consumed in our environment. Materials and Methods: Eleven beers (1 craft and 10 industrial) were analyzed for the simultaneous determination of aflatoxins G1, G2 and B1 (AFLA), fumonisin B1 and B2 (FB), ochratoxin (OTA), Deoxynivalenol (DON) by a QuEChERS based extraction procedure coupled to UPLC-MS/MS. Results: Of the 11 beers, seven (67.6%) gave detectable DON values with ranges between 1.6 – 6.4 ug/l. and an average of 3.2 ug/l, median of 1.7 ug/l. No detectable content of fumonisins (FB1 and FB2), ochratoxin and aflatoxins (AFG1, AFG2, AFB1 and AFB2) were found. Considering the results of beer consumption survey (average: 284,4 ml/day, age range:36-50 years) carried out the last year among the

population of Mar del Plata and Buenos Aires province, the estimated daily intake (EDI) of DON was calculated, being 0,02 ug DON/kg b.w. /day. This intake contributes to 2 % of the tolerable daily intake (TDI). Conclusion: The detectable levels of DON in 67,6 % of the beers analyzed, indicate that the beer may contribute to the consumption of this mycotoxin. It's necessary to expand the analysis of beer, to carry out a risk analysis, considering the different varieties of beer in a deterministic modelling approach.

777. (137) **PERINATAL EXPOSURE TO A MIXTURE OF AGROCHEMICALS INDUCES LESIONS IN THE VENTRAL PROSTATE IN POSTPUBERTAL RATS**

Ayelen Luciana Gomez [1, 2], Débora Gisel Reato [1], Gonzalo Schierano-Marotti [1], Laura Kass [1, 2], Gabriela Anahí Altamirano [1, 2]

[1] Instituto de Salud y Ambiente del Litoral (ISAL. UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2] Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Studies reported that there could be an association between agrochemicals exposure and prostate diseases. Here, our aim was to evaluate whether perinatal exposure to a mixture of the fungicide propiconazole (PRO) and the herbicide glyphosate (GLY) alters the ventral prostate (VP) development of postpubertal rats. Pregnant rats were orally exposed to vehicle (saline solution) or a mixture of PRO and GLY (each 4 mg/kg/day), from gestation day 9 until weaning. On postnatal day 60, male offspring were euthanized, and serum and VP samples were collected. Serum level of testosterone (T) and estradiol (E2) was measured. In the VP, the presence of lesions was examined. Also, the proliferation index (Ki67), androgen receptor (AR), estrogen receptor beta (ESR2) and alpha-smooth muscle actin (α-SMA) were assessed by immunohistochemistry. In PROGLY rats, serum level of T was decreased compared with controls, whereas the E2 level was similar between groups. Histologically, both foci of epithelial hyperplasia and atrophy were found in the VP. In this sense, PROGLY rats showed a higher percentage of prostatic ducts with hyperplasia (Control: 1.31±0.42% vs PROGLY: 4.44 ± 0.55%; $p<0.05$) and a higher incidence of atrophied ducts (60% of PROGLY rats presented two or more atrophied ducts compared with 11% in Control rats, $p<0.05$). In prostatic ducts without lesions, similar proliferation index and expression of AR, ESR2 and α-SMA were found between groups. In prostatic ducts with hyperplasia, a higher proliferation index and lower ESR2 expression were found in PROGLY rats than in controls. The focal prostatic atrophy observed in PROGLY rats was characterized by lower expression of ESR2 and higher expression of α-SMA expression than normal prostatic ducts from Control rats. In summary, perinatal exposure to a mixture of PROGLY decreases serum T levels and induces prostatic lesions such as hyperplasia and focal atrophy, impairing the normal development of the VP in postpubertal rats.

778. (144) **EFFECTS OF A GLYPHOSATE-BASED HERBICIDE ON THE OVARY FOLLICULAR DYNAMICS OF PREPUBERTAL LAMBS**

Ana Laura Alegre[1], Ramiro Alarcón[1], Lovera Lourdes[1], Rivera Oscar Edgardo[2], Dioguardi Gisela Haydee[2], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1*], Paola Inés Ingaramo[1*]

*[1] Instituto de Salud y Ambiente del Litoral (ISAL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Instituto de Investigación Agropecuaria, Ambiente y Salud (IIPAAS), Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora. *These authors contributed equally to this work.*

Glyphosate-based herbicides (GBH) are considered a risk to the environment and health and can act as endocrine disruptors. The aim of this study was to investigate if the exposure of neonatal ewe lambs to the reference dose of GBH (US-EPA) alters ovarian follicular development. Crossbreed ewe lambs orally received GBH (1 mg/Kg/day of glyphosate) or saline solution (vehicle) from postnatal day (PND) 1 to PND14. From PND 41 to 43 a group of lambs exposed to vehicle or GBH, was treated daily with pFSH conforming the FSH and GBH-FSH groups. On PND45 animals were weighted, the ovaries obtained, weighted, sectioned, and stored at -80 °C or paraffin-embedded. Molecules involved in the regulation of ovarian follicular development were studied by RT-PCR or immunohistochemistry (IHC). The weight of animals was not affected, ovaries weight increased in FSH but not in FSH lambs exposed to GBH. mRNA of steroid receptors (ESR1, ESR2, and PR), bone morphogenetic protein 15 (BMP15), Follistatin (FST) and Activin A Receptor Type 2A (ACVR2) were determined. β-actin was used as housekeeping gene. A significant reduction of mRNA of ESR1 (56%), PR (75%), ACVR2 (85%), and BMP15 (88%) was found in GBH lambs. FSH induced a downregulation of FST (81%), ACVR2 (77%), BMP15 (93%) and FSHr (72%); while in lambs exposed to GBH, FSH only decrease ACVR2 (68%) and BMP15 (81%). By IHC a decreased expression of antimullerian hormone (AMH) in antral follicles in GBH (54%) and in FSH (42%) was observed. In GBH animals, a reduction of BMP4 (31%) in primordial follicles was also found. These results demonstrated that GBH exposure alters expression of molecules involved in follicular development and interferes with FSH action, affecting its receptors or molecules involved in the action of this hormone in the ovary. Further studies will define whether the described effects have consequences in the adult ovarian function.

779. (161) **THIOREDOXIN OVEREXPRESSION SHOWS CARDIOPROTECTION AGAINST THE REDOX METABOLISMS ALTERATIONS INITIATED BY AIR POLLUTION EXPOSURE**

Mariana Garcés[1], Natalia Magnani[1], Virginia Perez[2], Lourdes Cáceres, Freire Agustina[1], Yeison Triguero[1], Silvia Alvarez[1], Ricardo Gelpi[2], D'annunzio Veónica[2], Pablo Evelson P[1].

[1]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica.

[2]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Medicina.

Epidemiological studies showed correlations between daily changes in particulate matter (PM) concentration and cardiovascular diseases such as heart failure. Reactive O_2 species (ROS) production triggered by PM exposure can initiate oxidative damage mechanisms. Thioredoxin (Trx) is part of an antioxidant system that maintains redox homeostasis; nevertheless, their role after air PM is still unknown. The aim of this work was to analyze the cardioprotective effect of selective overexpression of thioredoxin 1 (Trx1) in a mice model of acute exposure to residual oil fly ash (ROFA). Wild type (Wt) and Trx1 mice were intranasally instilled with a ROFA suspension (1 mg/kg body weight). Saline solution was used as a control. Impaired contractile reserve and lusitropic reserve were observed on Wt mice exposed to ROFA ($p<0.05$; $p<0.01$), while Trx1 overexpression prevented these changes. ROFA induced a significant decrease in heart O_2 consumption on Wt mice compared to saline group ($p<0.01$) while Trx1 overexpression significantly attenuated these changes when compared to saline. In addition, increased lipids and protein oxidation was observed on Wt mice exposed to ROFA ($p<0.01$; $p<0.05$). Our findings indicate that Nrf2 increased expression after ROFA exposure could be involved in protective effects by signaling pathway activation on Trx1 mice ($p<0.05$). Moreover, a decrease in NF-kB expression was observed in this group ($p<0.05$). Regarding the antioxidant system we observe increased GSSG levels on Wt ($p<0.01$) and Trx1 mice ($p<0.05$) exposed to ROFA compared to saline. Nevertheless, only the Wt group showed alterations on GSH/GSSG ratio ($p<0.05$). The increased activities of GR ($p<0.01$), GPx ($p<0.01$), GST ($p<0.01$), G6PDH ($p<0.05$) and increased expression of HO-1 ($p<0.01$) in Trx1-ROFA possibly prevented damage to macromolecules. These results provide direct evidence linking Trx1 overexpression and altered cardiac function prevention following an acute exposure to PM.

780. (164) **EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ALTERS THE GESTATIONAL PROCESS IN WISTAR RATS IN THE LONG TERM**

Ramiro Alarcón[1], Ximena Bidner[1], Ana Laura Alegre[1], Luz

Caglieris[1], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1], Paola Inés Ingaramo[1].
Instituto de Salud y Ambiente del Litoral (ISAL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral

It has been demonstrated that neonatal exposure of rats to glyphosate-based herbicides (GBH) alters fertility and uterine decidualization. Rats exposed during early postnatal days to GBH showed, in adulthood, post-implantation failures associated with a diminished decidualized area of implantation sites (IS). The present work aimed to investigate the effects of neonatal exposure to GBH on gestational day 15 (GD15), when placenta development is considered complete. Female Wistar pups received saline solution (control, C) or an environmentally relevant dose of 0,2 mg/kg of glyphosate (GBH0,2) or 2 mg/kg of glyphosate (GBH2) by sc injection on postnatal days (PND) 1, 3, 5 and 7. When pups (n=8/group) grew up, on PND90, were mated with fertile males. Pregnant rats were sacrificed on GD15. The IS were counted, and all fetuses and placentas were extracted, measured, and weighted. Two IS of each animal were used to investigate molecules associated with placental development by immunohistochemistry (IHC) or RT-PCR. Length of fetuses and number of implantation sites in the GBH0,2 group were decreased an 8% and 6%, respectively, without changes in placental weight. The protein expression of ER-alpha in decidua basalis decreased (53%) in GBH0,2 without changes in GBH2. The mRNA expression of iNOS decreased in both groups, 80% in GBH0,2 and 86% in GBH2. The mRNA expression of TNF-alpha also was lower in both groups: 58% in GBH0,2 and 53% in GBH2. Present results demonstrate that neonatal exposure to GBH interferes with the molecular pathway involved in decidualization and placental development showing more deleterious effects at the lower dose. Decreased TNF-alpha and iNOS would interfere with the relaxation of vascular smooth muscle impairing angiogenesis in early gestation. Deregulation of the studied molecules and ER-alpha expression during embryo development might explain reproductive failures observed in neonatal GBH-exposed rats.

781. (247) **G-PROTEIN-COUPLED ESTROGEN RECEPTOR GPER MEDIATES IMIDACLOPRID-INDUCED MAMMARY EPITHELIAL CELL MIGRATION**

M. Agustina Leguizamón[1], Sol Buján[1], Carolina Pontillo[1], Florencia Chiappini[1], Marianela Lasagna[2], Claudia Cocca[2], Andrea Randi[1], Noelia V. Miret[1]
[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Paraguay 2155, 5° piso, (CP 1121) Buenos Aires, Argentina, [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos, Junín 954, primer subsuelo, (CP 1113), Buenos Aires, Argentina.

Imidacloprid (IMI) is a neonicotinoid insecticide widely used in agriculture worldwide. It is detected in fruits and vegetables, as well as in human serum and urine. Although neonicotinoids are considered to be of low toxicity to mammals compared to other insecticides, increasing studies show the potential risk to humans of IMI exposure. IMI is an endocrine disruptor that enhances aromatase activity and estradiol secretion in breast cancer cells. G protein-coupled estrogen receptor (GPER) is activated by estradiol and estrogenic compounds, promoting cell migration, metalloprotease (MMP) activity, and epithelial-mesenchymal transition processes in breast cancer. Due to these antecedents, we hypothesize that IMI exposure produces alterations in the mammary gland that favor tumorigenesis. In order to evaluate the potential impact of IMI on mammary epithelial cells, we worked with the NMuMG cell line exposed to environmentally relevant doses of IMI (0.01-10 μM) or vehicle (DMSO) for 24 hours. First, the possible cytotoxic effect of IMI was studied (MTT assay), finding no effect on NMuMG cell viability. However, when examining the levels of the proliferation marker PCNA (Western blot, WB), we found a reduction in their levels at 1 μM (40% $p<0.05$). Next, we evaluated GPER expression (WB), observing an increment after 1 μM (66% $p<0.01$) and 10 μM IMI exposure (57% $p<0.05$). Considering that GPER is involved in promoting cell motility, we analyzed the effect of IMI on cell migration (wound healing assay) and GPER involvement by preincubating with the GPER specific inhibitor, G15. Results showed that IMI (1 and 10 μM) stimulates cell migration (90% $p<0.01$) and this effect is prevented in the presence of G15 ($p<0.001$). In addition, 10 μM IMI increases MMP-2 (121% $p<0.01$) and MMP-9 activities (43% $p<0.05$) in the supernatant of cells exposed to the toxic (gel zymography). In conclusion, results support our hypothesis and demonstrate the implication of GPER in the IMI-induced cell motility.

782. (249) **ACTION OF THE NEONICOTINOID INSECTICIDE IMIDACLOPRID ON MCF-7 BREAST CANCER CELLS**

Noelia V. Miret[1], M. Agustina Leguizamón[1], Sol Buján[1], Carolina Pontillo[1], Florencia Chiappini[1], Marianela Lasagna[2], Claudia Cocca[2], Andrea Randi[1]
[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Paraguay 2155, 5° piso, (CP 1121) Buenos Aires, Argentina, [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos, Junín 954, primer subsuelo, (CP 1113), Buenos Aires, Argentina.

The incidence of breast cancer is increasing globally and exposure to endocrine disruptors (EDs) has become a potential risk factor for this disease. Different studies have linked breast cancer risk with pesticide exposure. Imidacloprid (IMI) is a neonicotinoid insecticide widely used in agriculture and veterinary medicine. It acts as an ED by increasing aromatase activity and 17β-estradiol secretion in MCF-7 breast cancer cells. Given that most breast tumors are hormone-dependent, it is suggested that IMI exposure could promote their development. Due to the limited information on the effects of IMI on breast cancer, our objective was to evaluate its possible impact on cell viability, proliferation and estrogen receptor (ER)-α and G protein-coupled ER (GPER) protein expression in breast cancer. In addition, we examined metalloproteinases (MMP) activity, as they degrade the basal membrane and thus allow tumor cell migration and invasion. MCF-7 cells were exposed to environmentally relevant concentrations of IMI (0.01-10 μM) for 24 h or vehicle (DMSO). Results showed that IMI does not alter cell viability (MTT assay), but produces an increase in cell proliferation. The clonogenic assay showed an enhancement in the number of colonies at 0.01 µM ($p<0.01$), while the expression of the proliferation marker PCNA (western blot, WB) was increased at 0.1 ($p<0.05$), 1 ($p<0.01$) and 10 µM ($p<0.001$). On the other hand, WB results showed a reduction in the levels of ER-α at 0.01 µM ($p<0.001$) and GPER at 10 µM ($p<0.05$). Finally, an increase in the activity of MMP-9 was observed at all assayed doses ($p<0.05$, gel zymography). In summary, IMI exposure boosts cell proliferation and MMP-9 activity, which could contribute to breast cancer risk.

783. (253) **ARYL HYDROCARBON RECEPTOR AND HIF-1α PATHWAYS ARE INVOLVED IN THE INCREASE IN VEGF EXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER CELLS EXPOSED TO THE ENDOCRINE DISRUPTOR CHLORPYRIFOS**

Buján Sol[1], Pontillo Carolina[1], Miret Noelia[1], Leguizamón Agustina[1], Chiappini Florencia[1], Zárate Lorena[1], Cocca Claudia[2], Randi Andrea[1].
[1] Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina; [2] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica. Cátedra de Física, Laboratorio de Radioisótopos, Buenos Aires, Argentina

Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticides in agriculture. Breast cancer is the most frequently occurring malignancy in women, and in recent years the exposure to environmental pollutants has gained importance as a risk factor. CPF promotes cell migration and invasion in breast cancer, and binds to the aryl hydrocarbon receptor (AhR), a transcription factor

involved in vascular development and tumor progression. Hypoxia Inducible Factor-1α (HIF-1α) induces vital genes in tumor survival, such as Vascular Endothelial Growth Factor (VEGF), Nitric Oxide Synthase-2 (NOS-2) and Cyclooxygenase-2 (COX-2). COX-2 and NOS-2 are pro-inflammatory enzymes that contribute to tumor progression. Our aim was to analyze the CPF action on proangiogenic factors expression like the HIF-1α, VEGF, NOS-2 and COX-2 in triple negative breast cancer cells MDA-MB-231. In addition, we examined whether VEGF secreted by CPF-treated MDA-MB-231 could activate the endothelial cells. Tumor cells were exposed in a dose- (0.05; 0.5; 5 and 50 μM) and time-(6 and 24 h) curves to CPF. Our results showed that CPF (0.05 μM) enhances HIF-1α protein content at 6 and 24 h ($p<0.01$) (Western blot, WB). Also, CPF exposure (0.05; 0.5 and 5 μM) increases VEGF secretion at 6 h ($p<0.05$) (WB). Besides, CPF (0.05 μM) raises VEGF expression through AhR and HIF-1α pathways ($p<0.05$). In addition, the NOS-2 and COX-2 expression, at 6 and 24 h respectively, were enhanced with CPF (0.05 μM) ($p<0.001$) (WB). Moreover, conditioned medium (CM) from tumor cells exposed to CPF (0.05 μM) at 6 h, increases endothelial cell survival (MTT) ($p<0.05$). In conclusion, the pesticide CPF induces an enhancement in proangiogenic factors levels NOS-2 and COX-2, while increasing VEGF expression via AhR and HIF-1α pathways. Besides, CPF activates endothelial cells through CM from tumor cells, contributing with angiogenesis processes.

784. (258) **INCREASES IN CELL MIGRATION RATE AND AROMATASE EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS INDUCED BY A NEONICOTINOID INSECTICIDE IMIDACLOPRID**

Martina Leturia, Leandro Ceballos, Noelia Miret, Carolina Pontillo, Andrea Randi, Florencia Chiappini.
Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina

Under the modern lifestyle, humans are exposed to chemicals such as pesticide residuals. These compounds can mimic or influence the action of endogenous hormones acting as endocrine disrupting chemicals (EDCs). Imidacloprid (IMI) is a neonicotinoid insecticide; the most widely used agricultural insecticides. Several studies report that EDCs, such as IMI, affect aromatase expression and activity, and interfere with estrogen signaling. Endometriosis is a chronic disease hormone-dependent which is defined by the presence of endometrial tissue outside the uterus. The EDCs may be involved in the development and progression of disease. Aromatase is the key enzyme in the estrogen biosynthesis and it is essential for establishment and growth of endometriosis lesions. Moreover, the development of endometriosis lesions depends on factors that facilitate migration, adhesion, proliferation and invasion of endometrial cells. The aim of this work was investigate the IMI effect in human endometrial stromal cells (T-HESC) on: cell migration (wound healing assay) and proliferation (PCNA by WB), aromatase expression (WB), and Matrix metalloproteinases 2 and 9 (MMP-2,-9) activities (zymography). T-HESC cells were exposed to different doses of IMI (0.01, 0.1, 1 and 10 µM) for 7 and 24 h. The results showed that IMI increase cell migration ratio (15 and 30% IMI 1 and 10 µM) in a dose response manner. Moreover, we observed that IMI induce Aromatase and PCNA expression (74 and 67%; 30 and 38%; IMI 1 and 10 µM).We also examined the activity of MMP-2 and 9, which are involve in remodeling the extracellular matrix and participate in migration and invasion processes. IMI increased MMP-9 (17, 26 and 23% IMI 0.1, 1 and 10 μM) and MMP-2 activities (41 and 28% IMI 0.01, 1 μM). In conclusion, our results show that IMI exposure could contribute to endometriosis development, affecting migration and invasion parameters, proliferation and disrupting hormonal homeostasis in human endometrial cells.

785. (262) **URINARY GLYPHOSATE CONCENTRATION AND BREAST CANCER RISK: A CASE-CONTROL STUDY IN SANTA FE, ARGENTINA**

Florencia Doná[1], Virginia Lorenz[1, 2], Tamara Ricardo[3, 5], María Rosa Repetti[4], Luisina Demonte[4,5], María Mercedes Milesi[1, 2], Jorgelina Varayoud [1, 2]
[1]Instituto de Salud y Ambiente del Litoral (ISAL), CONICET-UNL, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, [2]Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, [3]Departamento de Cs. Naturales, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina, [4]Programa de Investigación y Análisis de Residuos y Contaminantes Químicos (PRINARC), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina, [5]CONICET, Santa Fe, Argentina.

Pesticide exposure has been associated with higher breast cancer (BC) risk. Glyphosate (GLY) is the most widely used pesticide worldwide and has been classified as a 2A "probable human carcinogen" by the International Agency for Research on Cancer. Since human exposures to GLY are rising rapidly and may also increase BC risk, we determined the urinary concentration of GLY and its degradation product, aminomethylphosphonic acid (AMPA) and we designed a case-control study to determine if residence or other lifestyle/medical factors could be associated with the BC risk. We analyzed 130 women (35 BC cases and 95 controls) who attended the gynecology service of J.M. Cullen Hospital in Santa Fe. Demographic data, lifestyle factors and residence were obtained by questionnaire. Medical outcomes and reproductive history were abstracted from medical records. We collected urine from 74 women (17 BC cases and 57 controls). Urine concentrations of GLY and AMPA were assessed by Ultra high performance liquid chromatography–Mass spectrometry. GLY was detected in 92.8% of urine samples (mean 0.48 μg/L, range 0.10-3.50 μg/L), and no differences were observed between cases and controls groups. AMPA was not detected in any of the analyzed samples. We found an association between increased risk of BC with older women (OR: 1.12, 95% CI: 1.06-1.18), older age at first delivery (OR: 1.18, 95% CI: 1.04-1.34) and residence near agricultural fields (OR: 9.36, 95% CI: 2.8-30.4). To our knowledge, this is the first study examining the urinary concentration of GLY in Argentina, indicating the ubiquitous presence of the pesticide in human samples. The novelty of the detection of GLY in urine, provides baseline information that is relevant for designing future decisions. In addition, our preliminary findings suggest an increased BC risk associated with the place of residence; however, these results require confirmation in a larger population to increase the power of the study.

786. (351) **NEUTROPHILS, IRON AND MICROCYSTIN: STUDY OF ENVIRONMENTAL TOXIC AGENTS AND OXIDATIVE STRESS IN AN IN-VITRO MODEL**

Iris Chiesa[1], Fabiana Lairion[1,2], Claudio Carbia[3], Cristian Saporito Magriñá[1,2], Marisa G. Repetto[1,2]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas, Química General e Inorgánica, Buenos Aires, Argentina, [2]Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL, UBA-CONICET), Buenos Aires, Argentina. [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Bioquímica Clínica II, Hematología, Instituto de Fisiopatología y Bioquímica clínica (INFIBIOC), Buenos Aires, Argentina,

Human polymorphonuclear cells (PMN) are involved in the resolution of infectious and inflammatory processes, in the regulation of spontaneous tumorogenesis and in the development of cancer. Excess inorganic iron (Fe^{2+}) and the presence of MC-LR/hepatotoxin (MC-LR) in drinking and recreational water are considered detrimental to human health and predispose to inflammatory and neoplastic processes associated with PMN-induced oxidative stress (OS). Therefore, our aim is to characterize the damage of both antigens by detecting OS and cellular respiratory burst in PMNs exposed to different concentrations of Fe^{2+} and MC-LR. Methodology: Enriched PMN fractions were obtained from venous blood of healthy volunteers to assess OS and cellular respiratory burst (RB). Each sample was separated into aliquots and incubated, one part with Fe^{2+} and

the other with MC-LR, at 37 °C for 15 minutes. OS was assessed by measuring spontaneous chemiluminescence (CL) of the cells with a scintillation photon counter, and oxygen consumption (OC) with a Clark-type oxygen electrode at 37 °C, in basal condition or after activation (exposure to agonist for 15 minutes). In order to determine the effects of Fe^{2+} and MC-LR on PMN function, increasing concentrations were evaluated, Fe^{2+}: 0, 6, 30, 300 μg/L and MC-LR: 0, 1, 25 μg/L. Results: The optimal cell concentration for assessing OS in PMNs was 8 x10^4 PMN/mL. OC decreased 40% with 6 and 30 μg Fe^{2+}/L ($p<0.01$) and 60% with 300 μg Fe^{2+}/L ($p<0.001$), but CL increased 100% with 30 μg Fe^{2+}/L ($p<0.01$). MC-LR increased OC 34% (1 μg MC-LR, $p<0.05$) and 60% (25 μg MC-LR, $p<0.01$) and CL 60% (1μg MC-LR/L, $p<0.001$) compared to control. Conclusion: Fe^{2+} does not activate RB mediated by NADPH oxidase in the range of concentrations allowed in water, however, it generates OS with the higher concentration allowed. MC-LR generates RB in the allowed range of concentration, and OS at the lower concentration, suggesting that OS is involved in the toxic mechanisms.

787. (392) **CANCER AND MATERNITY: NATURAL COMPOUND TO PRESERVE FEMALE FERTILITY AGAINST CHEMOTHERAPY**

Yamila Herrero 1, Candela Velázquez 1, Pomponio Valeria 1, May María 2, Dalhia Abramovich 1, Leopoldina Scotti 1,3, and Fernanda Parborell 1.

1 Lab. Estudios de la Fisiopatología del Ovario, Instituto de Biología y Medicina Experimental (IByME– CONICET), Buenos Aires, Argentina, 2 Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, 3 Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – CONICET - UNNOBA – UNSAdA, San Antonio de Areco, Argentina.

Chemotherapeutic drugs such as Doxorubicin (Doxo) cause severe damage to the ovary called premature ovarian failure (POF). Resveratrol (Res) is agent that has antioxidant, antiproliferative and antiapoptotic properties. The objective was to evaluate the effect of Res on ovarian function in a Doxo-induced POF model. Eight-week-old female F1 mice (Balb C x C57) were divided into four groups control, Doxo, Doxo+Res 7 mg/kg and Doxo+Res 15 mg/kg. Animals received seven IP injections of Res or physiological solution (SF). The animals were sacrificed 15 days after the administration of Doxo (10mg/kg) IP. Ovaries were isolated for histology, IHC and western blot. The uterine horn was isolated for histopathological analysis. Statistical analysis was performed using ANOVA followed by Tukey's test. Doxo decreased the % of antral follicles compared to the control while Res (both doses) increased it compared to Doxo ($p<0.05$). Doxo increased the % of atretic follicles while Res reversed it ($p<0.05$). Doxo incremented AMH expression, while Res increased it similar to Doxo ($p<0.05$). Doxo reduced the ovarian reserve and Res restored it ($p<0.01$). Doxo increased the apoptotic index and Res diminished it ($p<0.05$), without changes in BAX/BCL-2 expression between groups. Doxo increased genomic damage on follicles (γH2AX) and Res reversed it ($p<0.05$). Doxo decreased the proliferation index (PCNA) in follicles and Res increased it ($p <0.05$). Doxo decreased the peri-endothelial area (α-actin) while Res increased it ($p<0.05$). Doxo decreased VEGF expression while Res reversed this parameter ($p<0.05$). No diferences were observed in ANGPT1/ANGPT2 expression between groups. Doxo reduced SOD expression while Res increased it ($p<0.05$). 3-βHSD, STAR levels unchanged between groups. In addition, Doxo affected the tissue architecture while Res partially restores it. Conclusion: Res shows potential as an ovarian-protective agent, which may be able to preserve fertility in female cancer patients.

TUMOR IMMUNOLOGY

Friday, November 18, 14-15:30 hr

Chairs: Mercedes Fuertes - Romina Gamberale - Carolina Domaica - Daniela Papademetrio - Alejandro Cagnoni - Ana Colado

788. (55) **CLUSTERIN EXPRESSION IN GLIOMA IS ASSOCIATED WITH MACROPHAGE INFILTRATION AND WORSE PROGNOSIS**

Melina Sager[1], Guadalupe García[2], Alvaro López Malizia[1], , Antonela Merlotti[3], Agustina Cazala[2], Silvina Figurelli[2], Javier GARDELLA[4], Martín GUEVARA[4], Juan Sabatté[1]

[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, UBA-CONICET, [2]División Anatomía Patológica del Hospital General de Agudos Dr. Juan A. Fernández, [3]Institute Curie INSERM U932, Immunity and Cancer, [4]División Neurocirugía del Hospital General de Agudos Dr. Juan A. Fernández.

Introduction: Clusterin (CLU) production is increased in different tumors. Furthermore, the increased production of CLU is usually associated with a worse prognosis. However, the nature of this association has not been clearly defined. The expression of clusterin in gliomas has not been studied. We analyzed the expression pattern of CLU in gliomas and its relation with intra-tumoral macrophages. Results and Methods: We studied CLU expression using 98 glioma tumor samples by immunostaining on paraffin sections. Positivity was evaluated from 0 to 3, where 0 represents no staining, 1 focal and weak staining, 2 intermediate staining, and 3 scattered and strong staining. We detected clusterin expression in 91 of the 98 samples analyzed by immunohistochemistry (92.8%), with a positivity percentage of 97.5% for high-grade gliomas and 72.2% for low-grade gliomas. In the positive samples, a diffuse cytoplasmic expression pattern was demonstrated (64.1%), with paranuclear enhancement (27.2%) or mixed patterns (8.7%). High-grade gliomas showed a higher degree of positivity, according to the evaluation on the proposed scale ($p<0.001$). Using the TCGA (The Cancer Genome Atlas) database for low-grade gliomas, the 50% group with the highest expression of clusterin presented a lower Disease-Free Survival and a lower Overall Survival (Kaplan-Meier method p=0.00032 and p= 0.00074 respectively). We investigated the correlation of clusterin expression with tumor immune infiltration using the Tumor Immune Estimation Resource (TIMER) Database. Interestingly, we found a strong association between CLU expression and macrophage infiltration in low grade gliomas (Rho=0,421, p=5.8exp-22). A preliminary confirmation by immunostaining on paraffin sections using anti-CD68 antibodies was performed. Conclusion: clusterin expression in glioma is associated with macrophage infiltration and worse prognosis.

789. (69) **SOLUBLE TNF**A **BLOCKADE SENSITIZES HER2-POSITIVE BREAST CANCER TO TRASTUZUMAB AND PROMOTES MACROPHAGE-NK CELL COLLABORATION TO SUBVERT IMMUNOSUPPRESSION**

Sofia Bruni[1], Mara De Martino, Florencia L. Mauro[1], Mar**í**a Florencia Mercogliano[1], Rosalia Cordo-Russo[1], Roxana Schillaci[1]

[1]Instituto de Biologia y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina.

[2]Department of Radiation Oncology, Weill Cornell Medical College, New York, New York, USA.

Trastuzumab (Tz) resistance is an important clinical issue. We proved that soluble TNFα (sTNFα) upregulates mucin 4 (MUC4) expression, which shields Tz epitope on HER2, hindering its therapeutic effect. Since Tz efficacy relies on the immune response, we addressed the role of MUC4 on modulating the tumor immune infiltrate to foster immune evasion in sTNFα-induced Tz-resistant HER2+ breast cancer (HER2+ BC). Female nude mice bearing s.c. *de novo* Tz-resistant JIMT-1 and KPL-4 tumors with a doxycycline (dox) inducible MUC4 shRNA (~100 mm^3), were treated i.p with IgG, Tz (5mg/kg each), a dominant negative (DN) sTNFα inhibitor (10 mg/kg) or Tz+DN. At day 21, tumor-infiltrating immune cells were studied by immunofluorescence and flow cytometry. For macrophage and NK cell depletion, clodronate or anti-asialo GM1 Ab was used, respectively. Antibody-dependent cellular phagocytosis was studied using JIMT-1 WT cells pre-cultured for 48h with DN (10 µg/ml) or vehicle and then co-cultured with human macrophages for

1.5h. In dox+ tumors, MUC4 silencing reinstated Tz antitumor effect (80% or 85% tumor growth inhibition, JIMT-1-shMUC4 or KPL-4-shMUC4, respectively; p<0.0001). In dox- tumors, Tz+DN inhibited tumor growth, induced M1-like macrophage polarization (p<0.01) and NK cell degranulation (p<0.01). In MUC4-silenced tumors, Tz treatment alone mimics these phenomena. Depletion experiments revealed a macrophage-NK cell cross talk, necessary for Tz+DN antitumor effect. In MUC4-silenced tumors, Tz antitumor effect was lost upon macrophage depletion, but it was preserved when NK cells were absent. JIMT-1 cells pre-treated with DN were more susceptible to Tz-dependent cellular phagocytosis (p<0.05). In all, sTNFα blockade, together with Tz, triggers an effective antitumor immune response that relies on M1-macrophage-NK cell collaboration. Our findings provide rationale to combine sTNFα blockade with Tz or Tz drug-conjugates for MUC4+HER2+ BC patients to overcome Tz resistance.

790. (93) **CPG-ODN FORMULATED WITH COA-ASC16 REDUCES THE DEVELOPMENT OF LUNG METASTASIS: CONDITIONING OF THE PRE-METASTATIC NICHE**

Lucía Boffelli, Constanza Marin, Jeremías Dutto, Federico Ruiz Moreno, Belkys Maletto, Mariana Maccioni.
Centro de Investigaciones en Bioquímica Clínica e Inmunología, CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

CpG-ODN is being widely tested as a prophylactic approach against metastasis. Here we formulated CpG-ODN with a liquid crystal nanostructure of 6-Oascorbyl palmitate (COA-ASC16) to overcome CpG known limitations (short half-life, unfavorable biodistribution). COA-ASC16 has been reported to be a potent adjuvant favoring the CD8+ CTL response in other scenarios. To generate lung metastases, B16.F10 cells (5E4 cells) were injected i.v into C57BL/6 mice. The day before and the day after tumor cell inoculation, mice were treated s.c with CpG-ODN or CpG-ODN formulated with Coa-ASC16 (CpG-ODN/COA). We found that mice treated with CpG-ODN/COA exhibited reduced incidence of metastasis, lower number of metastatic nodules (p<0,05) and better survival compared to mice treated with CpG-ODN. To investigate if these treatments conditioned the lung as a metastatic niche, mice were immunized twice, every other day, with CpG-ODN or CpG-ODN/COA. 24h later, the lung immune cells were analyzed by flow cytometry. No significant differences were observed in the number of CD45+, CD3+ (neither CD4+ nor CD8+ T cells) and CD19+ cells present in the lungs of both experimental groups. The frequency of NK1.1+ cells was increased in mice treated with CpG-ODN/COA when compared with mice treated with CpG-ODN (p<0,05), particularly NK1.1+CD11B+ cells (p<0,05). Among this population, we found an increase in the frequency of NK1.1+CD11B+LY6C+ cells, which are considered to be a reservoir of potential NK cells upon reactivation, in mice treated with CpG-ODN/COA when compared with mice treated with CpG-ODN (p<0,001). Besides, mice treated with CpG-ODN/COA showed a reduced frequency of monocytes (Ly6C+CD11B+ cells) when compared with CpG-ODN treated mice (p<0,05). Thus, CPG-ODN/COA reduces the development of lung metastasis, reshaping the immune compartment present in the lung. Further studies should be done to decipher the immune mechanisms underlying the lung conditioning of CpG-ODN and CpG-ODN/COA.

791. (106) **DEFECTIVE NK CELL ACTIVATION, MACROPHAGE POLARIZATION AND TUMOR GROWTH CONTROL IN A SENESCENT ENVIRONMENT**

María Natalia Rubinsztain, María Victoria Regge, Mariana Gantov, María Cecilia Santilli, Adrián David Friedrich, Aldana Trotta, Jessica Mariel Sierra, Florencia Secchiari, Belén Candela Lozada Montanari, Julieta Erramouspe, Mercedes Beatriz Fuertes, Carolina Inés Domaica and Norberto Walter Zwirner,
Instituto de Biología y Medicina Experimental (IBYME-CONICET), Laboratorio de Fisiopatología de la Inmunidad Innata.

Ageing is associated with the accumulation of senescent cells, development of many types of cancer, and defective immune responses against infection, vaccines and neoplastic cells. However, the effect of such accumulation on the immune system remains ill-defined. Thus, this work aimed to analyze the effects of senescent non-immune cells (fibroblasts) on NK cell IFN-γ production, macrophage polarization and tumor growth. To generate senescent fibroblasts (SenFb), the IZA non-tumorigenic mouse fibroblast cell line was treated with 1μM of etoposide for 48 h. After removal of etoposide and culture for 24 h, SenFb and control fibroblasts (ConFb) were cocultured with spleens of normal syngeneic (BALB/c) mice in the presence of IL-12, IL-15 and IL-18 for 24 h or with bone marrow-derived macrophages (obtained by culture of bone marrow cells with M-CSF for 5 days) in the absence or in the presence of M1-, M2- and tumor-associated macrophage (TAM)-like polarizing conditions for 48 h (LPS and IFN-γ for M1 polarization, IL-4 and IL-13 for M2 polarization, and conditioned media from the CT26 syngeneic tumor cell line) for 48 h. SenFb led to a significant decrease in the frequency of IFN-γ-producing NK cells, as assessed by flow cytometry (FC; SenFb: media 44.3±4.4, ConFb: 66.7±2.4, n= 15, p<0.001). Also, SenFb induced an increase in the expression of the M2-associated marker CD206 in the presence of M1 polarizing conditions (CD206 MFI: SenFb: 204.3±13.3, ConFb: 52.0± 4.5, n=3, p<0.05). A similar trend was observed in M2 and TAM-like polarizing conditions. In line with these findings, the colon carcinoma cell line CT26 exhibited an accelerated *in vivo* growth when they were coinjected with SenFb compared to ConFb. Our results indicate that a senescent environment negatively affects NK cell IFN-γ production, and macrophage polarization, resulting in faster tumor growth, providing a possible explanation for the increased frequency of malignancies observed in the elderly.

792. (193) **AN Fc-ENGINEERED IgG MONOCLONAL ANTIBODY DISPLAYS INCREASED BINDING TO THE 158V AND 158F VARIANTS OF THE CD16a GENE (*FCGR3A*) ON NK CELLS**

Belén Candela Lozada Montanari, Adrián David Friedrich, María Victoria Regge, María Natalia Rubinsztain, María Cecilia Santilli, Jessica Mariel Sierra, Florencia Secchiari, Mariana Gantov, Aldana Trotta, Julieta Erramouspe, Mercedes Beatriz Fuertes, Carolina Inés Domaica and Norberto Walter Zwirner,
Instituto de Biología y Medicina Experimental (IBYME-CONICET), Laboratorio de Fisiopatología de la Inmunidad Innata.

NK cells play a crucial role during the treatment of cancer patients with monoclonal antibodies (mAb) against tumor cell surface molecules because they express CD16a, the type IIIA receptor for the Fc portion of IgG, which triggers antibody-dependent cellular cytotoxicity (ADCC). The CD16a gene (*FCGR3A*) exhibits a dimorphism in its extracellular domain (position 158 of the protein) due to a single nucleotide polymorphism (SNP), which generates the 158V and 158F variants. Variant 158V binds IgG with higher affinity than variant 158F. Thus, the genotype of an individual (V/V, V/F or F/F) may determine the ability of its NK cells to mediate ADCC in response to therapeutic mAb. Currently, it is possible to engineer mAb to promote increased binding to CD16a and enhanced ADCC. One possibility is to incorporate two mutations (S239D and I332E) in the heavy chain (H). Accordingly, our aim was to investigate the impact of the *FCGR3A* SNP on NK cell binding of a humanized mAb and its engineered S239D/I332E variant (DE variant) to establish whether Fc-enhanced engineered mAb can override the low binding ability of CD16a from individuals with the 158F allele. Healthy volunteers were genotyped for the *FCGR3A* SNP by PCR, and their NK cells were used for mAb binding assays. NK cells with V/V and V/F genotypes showed higher binding of the DE variant mAb than of the parental mAb (p< 0,0001 at 50 μg/ml). We did not detect F/F individuals so far. Also, the DE variant mAb exhibited significantly higher binding to NK cells with V/V genotype than to NK cells with V/F genotype in a dose-dependent manner (p< 0,0001 at 50 μg/ml), while there was a slight increased binding of the parental mAb to NK cells with V/V compared to NK cells with V/F genotypes (p< 0,001 at 50 μg/ml). Our results indicate that the DE variant mAb achieved adequate binding even to CD16a on NK cells from V/F individuals, which sustains the development of Fc-engineered mAb to trigger

ADCC for immunotherapy.

793. (243) **SMALL EXTRACELLULAR VESICLES FROM PANCREATIC DUCTAL ADENOCARCINOMA CELLS MODULATE NATURAL KILLER CELLS ACTIVITY**

Daniela L. Papademetrio; Maria Noé Garcia; Santiago Behr, Mariel Benítez, Daniel Grasso; Élida Álvarez.
Instituto de Estudios de Inmunidad Humoral (UBA-CONICET), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Pancreatic ductal adenocarcinoma (PDAC) induces immunotolerance where tumor-derived extracellular vesicles (EVs) play a crucial role bearing signaling mediators from tumor to immune cells. Despite its poor efficacy, Gemcitabine (Gem) is a first line agent for PDAC treatment. Type-I IFNs had been shown to possess antitumoral properties with a model-dependent behavior. We investigated the role of Gem, IFNα and IFNβ in tumor derived small EVs composition and their impact on NK cells activity.
Small EVs were collected from supernatants of MIAPaCa-2 and PANC-1 cells upon 2h of Gem or IFNs treatments. NK cells from Buffy-Coats of healthy donors were purified by magnetic MAbs negative selection. NK cytotoxicity was evaluated by CFSE/PI stain on K562 cells. We incubated NK cells with EVs for 2h. CFSE dyed-K562 cells were added in ratio 5:1 (NK:K562) for 4h. IFNγ release was measured in supernatants from cytotoxicity assays by ELISA. CD107a was analyzed in NK cells by flow cytometry.
EVs-Gem slightly increased NK cytotoxicity (19% and 21%, for MIAPaCa-2 and PANC-1, respectively ($p<0.05$)) without modulation of IFNγ release. EVs-IFNα2b decreased NK cytotoxicity (49% and 38%, for MIAPaCa-2 and PANC-1, respectively $p<0.01$). The EVs obtained by EVs-IFNα2b decreased IFNγ release only for MIAPaCa-2 cells (37% $p<0.01$). Contrasting, EVs-IFNβ increased NK cytotoxicity (36% $p<0.01$) without modulation of IFNγ release.
Our results show an opposite behavior, regarding NK activity response, of IFNα2b and Gem treatments to IFNβ. In consequence, data suggest IFNβ as a potential candidate to be tested in combined therapies with Gem to deal with PDAC.

794. (318) **RELATIONSHIP BETWEEN THE EXPOSURE OF MALARIA IN AN ENDEMIC ZONE AND ITS ASSOCIATION WITH CANCER INCIDENCE**

Mondragon, Leonel(1,2); Martinez Marignac, VL(2).
1.Universidad Adventista del Plata, Faculty of Medicine, Libertador San Martin, Entre Rios, Argentina. 2. CICYTTP, IBIOGEM, (CONICET, Prov ER & UADER). Diamante, Entre Rios, Argentina.

An inverse association between malaria infection and risk of cancer has been established worldwide, however nutritional, economical incomes, social parameters bias and cancer risk increased by year related to life style and other factors have not been studied nor have it formally estimated of their magnitude on risk for cancer in an endemic malaria region such as those in South America. The present analysis examines the association by malaria and cancer and neoplasm incidence, restricting analyses to studies performed by the Institute for Health Metrics and Evaluation (IHME) where data for South America for endemic countries (Suriname, Guyana, Ecuador, Peru, Venezuela, Colombia, Brazil, Bolivia) and non-malaria endemic countries were available (Argentina, Paraguay) while Uruguay and Chile were not included as per more than 10 years they have no reported cases of Malaria. The authors reviewed studies and analyzed online available databases through 1990 to 2019, examining both sexes, people of 25 year of age and olders, and analyzed the association between malaria, year of incidence, nutrition and risk of having different types of cancers and neoplasm, total cancer and neoplasm, respectively, as defined by Global Health Data Exchange (GHDx) and IHME. A total of 10 Latin and South American countries were included. An inverse association between malaria total cancer and a strong negative correlation with neoplasm was found in both sexes, and no association was found between male and women. This study confirms previous studies reporting an inverse association between malaria and cancer in both men and women, and provides quantitative estimates of the inverse association in South American countries which showed endemic cases of malaria. And suggest that two malaria non-endemic countries (Chile and Uruguay) have an elevated risk level of cancer incidence. We discuss possible mechanism of malaria infection as a counter to cancer or neoplasm development.

795. (368) **VEMURAFENIB-RESISTANT MELANOMA CELLS INDUCE AN IMMUNOSUPPRESSIVE RESPONSE IN MACROPHAGES**

Johinna Mons, Celia N Pérez, Cristian R Falcón, Federico Cuello Orlandi, Mercedes L Sangiacomo Ruiz, Sergio E Alvarez.
Universidad Nacional de San Luis and Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL) CONICET

Melanoma is the most aggressive form of skin cancer. Although targeted therapy with BRAF and MEK inhibitors (BRAFi/MEKi) has shown an impressive success, the occurrence of resistance and relapse reduces its efficacy. Many reports have established that the tumor microenvironment and infiltration of different immune cells have a crucial role in cancer progression and resistance. In that sense, expression of the immune checkpoint (IC) Tim-3 and its ligand Galectin 9 (Gal 9) are linked to immune exhaustion. The aim of this work was to elucidate how Vemurafenib (Vem, BRAFi) resistance affects Gal 9 expression in melanoma cells, thus modulating Tim-3 expression in macrophages. The expression of Gal 9 and Tim-3 was evaluated in sensitive and Vem-resistant A375 melanoma cells (A375S and A375R respectively) by RT-qPCR. Resistance was associated with increased expression of Gal 9 and other IC ligands such as PD-L1 and PD-L2. On the other hand, Tim-3 expression was evaluated by treating PMA-differentiated THP-1 cells with conditioned media (CM) derived from A375S and A375R cells. Remarkably, while differentiated macrophages exposed to CM from A375R cells showed an M2-like profile, CM from both cell types induced the down-regulation of Tim-3. To further study the regulation of these proteins, we performed a bioinformatic analysis using the open access web server GEPIA2. *In sílico* data show a higher expression of both Tim-3 and Gal 9 in melanoma compared to healthy tissue. Altogether, our results indicate that resistance to BRAFi increases Gal 9 expression in melanoma and induces an immunosuppressive response by macrophages. Furthermore, we suggest that secretion of Gal 9 by melanoma cells may modulate the expression of Tim-3 in macrophages.

796. (378) **INFECTION WITH *T. cruzi* AND THE GENERATED IMMUNOLOGICAL MEMORY INHIBIT TUMOR GROWTH IN C57BL/6 MICE CHALLENGED WITH THE B16-F10 CELL LINE**

Cintia D. Kaufman[1], Cecilia Farré[1,2], Lucía Biscari[1], Victoria Huhn, Ana Rosa Pérez[1], Andrés Alloatti[1]
[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER) – CONICET/UNR, [2]Centro de Investigación y Producción de Reactivos Biológicos (CIPReB)

Spontaneous regression of tumor is often associated with infections. To evaluate the antitumor effect of the infection with *Trypanosoma cruzi* and the associated immunological memory, C57BL/6 female mice were randomly divided into four groups: (G1) MEMORY received an intraperitoneal (IP) dose of 10,000 trypomastigotes (day 0) and mice were treated with Benznidazole (BZ). To boost the response, this scheme was repeated two months later; (G2) INFECTION received an IP injection of 500 trypomastigotes (day 110); (G3) BENZNIDAZOLE was not infected but was treated with BZ as G1; (G4) TUMOR was neither infected nor received BZ. All groups were challenged subcutaneously with B16-F10 tumor cells (day 125). Parasitemia and tumor growth were monitored. No differences were observed in the tumor volume and survival between G3 and G4, and thus G3 was excluded from the analyses. From day 7 post-inoculation (PI), differences in tumor volume were found among groups (G2 vs G4 - days 7 and 11-18 PI. – $p<0.01$ and $p<0.001$, respectively; G1 vs G4 - days 7 and 11-18 PI – $p<0.01$ and $p<0.05$, respectively; G2 vs G1 – day 18 PI $p<0.05$ - Kruskal-Wallis test). This trans-

lated into a significant difference in survival between G2 and G4 ($p<0.01$ - Log-rank test). The animals were sacrificed when tumors reached 1500 mm^3 and spleen, lymph nodes and tumors were harvested and analyzed by flow cytometry. Significant differences were found between G2 and G4 in spleen and lymph nodes, when the percentage of specific effector memory, central memory and effector $CD8^+$ T lymphocytes against *T. cruzi* were evaluated. In addition, the analysis revealed that specific T cells infiltrated the tumor in G1 and G2. We conclude that both, infection with *T. cruzi* and the generated specific immunological memory inhibit growth of the B16-F10 tumor, although at different degrees. The infection probably induces an antitumor response through various mechanisms, being the epitope cross-reactivity one of them.

797. (379) **IDENTIFICATION OF POTENTIAL SHARED EPITOPES BETWEEN** *T. cruzi* **AND THE B16-F10 TUMOR CELL LINE**

Cintia D. Kaufman[1,#], Ibel Carri[2,#], Leonel Bracco[2], Morten Nielsen[2,3], Fernán Agüero[2,#], Andrés Alloatti[1,#]

1Instituto de Inmunología Clínica y Experimental de Rosario (IDICER) – CONICET/UNR; 2Instituto de Investigaciones Biotecnológicas – CONICET/UNSAM; 3The Technical University of Denmark.

 # These authors contributed equally to this work.

Spontaneous regression of cancer is often associated with infections. Pre- existing memory T lymphocytes generated in response to microorganisms could recognize tumor antigens that share sequences with microbial epitopes. We have observed that both the infection with *Trypanosoma cruzi* – the etiological agent of Chagas Disease – and the immunological memory generated in response to the infection, inhibit tumor growth in C57BL/6 mice challenged with B16-F10 cells (murine metastatic melanoma). To predict and identify potential MHC class I epitopes shared between the parasite and the tumor, we downloaded sequences of the whole exome of B16-F10 cells and C57BL/6 mice from the European Nucleotide Archive. After initial quality control, data were processed to obtain BAM files for variant analysis with Mutect2. Peptides of 8 to 11 residues that are potential ligands of H2-Db and H2-Kb alleles were found employing Mupexi, a tool that uses NetMHCpan 4.0 for the prediction. Neoepitopes were confirmed with the last available version of NetMHCpan (4.1). Additionally, mimotopes were generated by changing the aminoacidic residues of the anchor positions that do not interact with the TCR but determine binding to the MHC, for only those that can be in those positions (L, I, V & M and L, I, V, M & F in position 8 of the ligands of H2-Db and H2-Kb, respectively). BLASTP was used to search at the 100% similarity level the tumor epitopes and mimotopes in the proteome of different strains of *T. cruzi*. We have observed that proteins which are key for tumoral progression, such as SOX-21, CENPF, CLIP, SPON1 and DIP2A, could be immunogenic and shared peptides with *T. cruzi*. Interestingly, among parasite antigens that shared peptides with tumor antigens we found *trans*-sialidase family members, an important virulence factor and promising target for vaccines.

798. (390) **$CD20^+$ T CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)**

Brenda Buonincontro 1, Valeria Judith Sarapura Martinez 1, Gregorio Cordini 1, 2, Ana Colado 1, Chiara Cassarino 1, Juliana Bernatowiez 1, Raimundo Fernando Bezares 3, Gonzalo Garate 4, Mercedes Borge 1, 5, Mirta Giordano 1, 5 and Romina Gamberale 1, 5.

1 Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM), Buenos Aires, Argentina.

2 Hospital de Clínicas, José de San Martín, UBA, Buenos Aires, Argentina.

3 Hospital General de Agudos Teodoro Alvarez, Buenos Aires, Argentina.

4 Hospital Alemán, Buenos Aires, Argentina.

5 Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, UBA, Buenos Aires, Argentina.

CD20 is classically a B cell marker, but dim expression of CD20 has been noted on 0.1 to 7% of circulating T cells from healthy donors (HD). Compared to $CD20^-$ T lymphocytes, $CD20^+$ T cells produce greater levels of IL-2, IL-4, IL-10 IL-17, TNF alfa and IFN gamma. They may have a pathogenic behavior in autoimmune diseases and $CD20^+$ T-cell malignancies or a protective role in ovarian cancer and HIV infection. Their role in CLL has not been evaluated yet. CLL is the most common adult B cell leukemia, in which T cells are key players in the protective tumor microenvironment of lymphoid tissues. We aimed to study the subpopulation of $CD20^+$ T cells in peripheral blood (PB) and bone marrow (BM) of CLL patients. $CD20^+$ T cells were defined by flow cytometry as $CD20^+CD19^-CD3^+$ cells in 53 PB and 9 BM samples from CLL patients and 27 PB samples from age and sex matched HD. The percentage (%$CD20^+$ T cells) was calculated considering $CD3^+$ T cells as 100%. We found higher %$CD20^+$ T cells in PB of CLL patients compared to HD (4.6±0.6 vs 2.3±0.3, $p<0.05$). Similar results were found when the total amount of $CD20^+$ T cells was evaluated ($p<0.05$). The %$CD20^+$ T cells in our CLL patients range from 0.1 to 21.7 %. No significant differences were observed in the %CD20+ T cells when patients were segregated based on the expression of the prognostic markers CD38, CD49d, the mutational status of the IGVH, their clinical stage, platelets count or hemoglobin, beta-2-microglobulin and lactate dehydrogenase values. Interestingly, higher %$CD20^+$ T cells were found in the BM compartment compared to PB of the same patient ($n=9$, $p<0.05$).

In conclusion, the %$CD20^+$ T cells is higher in PB from CLL patients compared to HD and even higher in BM. No association was found between the %$CD20^+$ T cells and clinical and biological data of the CLL patients. Functional studies are needed to evaluate the impact of $CD20^+$ T cells on leukemic cell survival and activation.

799. (407) **PROBIOTIC YEAST *Kluyveromyces marxianus* FOR PREVENTION OF LOW-GRADE INFLAMMATION DERIVED TUMORIGENESIS: PROOF OF CONCEPT IN A MURINE MODEL OF COLORECTAL CANCER**

Malena Ferreyra[1], Gerónimo Ducca[1], María Dolores Pendón[2], Graciela Garrote[2], David Romanin[1], Martín Rumbo[1]

1 Instituto de Estudios Inmunológicos y Fisiopatológicos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CONICET - Asociado CICPBA, La Plata, Argentina.

2 Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata -CONICET-CIC.PBA, La Plata, Argentina

Colorectal cancer (CRC) is the third most prevalent cancer worldwide. It is known that chronic inflammation increases the risk of tumor development. Our group has demonstrated that *Kluyveromyces marxianus* CIDCA 8154 strain (Km 8154) has immunomodulatory properties which could modify the context for tumor development. Therefore, here we evaluate its administration to impact the process of tumorigenesis in a murine model of colitis-associated colorectal cancer by administration of azoxymethane/dextran sodium sulfate (AOM/DSS). After intraperitoneal injection of AOM 10 mg/kg, the AOM/DSS group were provided with 2,5% DSS in drinking water for 5 consecutive days, followed by 16 days of regular drinking water. This cycle was repeated 4 times. Mice were administered 10mL of a yeast suspension on gelatin (1-3 10^8 UFC/cage) every 48 hours, control mice received gelatin without yeast. Mice were monitored and weighted daily and euthanized at week 13. General inflammation markers, such as colon length and liver weight, were measured. No difference in these markers nor in the weight fluctuation between the AOM/DSS groups with or without Km 8154 were found. We counted the presence of polyps in the colon to discover that there are fewer polyps in the group AOM/DSS+Km 8154 compared to the control AOM/DSS group ($p<0,05$). The distribution throughout the length of the colon and the characteristics of the polyps were the same in both groups. We could demonstrate that the administration of Km 8154 has a preventive effect on the development of polyps in the AOM/DSS model and we are further characterizing the model. Simultaneously, we have proven *in vitro* that Km 8154 has a protective effect on the viability of epithelial cells against oxidative stress caused by hydrogen peroxide that is dose dependent ($p<0,01$), de-

termined by flow cytometry. Our results indicate that dietary administration of Km 8154 could be a positive intervention in prevention of inflammation induced CRC.

800. (487) **CHOLINERGIC SYSTEM MODULATES ACTIVATION OF NEUTROPHIL GRANULOCYTES FROM GLIOBLASTOMA PATIENTS**

Paula Saibene[1*], Alejandra Infante[1*], Juan Valentín Coronel[1], Carolina Maiumi Shiromizu[1], Carolina Jancic[1,3], Micaela Rossato[1], Juan Iturrizaga[1,2], Mónica Vermeulen[1], Marcela Villaverde[4], Analía Trevani[1,3] and Gabriela Salamone[1,3].

[1] Instituto de Medicina Experimental (IMEX) CONICET – Academia Nacional de Medicina, Buenos Aires, Argentina.

[2] División Neurocirugía del Instituto de Investigaciones Médicas A Lanari. Universidad de Buenos Aires. Universidad de Buenos Aires.

[3] Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina.

[4] Unidad de Transferencia Genética, Área Investigación, Inst. de Oncología Ángel H. Roffo, Facultad de Medicina, UBA.

**These authors contributed equally.*

Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor in adults, with a mean survival of less than a year after diagnosis. This tumor is defined by hallmark features, such as a great proliferative capacity, diffuse infiltration in adjacent tissues, necrotic focus and increased angiogenesis. Acetylcholine (ACh) is a neurotransmitter from ganglionic and post-ganglionic parasympathetic nerves and a non-neuronal paracrine mediator produced by different cell types. It has been established that ACh exceeds its role as a neurotransmitter, being involved in a broad variety of non-neuronal systems, including the immune system and tumor progression. Polymorphonuclear (PMN) were observed in large numbers in necrotic areas, these infiltrating cells could promote the expansion of the glioma stem cell pool, and may play an important role in glioma pathology. The aim of this work is to evaluate the relevance of the cholinergic system in human PMN obtained from healthy donors (PMN-h) and glioblastoma patients (PMN-p).
Both PMN-h and PMN-p were isolated from peripheral blood by Ficoll–Paque gradient centrifugation and dextran sedimentation. PMN were incubated with agonist cholinergic for 18 h and the supernatants were collected, and cytokine were evaluated by ELISA. CD11b expression was evaluated by flow cytometry after activation by cholinergic system by 15min. An increase in the expression of CD11b ($p<0.05$) when neutrophils were treated with cholinergic agonists was observed in both PMN-h and PMN-p. Also, IL-8 and VEGF production were increased when the cells were stimulated with cholinergic agonists ($p<0.05$). By contrast, IL-1β secretion tend to decrease in PMN-p compared to PMN-h when they were pretreated with cholinergic agonists and stimulated for 5 h with lipopolysaccharide -LPS and adenosine tri-phosphate- ATP). In conclusion the cholinergic system modulates the function of the physiology of PMN that could impact in the progression of GBM tumor.

801. (496) **EXPOSURE OF TUMOR CELLS TO THE PARP-1 INHIBITOR OLAPARIB STIMULATES NK CELL AND MACROPHAGE EFFECTOR FUNCTIONS**

Aldana Trotta, María Victoria Regge, Adrián David Friedrich, Jessica Mariel Sierra, Belén Candela Lozada Montanari, Maria Natalia Rubinztain, Maria Cecilia Santilli, Mariana Gantov, Julieta Erramouspe, Florencia Secchiari, Carolina Inés Domaica, Mercedes Fuertes, Norberto Walter Zwirner.

Olaparib belongs to a novel set of drugs called PARP (poly ADP-ribose polymerase)-1 inhibitors (PARPi) that induce synthetic lethality in several types of tumor cells regardless its BCRA mutation status. Although the effect of PARP-1 inhibition in tumor cells is well known, the consequences of tumor cell exposure to PARPi on immune cells that usually constitute the tumor microenvironment (TME) remains ill-defined. Consequently, the aim of this work was to explore the effect of Olaparib on tumor cell elimination by NK cells and phagocytosis by macrophages. To broaden the potential Olaparib indications for tumor treatment and taking into consideration that effects on antitumor immunity has been reported beyond the induction of synthetic lethality, several human tumor cell lines were treated with subapoptotic doses (1 or 2.5 μM) of Olaparib for 48 h and NK cell degranulation was assessed by flow cytometry (FC). We observed that pre-treatment of Raji cells with Olaparib increased NK cell degranulation and this effect was not observed with the solid tumor cell lines HCT116 and 786- O. We assessed whether this effect was due to an upregulation of NKG2D ligands (NKG2DLs) upon treatment with Olaparib. By FC we observed that expression of NKG2DLs remained unaffected in 786-O, ACHN, SN12c, HCT116, HT-29, U937, SKBR3, MCF-7 and T47D cell lines but MICA and MICB expression was increased in K562 cells, while MICA, MICB, ULBP-3 and ULPB-4 expression was increased in Raji cells. Accordingly, NK cell degranulation in response to Olaparib-treated Raji cells was NKG2D-dependent. In addition, Raji cells treated with Olaparib for 48 h were also phagocytosed more efficiently by macrophages, as assessed by FC ($p<0,05$). Accordingly, Raji cells treated with Olaparib upregulated cell surface Calreticulin and Annexin V ($p<0,05$), two well known “eat-me” signals. We conclude that Olaparib induces undescribed effects that result in an enhanced NK cell and macrophage effector functions.

802. (583) **PLASMABLAST WITH HIGH EXPRESSION OF CD39 AND PD-L1 INFILTRATE TUMORS FROM B16F10-OVA TUMOR-BEARING MICE**

Brunotto V[1], Abrate C[1], Amezcua-Vesely MC[1], Gazzoni Y[1], Gareca Julio[1], Acosta Rodriguez E V[1], Gruppi A[1], Montes CL[1].

[1] Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina.

The role of B cells in the anti-tumor response is not completely elucidated. B cells may play a dual role promoting or inhibiting cancer progression depending on their phenotype and the microenvironment. In this work, we aim to study the phenotype of tumor- infiltrating (TI) B lymphocytes (LiB) in a murine melanoma model. Non SPF C57BL/6 mice were injected intraperitoneally (ip) with $4x10^5$ B16F10-OVA tumor cells or PBS (controls). On day 13, tumors and peritoneal lavage (PL) were collected and by flow cytometry, we studied different LiB subsets such as: B2, B1a, B1b. The frequency of LiB ($CD19^+B220^+$) in PL from tumor-bearing mice was significantly lower respect to PL from control mice ($p\leq0.0001$). Most of these B cells correspond to B2 subset; in fact, B1a and B1b were nearly absent. Surprisingly, B1a and B1b subsets were almost missing in the tumor microenvironment. Within the TI-LiB we observed that a 61,55 ± 6,48% correspond to B cells exhibiting an unswitched phenotype, 18,53 ± 2,82 % an activated phenotype and 18,08 ± 4,03% exhibited plasmablast/plasma cells phenotype ($CD138^+$). Most of the of the $CD19^+B220^+CD138^+$ cells expressed IgM (49,86 ± 7,85%) and 17,88 ± 1,60 % expressed Ki67. We also evaluated the expression of PD-L1 and CD39 molecules involved in the regulation of the immune response. CD39 is an ecto-enzyme, which participate in adenosine production, a potent immune-regulator. Almost 90% of the $CD19^+B220^+$CD138+ cells showed high expression of CD39 and PD-L1. Additionally they expressed activation markers such as MHC class II and Fas. Interestingly, TI- activated B cells show a lower expression of PD-L1 and CD39 and TI- unswitched B cells do not express neither CD39 nor PD-L1. Together, these results show that TI-$CD138^+$ cells express modulatory molecules that may be involved in the regulation of the immune response against tumors.

803. (629) **CD39+ CD4+ T CONVENTIONAL CELLS ACCUMULATE WITHIN TUMORS AND INVADED LYMPH NODES FROM BREAST CANCER PATIENTS AND EXHIBIT FEATURES OF EXHAUSTION**

Bossio, Sabrina N.[1]; Tosello-Boari, Jimena[2], Abrate, Carolina[1], Ramello, María Cecilia[1]; Canale Fernando P.[1]; Gruppi, Adriana[1]; Acosta Rodriguez[1], Eva V.; Piaggio, Eliane[2,3]; Montes, Carolina L.[1]

[1]Centro de Investigaciones en Bioquímica Clínica e Inmuno-

logía (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina. [2]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [3]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

The role of CD4+ T cells in the immune response against tumors is not completely elucidated. In this work, by flow cytometry, we explored the distribution of CD4+ T cells in tumor, adjacent non-tumoral mammary tissues (yuxtatumor) and metastatic and non-metastatic draining lymph nodes (dLNs) from untreated breast cancer (BC) patients. We first evaluated the presence of LiTreg (CD4+FOXP3+) and CD4+ conventional T lymphocytes (LiTconv) (CD4+FOXP3-) in primary tumors matched with non-tumoral mammary tissues. As previously described, tumors exhibited higher infiltration of LiTreg, than yuxtatumor. However, CD4+ infiltrating tumors and yuxtatumors are composed of a majority of LiTconv. Surprisingly, we detected that tumors exhibited higher % of CD39+ expressing LiTconv compared to yuxtatumor ($p \leq 0.01$). We observed that while CD39+ LiTconv were absent in peripheral blood, the % in metastatic dLNs was significantly higher than in non-metastatic dLNs ($p \leq 0.05$). The phenotypic analysis of tumor-infiltrating (TI) LiTconv population revealed that, within CD39+ population, there were higher frequency of PD-1+, TIGIT+ and BTLA+ cells compared to the CD39- population ($p \leq 0.01$). The effector function of TI-CD39+ LiTconv was assessed analyzing their ability to produce TNF, IFNγ and IL-2. Thus, TI-CD39+ LiTconv exhibited a lower frequency of TNF-producing cells than CD39- LiTconv ($p \leq 0.05$) and a higher frequency of CD107a+ cells ($p \leq 0.05$). There were no significant differences in IFNγ and IL-2 production between CD39+ and CD39- LiTconv. *In vitro* stimulation of sorted TI-CD39- LiTconv with anti-CD3/anti-CD28 from 5 BC patients, showed an increased frequency of CD39+ cells upon 72 hs of stimulation. This result highlights the relevance of the TCR-stimulation in the CD39 expression. Together these findings suggest that tumor microenvironment drives the acquisition of immunoregulatory molecules on LiTconv which may impact in tumor progression.

804. (630) **TUMOR-INFILTRATING CD8+PD-1^{HIGH} T LYMPHOCYTES FROM BREAST CANCER PATIENTS EXHIBIT A PHENOTYPE ASSOCIATED TO TERMINAL EXHAUSTION**

Abrate Carolina[1], Valentina Brunotto[1], Bossio Sabrina[1], Boccardo Santiago[1], Edith Borcoman[2,3], Gruppi Adriana[1], Acosta Rodriguez Eva[1], Eliane Piaggio [2,3] Montes Carolina[1].

[1] Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina.

[2] Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France.

[3]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

The focus in cancer immunotherapy has been mainly posed in CD8$^+$ T lymphocytes. Evidences suggest that CD8$^+$ T cell subsets characterized by distinct expression levels of PD-1 diverge in their state of exhaustion and potential for reinvigoration by PD-1 blockade. In this work, we aim to perform a comprehensive study of PD-1^{hi}CD8$^+$ and CD8$^+$PD-1^{low} T cells in untreated breast cancer (BC) patients. We included in our study different BC subtypes according the expression of estrogen, progesterone or HER2 receptors. We studied the presence and phenotype of the PD-1 expressing CD8$^+$ T cell subpopulations in tumor, juxta-tumor (JT), invaded and non-invaded tumor-draining lymph node (I-dLN and NI-dLN). By flow cytometry we evaluated the expression of inhibitor receptors (PD-1, TIGIT), proliferation, activation, and exhaustion markers (Ki-67, OX40 and CD39) and cytokine production on CD8$^+$ T cells. We observed higher % of CD8$^+$PD-1^{high} cells in Tumor than JT, I-dLN and NI-dLN ($p<0.01$, $p<0.001$ and $p<0.0001$ respectively). The frequency of CD8$^+$PD-1^{low} cells was higher in tumor compared to I-dLN and NI-dLN ($p<0.0001$ for all). The evaluation of CD39, OX40 and Ki67 revealed that CD8$^+$PD-1^{high} cells exhibited higher frequencies of these molecules than CD8$^+$PD-1^{low} cells in the tissues evaluated ($p<0.05$ for all). Accordingly, CD8$^+$PD-1^{high} cells from tumors, JT and d-ILN showed higher % of TIGIT$^+$ cells respect to PD-1^{low} counterpart ($p<0.01$ for all). We observed that compared to CD8$^+$PD-1^{low} T cells, CD8$^+$PD-1^{high} T cells from tumors and I-dLN exhibited an impairment of TNF and INF production. Our results suggest that, PD-1 expression level identifies T lymphocytes with distinct phenotype. CD8$^+$PD-1^{high} T cells from tumors, JT and I-dLN exhibit a phenotype associated to terminal exhaustion. The characterization of a distinct state of PD-1 expressing CD8+ T lymphocytes from BC patients provide novel potential avenues for therapeutic intervention.

805. (665) **ORAL SQUAMOUS CELL CARCINOMA (OSCC) TUMORS FROM HEAVY ALCOHOL CONSUMERS ARE ASSOCIATED WITH HIGHER LEVELS OF TLR9 AND A PARTICULAR IMMUNOPHENOTYPE**

Sabrina Dhooge[1], Nicolás Bolesina[2], Silvia López de Blanc[2], Gerardo Gatti[1], Darío Rocha[3], Elmer Fernandez[4], Ruth Ferreyra[2], Vanesa Palla[1], Verónica Grupe[1], Rosana Morelatto[2] and Mariana Maccioni[5]

[1] Fundación para el Progreso de la Medicina. Laboratorio de Investigación en Cáncer, Córdoba, Argentina, [2]Departamento de Patología Oral, Cátedra de Estomatología, Facultad de Odontología, Universidad Nacional de Córdoba, Córdoba, Argentina, [3]Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina, [4]Centro de Investigación y Desarrollo en Inmunología y Enfermedades Infecciosas, CIDIE-CONICET, Universidad Católica de Córdoba; [5]Centro de Investigaciones en Bioquímica Clínica e Inmunología, CIBICI-CONICET, Departamento de Bioquíimica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

Oral squamous cell carcinoma (OSCC) is one of the most frequent types of oral cancer in developing countries and its burden correlates with exposure to tobacco and excessive alcohol consumption. Toll like receptors (TLRs) are major sensors of inflammatory stimuli, from both microbial and sterile causes and as such, they have been related to tumor progression and metastasis. Here, we evaluated the expression of TLR2, 4 and 9 as well as CD3+, CD8+ and Granzyme B+ cell infiltration by immunohistochemistry in oral samples of 30 patients with OSCC, classified according to their consumption of alcohol. Our findings indicate that there is a significant association between heavy alcohol consumption and tumors with higher expression levels of TLR9. Moreover, patients with TLR9high tumors, as well as those who indicated high consumption of alcohol exhibited a diminished overall survival. TCGA data analysis indicated that TLR9high tumors express a significant increase in some genes related with the oral cavity itself, inflammation and tumor promotion. Our analysis of tumor infiltrating leukocytes demonstrated that the major differences perceived in heavy alcohol consumers was the location of CD8+ T cells infiltrating the tumor, which showed lower numbers intratumorally. Our data suggest the existence of a pathogenic loop that involves alcohol consumption, high TLR9 expression and the immunophenotype, which might have a profound impact on the progression of the disease.

806. (804) **SECRETORY LEUKOCYTE PROTEASE INHIBITOR: A NOVEL CANDIDATE FOR TUMOR IMMUNE EVASION MECHANISM IN PANCREATIC CANCER**

Carla Lucero Remolins[1], Kevin Israel Matamoros Alcivar[1], Paula Ayelen Lamas[1], José Ignacio Valenzuela[1], Diego Guerrieri[1], Olivier Blond[1], Nicolas Fraunhoffer Navarro[1,2], Damian Vanzini[3], Eugenia Fratantoni[3], Ornella Di Tulio[3], Gustavo Kohan[3], Horacio Romeo[4], Juan Iovanna[2], Eduardo Chuluyan[1]

[1]Centro De Estudios Farmacológicos y Botánicos (CEFyBo-CONICET), Buenos Aires, Argentina, [2]French Institute of Health and Medical Research (INSERM), Marsella, Francia, [3]Sanatorio de la Trinidad Mitre, Buenos Aires, Argentina, [4]Universidad Católica Argentina (UCA), Buenos Aires, Argentina

The immune surveillance failure reported in pancreatic ductal adenocarcinoma (PDAC) could be attributed to immune evasion factors (IEF) produced by cancer cells in the tumor microenvironment. We have described that secretory leukocyte protease inhibitor (SLPI) is an immunomodulator that downregulates the generation of monocyte-derived dendritic cells (moDCs), and it is produced by different PDAC tumor lines. However, it is not known if SLPI-producing PDAC affects the differentiation of monocytes to moDCs. The aim of the present work was to determine the effect of PDAC tumors on the generation of moCDs and the ability of these to produce SLPI. Samples of tumor and heparinized peripheral blood were obtained from patients bearing PDAC at the time of the surgical resection. Monocytes were isolated and cultured in differentiation medium (RPMI 1640, 10% FBS, 10 ng/ml IL-4, 36 ng/ml GMCSF, 1% penicillin-streptomycin) in the presence or absence of rhSLPI 320 ng/ml or co-culture with tumor explants (TE) in transwells. After 5 days, molecules CD14, CD1c and CD86 were measured by flow cytometry. Furthermore, the levels of SLPI in the plasma of the patients and in the tumor culture supernatants (TCS) were determined by ELISA. Plasma SLPI levels were detected in all patients but in only 4 out of 10 TCS. There was a tendency to impair CD1c expression by 81.8% (p= 0.0908; Kruskal-Wallis test) in SLPI-producing TE. In addition, there was a positive correlation between plasma levels of SLPI and the percentage of CD1c decrease in the co-culture (r= 0.7702; p=0.0366). On the other hand, plasma levels of SLPI strongly correlated with the CD14/CD1c ratio detected in co-cultures (r= 0.94; p= 0.008). These results suggest that SLPI-producing PDAC contributes to the immunosuppressive microenvironment of pancreatic cancer by acting as an IEF and that its plasma levels could be an indicator of the state of the local immune response.

807. (845) **EVALUATION OF THE EFFECT OF PROINFLAMMATORY CYTOKINES IN VIRTUAL MEMORY CD8⁺ T CELLS**

Nicolás L. Lidón, Constanza Savid-Frontera, María Estefanía Viano, Quentin Fontaine and María Cecilia Rodríguez Galán
Centro de Investigaciones en Bioquímica Clínica e Inmunología. CIBICI-CONICET. Facultad de Ciencias Químicas - UNC.

Virtual memory CD8⁺ T cells (T_{VM}) are a subset of T cells with a memory phenotype (CD44^{hi} and CD122^{hi}) without previous antigen encounter. T_{VM} can be distinguished from conventional memory T cells (T_{MEM}) by their low expression of CD49d. We evaluated the role of different cytokines reported as important for both survival and functionality of T_{VM}, such as IL-12, IL-18, IL-15, IL-4 and IFNγ. To determine the effect of IL-15 on T_{VM} and T_{MEM} survival, *in vitro* assays with variations in IL-15 concentrations demonstrated that the lowest IL-15 concentration (5ng/ml, IL-15^{lo}) increased the percentage of T_{MEM}, but this effect is not seen with the highest IL-15 concentration (20ng/ml, IL-15^{hi}). Moreover, double stimulation with IL-15^{hi} and plate-coated anti-CD3 induced a significant decrease in the number of T_{MEM}. For T_{VM}, both IL-15^{hi} and the IL-15^{hi}+anti-CD3 conditions induced the greatest increases in the percentage of this population.
Regarding the possible functional activity of T_{VM}, Fura-2 assays show that cells from lymph nodes in contact with KPC tumor cells induced higher intracellular calcium mobilization when they come from animals treated *in vivo* with IL-12 and IL-18 (enriched in T_{VM}) compared to same cells from control mice (p<0,05). A potential effector molecule of T_{VM} cells is NKG2D receptor. We evaluated the expression of this receptor in T_{VM} and T_{MEM} cells from IL4KO and IFNγKO mice in steady-state or IL-12+IL-18 *in vivo* stimulation. Our data demonstrate that the absence of IL-4 affects the expression (MFI) of NKG2D only in T_{MEM}, while the absence of IFNγ affects the expression of NKG2D in both cell populations. Furthermore, the percentage of T_{VM} cells that express NKG2D (% NKG2D⁺T_{VM}) is not affected by the absence of both IL-4 and IFNγ, while the percentage of NKG2D⁺T_{MEM} decreased in both KO mice. These results might contribute to understanding the potential involvement of T_{VM} and T_{MEM} cells in tumor growth control.

808. (847) **LEUKOCYTE-SPECIFIC PROTEIN 1 ABSENCE REDUCES CYTOTOXIC RESPONSE GENERATION BY AFFECTING DENDRITIC CELLS AND CD8⁺ T CELL FUNCTION**

María Mercedes Pascual[1,2], Nicolás Daniel Dho[1,2], Constanza Marín[1,2], María Inés Crespo[1,2], María Cristina Pistoresi[1,2], Belkys Angélica Maletto[1,2], Víctor Gabriel Morón.[1,2]
1. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. 2. Consejo Nacional de Investigaciones Científicas y Técnicas, Centro de Investigación en Bioquímica Clínica, Córdoba, Argentina.

LSP1 is an intracellular F-actin binding phosphoprotein expressed in leukocytes and endothelial cells from humans and mice. LSP1 polymorphisms or reduced expression are risk factors for some types of cancer. We demonstrated that B16-OVA tumors in LSP1 KO mice reach bigger sizes and grow faster than in wild type (WT) mice. Also, there is a reduced extravasation efficiency of LSP1 KO leukocytes into tumors harvested from WT and LSP1 KO mice. In dendritic cells (DCs), capture of tumoral antigen and its transport to draining lymph node (dLN) is affected by LSP1 absence. In addition, LSP1 KO DCs cannot activate and induce CD8⁺ T cell proliferation in an appropriate way. Taking into account these previous results, we aimed to investigate if intratumoral CD8⁺ T cell functionality is affected by LSP1 absence. We observed a reduced frequency of CD8⁺ T cell producing perforin and granzyme B in LSP1 KO tumors (p<0.01), as well as a reduced frequency of polyfunctional CD8⁺ T cell producing Lamp1 e IFN-γ simultaneously (p<0.05). To evaluate the impact of the reduced production of effector molecules in CD8⁺ T cell-mediated cytotoxicity, WT and LSP1 KO mice were immunized with OVA adjuvanted in CpG-CoA-ASC16 and after 7 days an *in vivo* CTL assay was performed. A lower lysis percentage was observed in LSP1 KO mice with respect to WT mice (p<0.001). To investigate the impact of the reduced cytotoxic function in tumor development, WT and LSP1 KO mice immunized as indicated above were inoculated with 1.10^5 B16-OVA cells and tumor size was followed up. Immunization reduced the tumor size in WT (p<0.001) and LSP1 KO mice (p<0.01). However, tumors in immunized LSP1 KO mice grew still bigger than in immunized WT mice. Immunization also improved mice survival in both animal groups (p<0.01). These results demonstrate that LSP1 absence reduces CTL response generation through affecting DCs and CD8⁺ T cell function and therefore tumor control development is impaired.

809. (856) **GALECTIN-1 REINFORCES THE IMMUNOSUPPRESSIVE ACTIVITY OF EXTRACELLULAR VESICLES RELEASED BY MYELOID-DERIVED SUPPRESSOR CELLS**

Camila A. Bach[1], Joaquín P. Merlo[2], Ramiro M. Perrotta[1], Marco A. Scheidegger[1], Ricardo Chaurio[3], Mate Z. Nagy[3], Montana N. Manselle Cocco[1], José R. Conejo García[3], Ada G. Blidner[1*], Gabriel A. Rabinovich[1*]
[1]Laboratory of Glycomedicine, Institute of Biology and Experimental Medicine, National Council of Scientific and Technical Investigations, 1428, Buenos Aires, Argentina
[2]Functional and Molecular Glycomics Lab, Institute of Biology and Experimental Medicine, National Council of Scientific and Technical Investigations, 1428, Buenos Aires, Argentina
[3]Department of Immunology, H. Lee Moffitt Cancer Center and Research Institute, 33612, Tampa, Florida, USA
**co-senior authorship*

Myeloid-Derived Suppressor Cells (MDSCs) represent a major hurdle for cancer immunotherapy by blunting antitumor T cell responses. Emerging evidence suggests that extracellular vesicles (EVs) shedding serves as an MDSC immunosuppression mechanism; however, the molecular mediators that trigger this effect are still elusive. We have recently demonstrated that Galectin-1 (Gal1), a β-galactoside-binding protein, enhances the MDSC immunosuppressive and pro-angiogenic activities of MDSCs. Here, we investigated the role of Gal1 in the biology of MDSC EVs. We differentiated MDSCs from mouse bone marrow *in vitro*, incubated them with recombinant Gal1, and isolated EVs released (control EVs or Gal1 EVs). We characterized EVs by imaging flow cytometry, transmission electron microscopy, cytokine array, and liquid chromatography-tandem

mass spectrometry (LC-MS/MS) for proteomic analysis. Regarding tetraspanins, well-established EV markers, CD9 but not CD63 or CD81 were detected on the surface of MDSC EVs, as evidenced by imaging flow cytometry and LC-MS/MS. Interestingly, Gal1 EVs displayed lower levels of CD206 compared to control EVs. LC-MS/MS showed differential modulation of proteins in control EVs vs. Gal1 EVs. The glycosylation signature of MDSC EVs was assessed by flow cytometry revealing higher levels of asialo-core-1-O-glycans, leading to higher Gal1 binding on Gal1 EVs compared to control EVs ($p<0.05$). To elucidate the contribution of Gal1 to the immunosuppressive capacity of MDSC EVs, we co-cultured mouse activated T cells with control or Gal1 EVs. Gal1 EVs inhibited T $CD4^+$ and $CD8^+$ proliferation and activation in a dose-dependent manner ($p<0.001$). Cytokine array and LC-MS/MS showed that Gal1 EVs carried higher amounts of IL-16 which has been reported to inhibit TCR/CD3 mediated T cell stimulation in mixed lymphocyte reactions. Notably, Gal1 enhanced PD-L1 expression on MDSC EVs surface ($p<0.05$). Thus, Gal1 enhances the immunosuppressive capacity of MDSC EVs.

810. (912) **IMPACT OF GALECTIN-1/GLYCANS AXIS ON THE COMPOSITION OF THE IMMUNE INFILTRATE IN HUMAN BREAST CANCER**

Emiliano Moreno[1], Natalia Musco[1], Dante Goy[2], Nicolás Sarbia[1], Delfina Loch[1], Juan Carlos Stupirski[1], Nicolás Torres[1], Ma. Del Rosario Vidal[2], Ma. Julia Daponte[2], Ana I. Alonso[2], Natalia Baltz Hehn[2], Erica López[2], Ana Kosir[3], Leticia Ramos[3], Lorena Soria[3], Alfredo Britos[3], Laura Patricia Scarpelli[4], Luis Adolfo Alposta[4], Diego Aníbal Jofre[4], Silvia Saucedo[4], Gabriel Rabinovich[1] and Mariana Salatino[1].

1Laboratorio de Inmunopatología, IBYME, CONICET, CABA Argentina, 2Departamento de Oncología, Hospital Municipal de Morón, Ostaciana B. de Lavignolle, Morón, Argentina 3Departamento de Mastología, Hospital Municipal de Morón, Ostaciana B. de Lavignolle, Morón, Argentina 4Departamento de Anatomopatología, Hospital Municipal de Morón, Ostaciana B. de Lavignolle, Morón, Argentina

Breast cancer is the type of cancer with the highest incidence among women. It is classified into four subtypes, Luminal A and B, HER2-positive and TNBC, with different treatment options and clinical outcomes. Galectin-1 (Gal1) is a glycan-binding protein associated with tumor progression. In this work, we sought to understand the Gal1/glycans axis's impact on human breast cancer. Thus far, we analyzed 38 patients subjected to surgery and collected samples of fresh breast tumors, adjacent normal tissues, and sera. We found a significant association between tumor subtypes and tumor size being 53.8%* of Luminal A and 81.8%* of Luminal B tumors classified as intermediate size (T2, 2-5cm). We then investigated the tumor immune infiltrate by flow cytometry (FC) and observed that even though T cells were the predominant infiltrating population, there was a higher frequency of B cells in malignant tumors compared to normal tissue (5,87% vs 0,48%)*. Importantly, not only Treg infiltration increase in tumors compared with normal tissue (7,74 vs 2,52)* resulting in a lower CD8+/Treg ratio, but it also increases Th2-like cells (2,1 vs 0,1%)**. Regarding myeloid cells, Luminal A tumors were slightly enriched in M-MDSCs compared to other subtypes or normal tissues. We then assessed Gal-1 expression (RT-PCR) in tumors and serum samples (ELISA) and found that Gal1 levels in tumors correlated with the frequency of several immune cell subsets in tumors, including Th2-like cells*** and CD4+CD8+ T*, and in serum with PMN-MDSC**, CD4+CD8+T** and CD25+CD127lowTregs*. As the cell glycophenotype determines the response to Gal1, we analyzed it using fluorescein-labeled lectins (FC). We found a differential glycosylation profile among breast cancer subtypes and normal tissues. We conclude that different breast tumor subtypes may present different Gal1 expression levels and glycosylation signatures that can influence immune infiltration and disease outcome. * $p<0.05$, ** $p<0,01$, *** $p<0,001$.

811. (926) **LYMPH NODES ORCHESTRATE ANTI-TUMOR CD4+ T CELL RESPONSE**

Jimena Tosello Boari[1,2,*], Wilfrid Richer[1,2,*], Elisa Bonnin[1,2], Sebastien Lemoine[3], Yoann Missolo-Koussou[1,2], Marco Antônio M Pretti[3], Rodrigo Nalio Ramos[1,2,§,*], Jordan Denizeau[1,2], Sylvain Baulande[4,5], Mylène Bohec[4,5], Sonia Lameiras[4,5], Marine Lefevre[6], Agathe Seguin-Givelet[6,7], Christine Sedlik[1,2], Saumya Kumar[8,9], Luis Graça[8,9], Olivier Lantz[1,2,10], Nicolas Girard[1,2,10,11], Joshua J Waterfall[2,12,#], Eliane Piaggio[1,2,3,#].

1. INSERM U932, PSL Research University, Institute Curie Research Center, Paris, France.

2. Department of Translational Research, PSL Research University, Institut Curie Research Center, Paris, France.

3. Egle Therapeutics, Paris, France.4. Institut Curie, PSL University, ICGex Next-Generation Sequencing Platform, 75005 Paris, France

5. Institut Curie, PSL University, Single Cell Initiative, 75005 Paris, France

6. Institut Montsouris, Surgery Department, Institut du Thorax Curie Montsouris, Paris, France.

7. Paris 13 University, Sorbonne Paris Cité, Faculty of Medicine SMBH, Bobigny, France.

8. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal.

9. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

10. Centre d'Investigation Clinique Biothérapie, Institut Curie, Paris, France.

11. Institut Curie, Institut du Thorax Curie Montsouris, Paris, France.

12. INSERM U830, PSL Research University, Institute Curie Research Center, Paris, France.

** Present address: Laboratory of Medical Investigation in Pathogenesis and Directed Therapy in Onco-Immuno-Hematology (LIM-31), Department of Hematology and Cell Therapy, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, University of São Paulo, São Paulo, Brazil; and Instituto D'Or de Ensino e Pesquisa, São Paulo, Brazil*

** Co-first author*

 # Co-last and co-corresponding author (eliane.piaggio@curie.fr, joshua.waterfall@curie.fr)

Tumor-draining lymph nodes (LNs) are the most important sites for the priming of adaptive immune responses, yet their role in the orchestration of the anti-tumor CD4+ T cell response remains little explored. With the aim to uncover the role of nodal CD4+ T cells in the cancer biology, we performed an integrative single cell analysis of transcriptome and T cell receptor (TCR) of CD4+ conventional (Tconv) and regulatory (Treg) T cells from matched blood, LNs and tumors of treatment naïve Non-Small Cell Lung Cancer (NSCLC) patients. We studied 48,383 CD4+ T cells from five individuals that clustered in 21 groups with distinct transcriptomic characteristics. We first observed that blood and tumor presented a preferential accumulation of naïve and effector phenotypes, respectively, while LNs contained the majority of CD4+ T cell subsets. Secondly, we found that LN and tumor present a similar TCR expansion pattern, while blood presented less clonality. Moreover, tumor shared with LNs expanded clones as well as clones bearing phenotypic characteristic associated with neo-Ag specific cells. Thirdly, by using the TCR as a molecular lineage, we showed that Tregs and Tconvs mainly transited between LNs and tumors and most of the migrating clones conserved their expression identity while trafficking. Even more, Treg/Tconv presented shared and unique molecular patterns associated with this movement. Finally, we analyzed local state changes and found that among transiting cells, effector Tconvs mainly shared clones with proliferating or activating states, while Tregs showed a high plasticity among different states, particularly in the LNs. Notably, Tconv/Treg interconversion was detected in LNs and tumor, but was more prominent in the LNs. Overall, our results enrich our basic knowledge of the diversity of CD4+ T cells in tumors and particularly in the TDLNs and provides novel cues for the therapeutic targeting of tumor-Tregs in patients.

812. (929) **GALECTIN-7, AN ENDOGENOUS LECTIN PREFERENTIALLY EXPRESSED ON THE SKIN, CONTROLS MYELOID-DERIVED SUPPRESSOR CELL ACTIVITY ON MYELOID DERIVED SUPPRESSOR CELLS**

Laporte, L* ;Pinto, N*; Sundblad V; Bach,C; Blidner,A; Rabi-

novich, GA
*estos autores contribuyeron igualmente

Background: Galectin-7 (Gal7), an endogenous lectin preferentially expressed in stratified squamous epithelium of the skin, controls keratinocytes proliferation and differentiation. Overexpression of this lectin in Tg-46 transgenic mice leads to expansion of the myeloid-derived suppressor cells (MDSCs) in in vivo models of non-melanoma skin cancer (NMSC), compared to Gal-7-deficient (*Lgals7*$^{-/-}$) and control mice. Objectives: We aimed to investigate glycan-dependent binding of extracellular Gal7 to MDSCs and to explore its role in the differentiation and function of MDSCs. Materials and Methods: We differentiated and activated wild-type (WT) bone marrow (BM)-derived MDSCs with LPS in the presence (LPS+Gal7) or absence (LPS) of recombinant Gal7 (rGal7) and analyzed the frequency of MDSCs subpopulations by studying Ly6C and Ly6G in CD11b+ gated cells by flow cytometry. Using fluorescently labeled lectins, we investigated binding of rGal7 to BM-MDSCs activated or not with LPS in the absence or presence of the competitive disaccharide lactose by flow cytometry. Finally, we analyzed the frequencies of MDSCs subpopulations in BM-MDSCs from mice lacking complex branched N-glycans (*Mgat5*$^{-/-}$) or core 2-O-glycans (*C2gnt1*$^{-/-}$), activated with LPS in the presence or absence of r-Gal7.- Results: Flow cytometry data revealed association of Gal7 with the surface of activated MDSCs and their polarization toward a monocytic (CD11b^{+}Ly6C^{hi}Ly6G^{lo}) profile. (P=0.0008) Both, Gal7 binding to MDSCs, and their phenotype were inhibited by lactose, a galectin-specific disaccharide, demonstrating the involvement of protein-glycan interactions in this effect. Furthermore, Gal7 effects were abolished when BM progenitors from *Mgat5*$^{-/-}$ or *C2gnt1*$^{-/-}$ mice were evaluated, compared to WT MDSCs (P>0.05), suggesting that glycosylation-dependent Gal7-driven programs participate in of the control of MDSCs fate. Conclusion: Our study identify a glycosylation-dependent Gal7-driven circuit regulated by both N-glycans and O-glycans that controls polarization of immature myeloid cells into a monocytic profile.

VACCINES *Friday, November 18, 14-15:30 hr*

Chairs: Andrés Sánchez Alberti - Augusto Bivona - Mariana Ferrero - Ana Rosa Pérez - Florencia González - Gabriel Cabrera - Samanta Funes - Ana Rodríguez - Marina Biedma - Guillermo Docena

813. (33) **SHORT AND LONG TERM EVALUATION OF THE ANTI-SARS HUMORAL RESPONSE IN VOLUNTEERS GIVEN ANTI-COVID VACCINES**

Gretel Naidich1, Natalia Santucci2 3. Eduardo Ceccarelli4, Oscar Bottasso2 3, Mario Perichón1.
1CUDAIO (Centro Único de Donación, Ablación e Implante de Órganos). 2IDICER-CONICET-UNR. 3Facultad de Ciencias Médicas de Rosario. 4IBR-CONICET-UNR.

Anti-COVID vaccination showed some delays between the administration of the first and second doses along with a combination of different vaccine platforms. Considering the relevance of the antibody response in viral infections, we analyzed anti-S IgG, at different time points following Sputnik immunization, in healthy people attending the vaccination centers of Rosario city, who had shorter versus longer intervals between both doses. A total of 1035 adults with no COVID-compatible symptoms (throughout the study period) were separated according to the length between both vaccine doses: 21 (Group A, n=528), 30 (Group B, n=147), and 70 days (Group C, n=82), as well as an additional group of individuals with heterologous vaccination (Sputnik/Moderna, separated by a 107-day interval, n=278). While there were no between-group differences in baseline levels of specific antibodies, data at the time of administering the second dose showed that group D had the highest amounts of specific antibodies followed by values recorded in Groups C, A, and B (p<0.0001). The same trend of group differences was seen when measuring anti-S antibodies at 21 or 180 days following the first and second doses, respectively (p<0.0001). Delayed between-doses interval coexisted with higher antibody titers, even more, when employing a heterologous schedule.

814. (105) **A PROTOCOL BASED ON TSf-ISPA VACCINE AND 5-FLUOROURACIL ADMINISTRATION PROVIDES PROTECTION AGAINST DIFFERENT *TRYPANOSOMA CRUZI* STRAINS ASSESSED IN BALB/c AND C57BL/6 MICE**

Eliana Borgna1, Estefanía Prochetto1, Juan Cruz Gamba1, María Azul de Hernández2, Carolina Poncini3, Pamela Cribb2, Ana Rosa Pérez4, Florencia González4, Iván Marcipar1, Gabriel Cabrera1
1Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. 2Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Rosario, Argentina. 3 IMPaM, UBA-CONICET, Facultad de Medicina, UBA. 4Instituto de Inmunología Clínica y Experimental de Rosario, IDICER-CONICET and Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina

Introduction: We have previously reported that immunization with a trans-sialidase fragment (TSf) formulated with a cage-like particle adjuvant (ISPA) protects BALB/c mice against a challenge with *Trypanosoma cruzi* (*T. cruzi*) of Tulahuen (Tul) strain. In addition, pretreatment with 5-fluorouracil (5FU) increased the protective capacity of TSf-ISPA vaccine. Aim: to study whether the TSf-ISPA vaccine combined with 5FU provides protection in additional mouse models of *T. cruzi* infection. Methods: 3 doses of TSf-ISPA were given to BALB/c or C57BL6 mice. 5FU (50mg/kg) was given 1 day before and 8 days after each immunization dose (vaccinated group). Mice were challenged intraperitoneally with 1500-3000 Tul *T. cruzi* (DTU VI) or 20000 Dm28c *T. cruzi* strain (DTU I). Parasitemia was monitored on different days post-infection (p.i.). Survival was assessed until day 40 (p.i.). Since 5FU can deplete selectively myeloid-derived suppressor cells (MDSCs), flow cytometry was used to measure CD11b+Ly6G+Ly6C+/low, (G-MDSCs) and CD11b+Ly6G-Ly6C+ (M-MDSCs) splenocytes at different days p.i. Results: After a challenge with Tul or Dm28c parasites, vaccinated BALB/c and C57BL/6 mice always showed lower parasitemias than PBS-treated (PBS) and infected (Tc+) mice. For instance, the Tul mean parasitemia in 20 fields at day 14 p.i. in vaccinated and Tc+ BALB/c mice was 20,6±16 vs 72,8±32,5 in PBS Tc+ mice (p<0.05). In the analyzed models, vaccinated mice always had equal or significantly higher (p<0,05) survival than PBS Tc+ mice. At day 20 p.i., vaccinated Tc+ mice always had a lower absolute number of G-MDSCs than PBS Tc+ mice. For example, after Tul infection of C57BL/6 mice, the mean number of G-MDSC splenocytes (x10^{6}) in vaccinated Tc+ mice was 21,5±3.05 vs 55,6±26,4 in PBS Tc+ mice (p<0.05). Discussion: The TSf-ISPA vaccine combined with 5FU administration provided protection in BALB/c and C57BL/6 mouse models of *T. cruzi* infection, even using parasites from different DTUs.

815. (112) **THE USE OF ASCORBYL PALMITATE AS VACCINE PLATFORM DOES NOT MODIFY THE IMMUNE RESPONSE UNDER DIFFERENT STORAGE CONDITIONS**

Federico N. Ruiz Moreno[1,2], Constanza Marín[1,2], Giuliana Vedelago[1,2], Daniel Allemandi[3,4], Santiago D. Palma[3,4], María C. Pistoresi-Palencia[1,2], Gabriel Morón[1,2], Belkys A. Maletto[1,2]
1. Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Bioquímica Clínica. Córdoba, Argentina.
2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI). Córdoba, Argentina.
3. Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Ciencias Farmacéuticas. Córdoba, Argentina.
4. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Unidad de Tecnología Farmacéutica (UNITEFA). Córdoba, Argentina.

Previously we demonstrated that the nanoformulation of OVA and CpG-ODN with a nanostructure formed by self-assembly of 6-O-ascorbyl palmitate (Coa-ASC16) elicited antigen-specific antibody and cellular responses superior to those induced by the solu-

ble counterpart. Here, we study the stability of the nanoformulation OVA/CpG-ODN/Coa-ASC16 keeping it for 2 days at room temperature and for 30 days at 4°C, using the newly prepared nanoformulation as a control. Mice were subcutaneously immunized with a single dose of OVA and CpG-ODN nanoformulated with Coa-ASC16 (OVA/CpG-ODN/Coa-ASC16) maintained under the different storage conditions. Then, we evaluate the SIINFEKL-Kb tetramer+ $CD8^+$ T cells, OVA-specific antibody response and INFɣ produced by spleen cells upon rechallenge in vitro with OVA. ELISA and flow cytometry techniques were used. The results reveal that the OVA-specific antibody and cellular responses elicited were similar among the three groups of mice regardless of the formulation used (p:NS). These data showed that the ambient temperature as well as the conservation short time of the nanoformulation does not produce changes on immune response elicited, an indicator of its biological function.

816. (141) **EFFECT OF THE BIOSURFACTANT SURFACTIN ON THE ADJUVANT POTENTIAL OF NANOENCAPSULATED *MINTHOSTACHYS VERTICILLATA* ESSENTIAL OIL**

María de Lourdes Vancolli[1], María Eugenia Cecchini ME[2], Ivana Dalila Montironi [3], Sofía Arsaute [1], Nadia Lorena Azcurra [1], Romina Valeria Bellingeri [4], María Laura González Pereyra[5], Laura Noelia Cariddi [2]

[1] Departamento de Microbiología e Inmunología-UNRC, Río Cuarto, Córdoba, Argentina. [2] INBIAS-CONICET-UNRC, Río Cuarto, Córdoba, Argentina. [3] Cátedra de Farmacología-UNRC, Río Cuarto, Córdoba, Argentina. [4] IITEMA-CONICET- UNRC, Río Cuarto, Córdoba, Argentina. [5] INCIVET-CONICET-UNRC, Río Cuarto, Córdoba, Argentina.

In previous studies, the adjuvant effect of the *Minthostachys verticillata* essential oil (EO) was demonstrated by increase in the immunogenicity of different antigens. The aim of this work was to evaluate the effect of the biosurfactant surfactin, isolated from *Baccillus subtilis*, on the adjuvant potential of nanoemulsified *M. verticillata* EO (NEOS). The NEOS was synthetized with 20% v/v of EO, 1% of surfactin (0.1 mg/mL), 1% of Tween 80 and 78 v/v of distillated water; the nanodroplet size was 143 nm (PDI=0,21). The vaccine formulations were performed with inactivated enterotoxigenic *Escherichia coli* (ETEC) as antigen ($1x10^9$ UFC/mL), with either safe concentrations of NEOS (1; 0.75 and 0.5 mg/mL), incomplete Freund's adjuvant (IFA) (50%), EO (1 mg/mL), surfactant mixture (Tween80/surfactin 1mg/mL) or saline solution. Saline-treated animals were included as a negative control. Mice were divided into eight groups of four animals each and vaccines were administrated subcutaneously on days 1, 14, 28 and 42. Animals were kept under controlled temperature, provided ad libitum access to food and water and were sacrificed 7 days after the last inoculation. For each group, a pool of sera was obtained by centrifugation and anti-ETEC IgG titers were determined through the indirect ELISA method. Assays were performed by triplicate. The results showed that EO administered as adjuvant increased the IgG titers compared with ETEC group ($p<0.05$) though the levels of antibodies induced by EO were lower than IFA ($p<0.001$). Formulations containing surfactin (NEOS) failed to induce a specific anti-ETEC antibody response. Moreover, the IgG titers induced by NEOS was significant lower compared to EO ($p<0.01$) suggesting that surfactin decreased the adjuvant potential of EO. These results are interesting since they open a possibility to investigate the anti-inflammatory effect of NEOS.

817. (142) **INACTIVATED *ENTEROTOXIGENIC Escherichia coli (ETEC) VACCINE WITH A NATURAL-BASED NANO-ADJUVANT ENHANCES SPECIFIC IgG SERUM LEVELS IN MICE***

María Eugenia Cecchini[1], Ivana Dalila Montironi[2], Sofía Arsaute[1], José Raviolo[3], Nahuel Matías Camacho[4], María de Lourdes Vancolli[5], Santiago Daniel Palma[4], Fernando Javier Mañas[6], Romina Valeria Bellingeri[7], Laura Noelia Cariddi[1]

[1] INBIAS, CONICET- UNRC, Río Cuarto, Córdoba, Argentina. [2] Cátedra de Farmacología, FAV, UNRC, Río Cuarto, Córdoba, Argentina. [3] Departamento de Producción Animal, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina. [4] UNITEFA, CONICET- UNC, Córdoba, Argentina. [5] Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Río Cuarto Córdoba, Argentina. [6] INCIVET, CONICET- UNRC, Río Cuarto, Córdoba, Argentina. [7] IITEMA, CONICE-TUNRC, Río Cuarto, Córdoba, Argentina.

The immunoadjuvant potential of *Minthostachys verticillata* essential oil (EO) has been previously demonstrated. Therefore, this study aimed to evaluate the adjuvant effect of the EO-based nanoemulsion (NEO) incorporated in an experimental inactivated vaccine against enterotoxigenic *Escherichia coli* (ETEC). The NEO was synthetized with 20% v/v of EO, 1% v/v of a surfactant mixture (0.75% Tween 80 and 0.25% Span 60) and 79% v/v of distillated water; the nanodroplet size was 105.6 nm (PDI=0,301). Binding between the NEO and ETEC was assessed by SEM. Experimental vaccines were prepared under aseptic conditions with formaldehyde-inactivated ETEC at $1x10^9$ UFC/mL with either safe concentrations of NEO (1; 0.75 and 0.5 mg/mL), incomplete Freund's adjuvant (IFA) (50%), EO (1 mg/mL), surfactant mixture (Tween80/Span60 1 mg/mL) or saline solution. Saline-treated animals were included as a negative control. Mice were divided into eight groups of four animals each and vaccines were administrated subcutaneously on days 1, 14, 28 and 42. Animals were kept under controlled temperature, provided *ad libitum* access to food and water and were sacrificed 7 days after the last inoculation. For each group, a pool of sera was obtained by centrifugation and anti-ETEC IgG titers were determined through the indirect ELISA method. Assays were performed by triplicate. Results showed that IgG titers of the groups that received the vaccines formulated with NEO as adjuvant were significantly increased ($p<0.05$) compared to control groups without adjuvant, reaching similar values of those in IFA group. Moreover, IgG titers produced by the NEO at concentrations of 1 and 0.5 mg/mL were significantly higher ($p<0.05$) than those that received the EO-adjuvanted formulation, suggesting that the process of nanoencapsulation enhanced the immunomodulating activity of the EO.

818. (211) **KINETIC AND SUPPRESSOR FUNCTION OF CD11b+ GR-1+ SPLENOCYTES IN THE PRECLINICAL ASSESSMENT OF THE TSf-ISPA VACCINE AGAINST *TRYPANOSOMA CRUZI***

Estefanía Prochetto1, Eliana Borgna1, Juan Cruz Gamba1, Florencia Pacini2, Mónica Vermeulen3 Diego Arias4, Ana Rosa Pérez2, Iván Marcipar1, Gabriel Cabrera1

1Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. 2Instituto de Inmunología Clínica y Experimental de Rosario, IDICER-CONICET and Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina. 3 Laboratorio de Inmunología Oncológica, Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires Argentina. 4Laboratorio de Enzimología Molecular, Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Santa Fe, Argentina

Introduction: We have previously reported that a vaccine based on a trans-sialidase fragment (TSf) formulated with a cage-like particle adjuvant (ISPA) protects BALB/c mice against *Trypanosoma cruzi* (*T. cruzi*) infection. Alterations of CD11b+Gr-1+ (Ly6G+/Ly6C+) cells, with a phenotype compatible with myeloid-derived suppressor cells (MDSCs), were observed during both the immunization and the acute phase of infection. Aim: To study the kinetic and suppressor function of CD11b+ Gr-1+ splenocytes in the preclinical assessment of the TSf-ISPA vaccine against *T. cruzi*. Methods: 3 doses of TSf-ISPA were given to BALB/c mice. For the challenge, immunized mice were intraperitoneally infected with 1000 *T. cruzi* (Tulahuen strain). Flow cytometry was used to measure CD11b+ GR-1+ splenocytes at days 2, 7 and 15 post-immunization (p.imm.), and at days 15 and 21 post-infection (p.i.). Magnetic cell sorting was used to purify Gr-1+ cells. Suppressive capacity of purified cells was assessed *in vitro* by measuring the inhibition of proliferation of responder cells labelled with 5,6-carboxifluorescein diacetate succinimidyl ester (CFSE). Results: CD11b+GR-1+ splenocytes in-

creased p.imm. For instance, at day 7 p.imm. the mean number of CD11b+GR-1+ cells x10^6 ±standard deviation (SD) in PBS-treated mice was 1,4±0,1 vs 4,1±0,6 in TSf-ISPA mice ($p<0.05$). GR-1+ cells purified at day 7 p.imm. or at day 21 p.i. suppressed the proliferation of stimulated CD4+CFSE+ cells (responders) *in vitro.* Example: in the p.imm. analysis, the % of CD4+CFSE+ cell proliferation ±SD in control responder cells was 10,8±1,8 vs 6,4±0,1 in responders + GR-1+ cells ($p<0.05$). Discussion: CD11b+GR-1+ splenocytes were increased p.imm and at day 21 p.i., showing suppressive capacity *in vitro.* Since MDSCs may play an important role in both stages: during immunization and then in the outcome of the infection, targeting that population may represent a valuable strategy to improve a vaccine candidate against *T. cruzi.*

819. (280) **CHARACTERIZATION AND OPTIMIZATION OF A NANOPARTICLE-BASED VACCINE**

Camila Chavero[1], Daiana Bianchi[1], Gastón Rizzo[1], Eugenia Apuzzo[2], Santiago Esteban Herrera[2], Maximiliano Luis Agazzi[2], María Lorena Cortez[2], Waldemar Marmisollé[2], Omar Azzaroni[2], Guillermo Docena[1], Paola Smaldini[1]

[1] Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP-UNLP-CONICET);

[2] Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA-UNLP-CONICET).

Nanotechnology is a significant breakthrough and represents an area of research that has been expanding in recent decades. Nowadays, nanoparticles play an important role in immunology and are being exploited to develop novel vaccines. Our group is working in the nanoscience field and we aimed to characterize different nanoparticles (Np) as adjuvants for mucosal and systemic vaccines. Different Np were characterized in terms of cell activation and production of cytokines by ELISA and flow cytometry and capacity of immune activation in mice. Balb/c mice were grouped and intraperitoneally immunized with OVA in combination with Np1 (VAC1) or Np2 (VAC2) at two different concentrations with a 3-week interval (Group 1 OVA; Group 2 VAC1 150 ug/ml; Group 3 VAC 1 60 ug/ml; Group 4 VAC2 150 ug/ml; Group 5 VAC2 60 ug/ml). Twenty-one days after the second dose, humoral (IgG, IgG1, IgG2a) and cellular (CD4, CD8, IFNγ) immune responses were evaluated by ELISA and Flow Cytometry, respectively. Cell line experiments showed that Np2 promoted significantly greater cell activation, with higher IL-1β secretion than Np1 and the positive controls for inflammasome activation. *In vivo* experiments showed that mice immunized with VAC2 presented higher levels of serum and bronchoalveolar lavage specific-OVA IgG than the other groups ($p<0.05$), with predominant production of specific IgG2a ($p<0.05$). Also, VAC2-vaccinated mice showed a higher frequency of LT CD4+IFN-γ+ and LT CD8+IFN-γ+ cells than the other groups ($p<0,05$). In conclusion, Np2 showed a superior profile of inflammasome activation compared to Np1 and VAC2 promoted immune induction at lower doses. These results are promising for developing therapeutic or preventive systemic and mucosal vaccines.

820. (334) **EVALUATION OF CELL WALL-DERIVED PARTICLES FROM *LACTOCOCCUS LACTIS* WITH FLAGELLIN AS AN ADJUVANT FOR MUCOSAL IMMUNIZATION**

Dalila Silvestre[1,2], Griselda Moreno[3], Marcelo H Argüelles[1], Marina E Biedma[3], Julieta Tomás Fariña[1,2], Estefania S Peri Ibañez[1,2], Marcelo G Mandile[1,2], Graciela Glikmann[1], Alejandro A Castello[1], Martín Rumbo[3], C Facundo Temprana[1,2]

[1]Laboratorio de Inmunología y Virología, UNQ, Argentina. [2]CONICET, Argentina. [3]Instituto de estudios Inmunológicos y Fisiopatológicos-CONICET, UNLP, Argentina.

Lactic acid bacteria have emerged as a potential antigen delivery system for mucous surfaces. In particular, our aim is to develop a platform based on *Lactococcus lactis* cell wall-derived particles (CWDP). We previously generated CWDP that contained rotavirus VP6 protein, which conferred protection against infection when co-administered intranasally with dmLT in a murine model. Because flagellin has been proposed as a mucosal adjuvant, our goal is to increase the immunogenicity of CWDP using FliC131, a mutant of *Salmonella* flagellin. We generated *L. lactis* that express FliC131 on their cell wall. The CWDP-FliC131 obtained were evaluated by SDS-PAGE, Western blot, and flow cytometry. Moreover, the concentration of FliC131 was determined by SDS-PAGE and bands densitometry. The ability of CWDP-FliC131 to activate the TLR5 receptor was evaluated *in vitro* with the HEK-hTLR5 reporter cell line. The results confirmed the identity of FliC131 and showed a concentration of 2,1 mg/ml. Additionally, CWDP-FliC131 stimulated TLR5 under the tested conditions. To evaluate the adjuvanticity of CWDP-FliC131 *in vivo*, groups of five mice were immunized intranasally with PBS, OVA, CWDP-FliC131, OVA plus CWDP-FliC131 or OVA plus CWDP-NZ9000 (derived from non-recombinant *L. lactis* NZ9000). After three doses, samples of serum, bronchoalveolar lavage (BAL), and intestinal lavage were collected. Both CWDP-FliC131 plus OVA (CFO) and CWDP-NZ9000 plus OVA (NZO) induced anti-OVA IgG and IgA detectable by ELISA in serum and BAL. Even though there were no statistically significant differences, four mice in the CFO group tested positive for serum IgA versus only one in the NZO group. In conclusion, CWDP were immunogenic when administered intranasally, although the adjuvant effect of FliC131 needs to be further tested using different doses of CWDP-FliC131 and increasing the number of mice to reduce the variability associated with the intranasal immunization and the biological response of each mouse.

821. (358) **COMBINING THE FC REGION WITH AN ANTI-CD11C NANOBODY FOR SYNERGISTIC ADJUVANT EFFECT IN CHIMERIC ANTIGENS**

Carolina Padula[1], Martín Rossotti[2], Gabriel Lassabe[1], Gualberto González-Sapienza[1].

[1]Área Inmunología, DEPBIO, Facultad de Química, Universidad de la República, Montevideo, Uruguay, [2]Human Health Therapeutics Research Centre, National Research Council Canada, Ottawa, Canada.

The Fc region of antibodies enhances the immune response against coupled antigens; on the other hand, delivering antigens to dendritic cells (DCs) by targeting specific receptors with antibodies, also has proven effective in increasing the immune response in mice. Our group has developed single-domain antibodies or nanobodies (Nbs) against the murine receptor CD11c, a distinctive marker for DCs, which has already been studied as a target for delivering antigens coupled to conventional antibodies, so we aim to explore the potential of our Nbs for this purpose. Initially, the targeting ability of our anti-CD11c Nbs was explored by generating a chimeric protein with the Fc region of human IgG1 (Nb-hFc), which was expressed in HEK293T cells as an homodimer. In C57BL/6 mice immunized with a single 2 µg dose of these chimeric protein without adjuvant, the IgG titer against hFc was ≈1/10000 at day 7, hundred times higher than that obtained with an irrelevant Nb instead of an anti-CD11c one. Even more, this response increased an additional 10-fold when including a goat anti-human antibody as a crosslinking agent. When trying to apply the potential of our anti-CD11c Nbs to chimeric proteins with model antigen ovalbumin, it was found that not only multivalence was an important aspect to obtain a high antibody response with a single-low-dose immunization —since monomeric anti-CD11c-ovalbumin fusion protein failed to induce a significant response—, but also the presence of the hFc, showing that the adjuvant effect of the Fc region and the targeting ability of the anti-CD11c Nb work synergistically and have great potencial for boosting the immune response against vaccination antigens. Currently, we are working in the development of new chimeric antigens that include both the Fc and the anti-CD11c domain, and in the introduction of CD40-agonist nanobodies as activating domains, for which several potential candidates have been isolated by the phage display technique.

822. (361) **REVERSE VACCINOLOGY APPROACH FOR IDENTIFICATION AND PRIORITIZATION OF ANTIGEN CANDIDATES FOR A VACCINE AGAINST A POULTRY PATHOGEN**

M. Esperanza Felici [1,2], Yosef D. Huberman [3], Belkys A. Maletto* [1,2], Rodrigo Quiroga* [4,5]

1 Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Bioquímica Clínica, Córdoba, Argentina; 2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI), Córdoba, Argentina; 3 Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Balcarce, Área de Investigación y Producción animal, Bacteriología, Balcarce, Buenos Aires, Argentina; 4 Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Química Teórica y Computacional, Córdoba, Argentina; 5 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC-CONICET). Córdoba, Argentina.
**This authors contributed equally*

Avibacterium paragallinarum is the causative agent of infectious coryza, an acute disease that affects the upper respiratory system of chickens. This Gram-negative bacteria is widely distributed in poultry production systems all over the world, causing significant economic losses. Despite vaccination being the main form of prevention, commercially available vaccines show incomplete protection against strains not included in the formulation. As a first step to develop a more effective vaccine, we implemented Reverse Vaccinology strategies to identify potential antigens present in 28 *Av. paragallinarum* strains. Comparative and subtractive genomics and prediction of several protein attributes (sequence conservation, antigenicity, essentiality, homology to the host, subcellular localization and adhesin probability, among other features) were used to narrow down and prioritize proteins with ease of expression and protection potential. To validate this computational workflow, the same pipeline was applied to a dataset of 1085 proteins with experimentally known antigenicity, collected from published literature and various antigen and/or epitope databases. Among the possible antigens (~4% of each strain proteome), 25 proteins were conserved in all strains. Outer membrane protein assembly factor BamA, porin OmpA, TolC family protein, translocation-assembly module TamB and LPS assembly protein LptD were classified as the most prominent candidates qualifying all the set criteria. Furthermore, more than 27% of the positive control dataset were correctly identified as potentially protective antigens. Despite the imposed restrictions lead to the loss of many experimentally verified antigens, the applied restrictive criteria proved to be useful for our goal of protein prioritization. Although *in vivo* testings are still needed, this study provides a basis for the development of a novel subunit vaccine against *Av. paragallinarum*.

823. (415) **VACCINATION STRATEGY BASED ON LPS-ACTIVATED DENDRITIC CELLS INDUCES CD8⁺ T CELL RESPONSE CAPABLE OF CONFERRING PARTIAL PROTECTION AGAINST *TRYPANOSOMA CRUZI* INFECTION**

Lucía Biscari[1], Cintia D. Kaufman[1], Cecilia Farré[1,2], Victoria Huhn[3], María Florencia Pacini[1], Camila Bulfoni Balbi[1], Karina A. Gómez[4], Ana R. Pérez[1], Andrés Alloatti[1]
[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER/CONICET- UNR).
[2]Centro de Investigación y Producción de Reactivos Biológicos (CIPREB/FCM-UNR).
[3]Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario
[4]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular Dr. Héctor N. Torres (INGEBI/CONICET).

CD8⁺ T cells are key components of the immune response against *Trypanosoma cruzi*, and hence, the design of vaccines able to induce such responses is a promising strategy. In this work, a vaccination strategy based on mouse bone marrow-derived dendritic cells (BMDCs), incubated with a parasitic epitope named TsKb20 – derived from the transialidase protein of *T. cruzi* – and activated with LPS, was designed in order to induce a TsKb20-specific CD8⁺ T cell response. The experimental scheme was as follows: C57BL/6 mice were immunized intravenously with 50,000 TsKb20-loaded BMDCs activated with LPS, followed by a boost two weeks later. One group of animals was immunized with LPS-activated BMDCs not loaded with peptide (negative control). Fifteen days after the boost, cell suspensions derived from lymph nodes were cultured for 15 h with 50 µM TsKb20. The specific CD8⁺ T cell response was measured by flow cytometry evaluating CD25⁺ and CD69⁺ activation markers in the CD8⁺ T cell population. Through non-parametric Mann-Whitney test, it was found that the TsKb20- specific CD8⁺ T cell response in mice immunized with peptide-loaded BMDCs was significantly higher than in negative control animals. The same results were obtained by measuring IFN-γ production by ELISPOT after restimulation with the peptide, or by staining with specific tetramers. Subsequently, another pool of animals was immunized and then challenged with 2000 *T. cruzi* trypomastigotes. Female mice, but not male mice, showed lower parasitemia and increased survival compared to negative control animals. These results suggest that the adoptive transfer of BMDCs could be used as a strategy to induce anti-*T. cruzi* CD8⁺ T cell responses, although these appear to be protective only in female mice possibly due to sexual dimorphism in the immune response generated upon infection with *T. cruzi*.

824. (436) **IMMUNIZATION WITH THE NOVEL VACCINE CANDIDATE BASED ON OUTER MEMBRANE VESICLES IMPROVE RESISTANCE OF MICE TO *BORDETELLA PERTUSSIS* INFECTION**

Erika Rudi, Emilia Gaillard and Daniela Hozbor
Laboratorio VacSal. Instituto de Biotecnología y Biología Molecular (IBBM) Facultad Ciencias Exactas UNLP-CONICET

Pertussis is a severe respiratory disease that can be fatal, particularly in infants. Despite high vaccine coverage, the disease remains a problem because the currently used acellular vaccines (2nd-generation) do not completely prevent infection or transmission. Because of this, a 3rd-generation of vaccine capable of overcoming the weaknesses of current formulations is needed. Preclinical studies using the intranasal-challenge mice model showed that our vaccine candidate based on outer-membrane-vesicles (OMVvac) is an attractive candidate because of its safety and immunogenic properties. In this study we evaluated the ability of the OMVvac to prevent *B. pertussis* nasal colonization (infection). We evaluated 3 doses-OMVvac schemes in 3 different immunization routes in Balb/C (Th2/M2-dominant immune response) and C57BL6 (Th1/M1-dominant immune response) mice strains: intramuscular (IM); intranasal (IN) and sublingual (SL). After the challenge with sublethal doses of *B. pertussis* ($5x10^7$CFU/40µl) we detected that while in both mice strains SL route did not prevent nasal colonization, the use of OMVvac in both IN and IM scheme was capable to reduce nasal bacterial colonization compared to that detected in non-immunized animals ($p<0.05$). In comparison with Balb/C strain, the IN OMVvac immunization in C57BL6 mice was more efficient in controlling nasal colonization (reduction respect to the control: 1.3 *vs* 0.58 orders; $p<0,05$). Of interest, in the C57BL6 mice strain IN route induced the highest nasal IgA levels ($p<0.05$). In both mice strains higher levels of IL-17 (C57BL6 IN: 10322pg/ml± 105pg/ml IM: 5821pg/ml± 93pg/ml; Balb/C IN: 10364pg/ml± 276 pg/ml IM: 9428 pg/ml± 1012pg/ml) were detected either in IN or IM OMVvac scheme in comparison to those detected in SL route. INF-ɣ levels were also higher in both strains for these routes ($p<0.05$). The results obtained support the use of IN or IM OMVvac scheme to reduce *B. pertussis* transmission.

825. (519) **A MULTIANTIGENIC PROTEIN VACCINE PROVIDES PARTIAL PROTECTION AGAINST CHRONIC TOXOPLASMA INFECTION IN MICE**

PASTOR Victoria[1], ARCON Nadia[1], FENOY Ignacio[1], SANCHEZ Vanesa[1], GOLDMAN Alejandra[1], MARCIPAR Iván[2], MARTIN Valentina[1]
[1]Laboratorio de Vacunas y Alergia. Centro de Estudios en Salud y Medio Ambiente, ITECA, EcyT, UNSAM-CONICET.
[2]Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

Toxoplasmosis is a disease that affects 30% of the world's population. At present, there are no pharmacological treatments that eliminate the parasite or vaccines that confer protection to the host. The aim of the present work was to study the immunogenicity of vaccines

based on the mix of recombinant proteins combined with the new cage-like particle adjuvant (ISPA). Methods: C57BL/6 mice were intradermally immunized 2 or 3-times with a 2-week interval with different combinations of rGRA7 (G), rTgPF (T) or rROP2 (R) proteins along with ISPA adjuvant. Control groups received ISPA or PBS. Fourteen-days later, mice were orally challenged with *T. gondii* cysts and after one month, brain cyst numbers were determined under optical microscope. *In vitro* antigen-specific responses were characterized in sera and spleens (S) from vaccinated mice. Results: Significant reduction in brain parasite load was obtained with the mix of the three proteins R+G+T+ISPA (*p<0.05)*. All experimental groups (R+G, R+T and R+G+T) elicited strong humoral responses *(IgG:p<0.005)* with a mixed profile (Th1/Th2). R+G+T+ISPA formulation induced increased levels of IFN-γ producing CD4+ and CD8+ cells in spleen *(p<0.05).* On the other hand, when the Ag-specific responses were studied in spleen, it was observed that rTgPF induced a Th1 response in all experimental groups since only IFN-γ production was detected *(p<0.05)*. ROP2 induced a Th1/Th2 response with IFN-γ and IL4 production *(p<0.05)* in all vaccinated groups, and GRA7 generated a response with a mixed profile only in the R+T+G+ISPA group *(p<0.05)*. Additionally, all the antigens induced IL-10 secretion in R+T+G+ISPA group while only rTgPF induced its production in R+T+ISPA group *(p <0.01)*. Conclusion: The present results indicate that R+G+T formulation in combination with ISPA adjuvant was able to enhance and modulate the specific responses generating partial protection against chronic *Toxoplasma* infection.

826. (562) **IMMUNOGENICITY OF SPUTNIK V, BBIBP-CorV and ChAdOx-1S VACCINES IN NAÏVE AND PREVIOUSLY INFECTED INDIVIDUALS FROM TANDIL, ARGENTINA**

Silvina Elena Gutiérrez[1,2], Mariana Rivero[1,2], Marcelo Rodríguez[1], Silvia Marcela Estein[1,2]

Departamento de Salud Animal y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA); Centro de Investigación Veterinaria de Tandil (UNCPBA-CICPBA-CONICET), Tandil, Buenos Aires, Argentina.

Mass vaccination has been the key element in controlling the COVID-19 pandemic. Vaccination in Tandil began in January 2021 for health care workers, which mainly received Sputnik V (SpV). The inactivated vaccine from Sinopharm (Si) and the ChAdOs-1S vaccine from AstraZeneca (AZ) were available since March 2021. We aimed to measure and compare anti-SARS CoV-2 spike (S) antibodies in naïve and seropositive individuals after receiving first and second dose of different vaccines. A total of 141 individuals were recruited between January and August 2021. Serum samples were obtained before vaccination and after each vaccine dose. Specific anti-S antibodies were quantified using the COVIDAR IgG ELISA. Results were log transformed and a repeated measures ANOVA was adjusted. Seventy-one individuals had detectable anti-S antibodies due to previous infection while 70 were seronegative before receiving the first dose (initial status). Fifty-four from 71 (76%) of the initially seronegative participants seroconverted after the first dose. All but one of the non-seroconverters received the Si vaccine. After the second dose 95.8% of the participants seroconverted. Differences were observed depending on the initial status, type of vaccine and number of doses received (p= 0.0017). The titer of antibodies elicited against the first dose was influenced by the initial status of the individual for the 3 vaccines. After the vaccination scheme was completed, there were also differences in the level of specific antibodies achieved depending on the initial status in those who received the adenovirus vectored vaccines. Both in naïve and sensitized participants SpV and AZ induced higher levels of antibodies compared to Si (p> 0.05). Sinopharm resulted the less immunogenic vaccine. This result may be due to the interval between shots, which was 47 days mean, instead of the recommended 21-30 days. Therefore, individuals vaccinated with 2 doses of Si may be benefited from an heterologous booster.

827. (595) **NANOPARTICLE-BASED VACCINES TRIGGER Th1-DEPENDENT HUMORAL AND CELLULAR INMUNE RESPONSES TO TREAT OR PREVENT INFECTIOUS AND NON INFECTIOUS DISEASES**

Bianchi Daiana Stephanie[1], Chavero Camila[1], Rizzo, Gaston.[1], Apuzzo Eugenia[2], Herrera Santiago Esteban[2], Agazzi Maximiliano Luis[2], Cortez Lorena Maria[2], Marmisolle Waldemar[2], Azzaroni Omar[2], Smaldini, Paola[1], Docena Guillermo[1].

1 Instituto de estudios inmunológicos y fisiopatológicos, (IIFP-UNLP-CONICET), La Plata, Argentina. 2 Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas, Universidad Nacional de La plata (INIFTA-UNLP-CONICET), La Plata, Argentina.

** E-mail: bianchids@hotmail.com*

Nanoparticle-based vaccines are used as novel immunointervention strategies to prevent or treat infectious and non-infectious diseases through the induction of T-dependent humoral and cellular effector mechanisms. We aimed to optimize a vaccination strategy and compare nanoparticles (Np) with other commonly used adjuvants. BALB/c mice were intraperitoneally administered with Np, Alum and Alum-CpG, and different concentrations of OVA as immunogen. The humoral (IgG, IgG1, IgG2a) and cellular immune responses were evaluated by ELISA and Flow Cytometry, respectively. Different control groups that received only the adjuvant or immunogen were included. We observed that Np triggered higher OVA-specific IgG antibodies in serum and bronchoalveolar lavage (BAL) than alum-based adjuvants (p<0.05), with higher levels of OVA-specific IgG2a (3595±717,1; 359,1 ± 67,78 and 618,7 ± 222,5 for Np, alum and alum+CpG, respectively). The analysis of the cellular immune response showed that splenocytes from mice vaccinated with OVA/Np showed the most prominent production of IFN-γ. We also observed that the cell source of IFN-γ was T cells, as the frequency of $CD4^+IFN\text{-}\gamma^+$ and $CD8^+IFN\text{-}\gamma^+$ cells were significantly increased only in Np/OVA mice (p<). In conclusion, our findings showed that the nanoparticles, which could be used as a carrier for mucosal vaccines, are potent adjuvants that promoted Th1-dependent humoral and cellular immune responses (p<0.05), with mucosal IgG and high production of IFN-γ. This Np-based vaccine could be exploited in systemic and mucosal vaccines for infectious and non-infectious vaccines as preventive (viral infectious) or therapeutic (allergy and tumors) immunointervention strategies.

828. (611) **STUDY OF ANTIGEN-FREE IMMUNOSTIMULANTING COMPLEXES IN A MURINE MODEL**

Florencia S. Satler[1], Ivana G. Reidel[1,2], Noelia Flor[1], María I. Garcia[1], Cristina N. Diez[1], Álvaro Siano[3], Verónica Húmpola[3], Carolina M. Veaute [1]

[1]Laboratorio de Inmunología Experimental, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Department of Immuno-Oncology, Beckman Research Institute of City of Hope, Duarte, CA, USA.[3]Laboratorio de Péptidos Bioactivos, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

The non-specific stimulation of the immune system is an interesting strategy for the prophylaxis and early treatment of infections in circumstances where there is a lack of specific treatments such as vaccines available. This work aimed to evaluate different formulations as non-specific immunostimulants in a murine model. The formulations were based on an ODN-CpG combined with 4 gemini-type lipopeptides: AG2-C16, AG2-C18, AG10-C16 and AG10-C18. The optimal *gemini*:ODN-CpG ratio to achieve the ODN-CpG encapsulation was determined using agarose electrophoresis. The immunostimulatory capacity was studied in vitro and in vivo by qPCR. Total splenocytes collected from BALB/c mice, without prior treatment, were stimulated in vitro with the formulations or the individual components. No stimulation was used as basal condition (control). Formulations based on AG2-C16 were evaluated in vivo using BALB/c mice immunized with 3 doses. Control group was immunized with buffer. Lymph nodes were harvested 72hs after last dose for RNA purification. In both studies, the expression levels of these genes were measured: IFN-γ, TNF-α, IL-6 and CD-80. Results were expressed as ΔΔCT compared to the corresponding control, using GAPDH as housekeeping. Stat. anal.: Mann-Whitney test. In

the evaluated ratios, 3/4 *gemini* were able to encapsulate the ODN-CpG: AG2-C16, AG2-C18 and AG10-C16. In vitro studies, demonstrated that the *gemini*+ODN-CpG formulations, mainly those with AG2-C16 and AG2-C18, are capable of increasing the expression of IFN-γ, TNF-α and IL-6 (median $2^{-\Delta\Delta CT} \geq 2$). In vivo, the concomitant use of AG2-C16 and ODN-CpG was associated with an increase in IFN-γ levels (median $2^{-\Delta\Delta CT} \geq 2$). Even no significant differences were detected, a clear stimulation tendency was observed in both experiments. These results represent the first evidence that formulations based on these novel *gemini* lipopeptides and ODN-CpG, could be used as non-specific immunostimulants.

829. (657) **COMBINATION OF BACULOVIRUS AND FLAGELLIN AS A VACCINE FORMULATION**

Francisco Carriquiriborde*, Guido Molina**, Paula Molinari**, Oscar Taboga**, Martin Rumbo*, Marina Biedma*.
**Instituto de estudios Inmunológicos y Fisiopatológicos (IIFP)-CONICET-UNLP-CIC*
***Instituto de Agrobiotecnologia y Biologia Molecular (IABIMO)-CONICET-INTA Castelar*

Viral vectors are a relatively new vaccine platform that relies on recombinant viruses to deliver selected immunogens into the host. Among them, *Autographa californica multiple nuclear polyhedrosis* baculovirus (BV), categorized as a nonhuman viral vector with versatile features, has been positioned as an excellent vaccine vector. The final goal of the project is to combine flagellin (FliC), a TLR-5 ligand, with BV, in a single structure aiming to generate a synergistic immune response. To try out this hypothesis, in this study we combine FliC and BV as a vaccine formulation and analysed both in vitro and in vivo immune responses. Using TLR5 reporter cell lines we observed that FliC agonist activity is not impaired between 0-100 MOI of BVs (p<0,0001). At this MOI BVs showed a strong production of IL-1 β in THP-1 cell lines and interferon type I in α/β interferon reporter B16 cell line (p<0,0001). For in vivo studies, four groups of female Balb/c mice were intranasally immunized with different doses ($1,5x10^5$ or $1,5x10^7$ pfu/dose) of either BV or coadministered with a unique dose of 2μg of FliC. The vaccinations were repeated 3 times every 15 days. Treatments turned out to be safe according to WHO approved mouse weight gain test and no rise of IL-6 in serum at 4hs post first vaccination. BV and FliC specific serum IgG and mucosal IgA antibodies were quantified by indirect ELISA. The results revealed that mice co-administered with low dose of BV and FliC had an enhanced serum IgG immune response against BV compared with mice administered with other combinations (p<0,0001). . An increased mucosal IgA response against BV was also observed in the presence of FliC (p<0,0001). Our findings are indicative that the addition of flagellin improve the immune response exhibited by the viral vector alone. These results encourage us to develop a new vaccine platform based in BVs and FliC expecting to obtain a more powerful immunostimulatory properties than its components.

830. (680) **INNATE IMMUNITY AGAINST *TRYPANOSOMA CRUZI* IN IMMUNIZED ANIMALS IS AFFECTED BY SEXUAL DIMORPHISM**

Camila Bulfoni Balbi[1], Ma. Florencia Pacini[1], Brenda Dinatale[1], Florencia González[1], Eliana Borgna[3], Estefania Prochetto[3], Cecilia Farré[1,2], Gustavo Chapo[2], Iván Marcipar[3], Gabriel Cabrera[3], Ana Rosa Pérez[1,2].
[1] Instituto de Inmunología Clínica y Experimental de Rosario (IDICER CONICET UNR), [2] Centro de Investigación y Producción de Reactivos Biológicos (CIPReB) Facultad de Cs. Médicas (UNR), [3] Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas (UNL).

Vaccine efficacy may be modulated by different parameters like sex, among others; so, the inclusion of both sexes becomes relevant. Typically, vaccine studies against *T. cruzi* (Tc) have been focused on adaptive response evaluation. We previously showed that a nasal vaccine formulated with Trans-sialidase (TS) plus c-di-AMP was immunogenic, in terms of adaptive response, while females (F) showed better responses than males (M). Here we aim to evaluate possible variations in the innate response induced in F and M infected BALB/c mice previously immunized with our vaccine. We mainly focused on cells resembling myeloid-derived suppressor cells (rMDSC). For this, mice (n=5/group) were intranasally immunized in 3 doses, one every 2 weeks with 10 μg of TS emulsified in c-di-AMP adjuvant (TS+c-di-AMP). Other groups of mice were treated with saline (S), TS or c-di-AMP alone. Fifteen days after the last immunization, mice were orally challenged with 3000 Tc. Both rMDSC lineages (rG-MDSC: $CD11b^+Ly6C^{+/low}Ly6G^+$; rM-MDSC: $CD11b^+Ly6C^{+/low}Ly6G^-$) were evaluated at 21 days post-infection (dpi) by flow cytometry. Parasitemia and clinical score were recorded until 50 dpi. Overall, M mice showed increased parasitemias compared to the analogous groups of F (i.e.,17 dpi TS+c-di-AMP, p<0.05 M vs F), while clinical involvement was less evident in F than in M (p<0.05). In F, rM-MDCS frequencies were diminished in TS+c-di-AMP and TS groups compared with Tc (p<0.05); while in both sexes, rG-MDCS frequencies were significantly lower in S and TS+c-di-AMP animals than Tc (p<0.05). When comparing between sexes, M showed higher percentages of rG-MSDC than F in TS and TS+c-di-AMP (p<0.05 in all cases), but not in infected groups. We can conclude that there are quantitative differences in the response of rMDSC cells between M and F after the administration of a TS-based vaccine formulation. Functional studies may help to understand the role of MSDCs in the context of this experimental vaccine.

831. (684) **MUCOSAL VACCINE BASED ON TRANS-SIALIDASE PROTECTS AGAINST ACUTE AND CHRONIC DAMAGE AFTER ORAL INFECTION WITH TRYPANSOSOMA CRUZI**

Pacini MF[1], Bulfoni Balbi C[1], Dinatale B[1], González FB[1], Farré C[1,2], Chapo G[2], Bottasso O[1], Cacik PI[3], Prochetto E[3], Marcipar I[3], Perez AR.[1,2].
1. Instituto de Inmunología Clínica y Experimental de Rosario (IDICER CONICET UNR),
2. Centro de Investigación y Producción de Reactivos Biológicos (CIPReB) Facultad de Cs. Médicas, Universidad Nacional de Rosario.
3. Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Oral Chagas disease is a frequent form of infection in some countries of Latin America. Although there are drugs for its treatment, currently there are no prophylactic vaccines to combat the disease. We previously showed that a nasal vaccine formulated with a N-terminal fragment of Trans-sialidase (TS) and combined with c-di-AMP(A) generated immunogenicity at mucosal and systemic levels, in terms of cellular and humoral response. Here, we evaluated prophylactic efficacy during acute (17 dpi) and chronic (110 dpi) phases. Thus, female BALB/c mice (n=4-7/group) were intranasal immunized (3 doses, one every 2 weeks). As control groups, we used mice not immunized (NI) or only treated with TS or c-di-AMP. After immunization, animals were orally challenged with 3000 Tulahuen strain/mice (sub-lethal challenge). Parasitemia and clinical affectation (score) were recorded during the course of experiments. Muscle and liver damage (plasma CK, GOT, GPT) were assessed during the acute phase. Parasite load (qPCR), cytokines (TNF-α and INF-γ by ELISA and CBA) and myocarditis were evaluated at acute and chronic phases by histopathology. Heart functional impairment was evaluated by electrocardiogram (ECG) at 110 dpi. Clinical affectation, parasitemia and, acute muscle and hepatic damage were less evident in TS+A [e.g. GPT (17 dpi, AU, meanESM), Tc: 1880958, TS:12481200, TS+A 25035, NI 10725] compared to the rest of the groups (in all cases, p<0.05). Mild acute and chronic myocarditis were recorded in TS+A, while Tc, TS and A groups showed moderated damage (overall, p<0.05). Plasma cytokines were also diminished in TS+A mice at 17 dpi. ECG showed less alterations (both in QRS duration and corrected QT interval) in TS+A compared with Tc, TS and A, but similar to NI mice. Taken together, these results showed that TS+c-di-AMP formulation may be a good vaccine candidate for the development of a prophylactic mucosal vaccine against oral *T. cruzi* infection.

832. (743) **IMMUNOLOGICAL STUDY OF COVID-19 VACCINE CANDIDATE BASED ON RECOMBINANT SPIKE TRIMER**

PROTEIN FROM DIFFERENT SARS-COV-2 VARIANTS OF CONCERN

Erika Rudi[1#], Pablo Martin Aispuro[1#], Eugenia Zurita[1], Maria M. Gonzalez Lopez Ledesma[2] Daniela Bottero[1], Juan Malito[3], Magali Gabrielli[1], Emilia Gaillard[1], Matthew Stuible[4], Yves Durocher[4], Andrea V. Gamarnik[2], Andrés Wigdorovitz[3], Daniela Hozbor1*

1- Laboratorio VacSal, Instituto de Biotecnología y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Centro Científico Tecnológico – Consejo Nacional de Investigaciones Científicas y Técnicas (CCT-CONICET), La Plata, Argentina

2- Fundación Instituto Leloir-IIBBA CONICET. Buenos Aires, Argentina IVIT.

3- INCUINTA INTA, CONICET, HURLINGHAM, Buenos Aires, Argentina, INTA Castelar, Buenos Aires, Argentina.

4 Human Health Therapeutics Research Center, National Research Council Canada, Montreal, Quebec, Canada.

The emergency of new SARS-CoV-2 variants that feature increased immune escape marks an urgent demand for better vaccines that will provide broader immunogenicity. Here, we evaluated the immunogenic capacity of vaccine candidates based on the recombinant trimeric spike protein (S) of different SARS-CoV-2 variants of concern (VOC), including the ancestral Wuhan, Beta and Delta viruses. In particular, we assessed formulations containing either single or combined S protein variants. Our study shows that the formulation containing the single S protein from the ancestral Wuhan virus at a concentration of 2 μg (SW2-Vac 2 μg) displayed in the mouse model the highest IgG antibody levels against all the three (Wuhan, Beta, and Delta) SARS-CoV-2 S protein variants tested. In addition, this formulation induced significantly higher neutralizing antibody titers against the three viral variants when compared with authorized Gam-COVID-Vac-rAd26/rAd5 (Sputnik V) or ChAdOx1 (AstraZeneca) vaccines. SW2-Vac 2 μg was also able to induce IFN-gamma and IL17, memory CD4 populations and follicular T cells. Used as a booster dose for schedules performed with different authorized vaccines, SW2-Vac 2 μg vaccine candidate also induced higher levels of total IgG and IgG isotypes against S protein from different SARS-CoV-2 variants in comparison with those observed with homologous 3-dose schedule of Sputnik V or AstraZeneca. Moreover, SW2-Vac 2 μg booster induced broadly strong neutralizing antibody levels against the three tested SARS-CoV-2 variants. SW2-Vac 2 μg booster also induced CD4+ central memory, CD4+ effector and CD8+ populations. Overall, the results demonstrate that SW2-Vac 2 μg is a promising formulation for the development of a next generation COVID-19 vaccine.

833. (752) **MATERNAL PRIMING IN INFANCY WITH WHOLE CELL BUT NOT ACELLULAR PERTUSSIS VACCINE LEADS TO A MORE DURABLE AND ROBUST PROTECTIVE RESPONSE IN NEONATAL MICE**

Pablo Martin Aispuro, Daniela Bottero, Daniela Hozbor

Laboratorio VacSal, Instituto de Biotecnología y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Centro Científico Tecnológico – Consejo Nacional de Investigaciones Científicas y Técnicas (CCT-CONICET), La Plata, Argentina

The introduction of massive vaccination in the 50s dramatically reduced the mortality associated with pertussis. In the last years, however the incidence rates have increased. Since in many countries the use of cellular vaccines (wP) to cover the primary doses was changed to acellular vaccine (aP), the controversy regarding the convenience of using one or another vaccine arose. To generate evidence in this regard and to determine immunological differences between aP and wP priming in neonates born to mothers vaccinated with aP during pregnancy (aPpreg), we used the intranasal challenge mouse model. We vaccinated female mice with wP-wP-aPpreg or aP-aP-aPpreg and measured offspring protection against *B. pertussis* challenge and specific-antibody levels with or without revaccination. Both immunization schemes protected the offspring against pertussis as evidenced by a reduction (5-6 logs,p<0.001) in CFUs recovered from lungs. Immunity acquired by wP-wP-aPpreg vaccination conferred offspring protection in all pregnancies occurred up to 22 weeks after receiving aPpreg-dose. The immunity induced by aPaPaPpreg schedule began to decline in births occurred 8 weeks after receiving the aPpreg-dose. This protective-capacity loss was overcome by either aP or wP neonatal vaccination. In puppies born to mothers with aP-aP-aPpreg-scheme, total a-HK-IgG levels were lower compared to those detected in puppies born to wP-wP-aPpreg mothers (p<0.05). Of interest, pups born to aP-aP-aPpreg dams had IgG1 (a-PT-IgG1=28.34±2.95) but not IgG2a, while puppies born to mothers with a wP-wP-aPpreg exhibited an IgG2a/IgG1 ratio close to 1. Th1 profile was confirmed by evaluating levels of INFY which was strongly increased after receiving neonatal booster with the wP (7 times of increase,p<0.05). Overall, the results show that, while short-term protective capacity induced by aPpreg boost overlap between aP- and wP-primed individuals, a subset of aP-primed individuals present a divergent response.

834. (760) **EVALUATION OF THE HUMORAL RESPONSE TO THE THIRD DOSE OF SARS-COV-2 VACCINES IN LIVER TRANSPLANT PATIENTS**

Nicolás Ducasa[1*], Paula Benencio[1*], Margarita Anders[2], Manuel Barbero[4], María Florencia Quiroga[1], Carolina Berini[1], Patricia Etcheves[5], Giampaolo Scarton[5], Ezequiel Mauro[3], Manuel Mendizabal[6*]y Mirna Biglione[1*]

*** Comparten autoría*

[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.[2]Hospital Alemán, Buenos Aires, Argentina. [3]Hospital Italiano, Buenos Aires, Argentina. [4]Hospital El Cruce, Florencio Varela, Buenos Aires, Argentina. [5]Bioars S.A. Buenos Aires. [6]Hospital Universitario Austral, Pilar, Argentina.

Liver transplant recipients (LTR) mount lower humoral responses to two doses of SARS-CoV-2 vaccines when compared to immunocompetent individuals (IC). We have previously evaluated the humoral response in LTR who received two doses of vaccines against SARS-CoV-2, and reported that 38% (46/120) of LTR do not produce anti-Spike antibodies, and that the heterologous regime rAd26/mRNA-1273 elicited a higher humoral response in LTR than ChAdOx-1, rAd26/rAd5 and BBIBP-CorV homologous regimes (Mendizabal M, et al, 2022, *Hepat. Commun.*). Thus, we set out to evaluate the humoral response after three doses of SARS-CoV-2 vaccines. Blood samples were extracted between 21 and 90 days after the third dose, from 81 RTH and 28 IC. Positive serology for anti-Spike (S1&S2) IgG antibodies and neutralizing antibody efficiency against ACE2/RBD binding were evaluated by ELISA. In addition, we evaluated the humoral response of LTR and IC that had acquired SARS-COV-2 infection after receiving the third dose. SARS-CoV-2 infection was confirmed by anti-N IgG ELISA. We found that 18% (12/68) of LTR were negative for anti-Spike IgG, while all of the IC mounted a measurable response. LTR who responded to the third dose showed significantly lower anti-Spike IgG levels compared to IC (p<0.05). Nonetheless, LTR who were infected with SARS-CoV-2 showed similar antibody levels compared to IC, whether they had been infected or not. When analyzing the immunosuppressive treatments received by LTR, we found that mycophenolate correlated with a lower IgG concentration than the other treatments (p<0.05). Regarding vaccine regimes, the heterologous regime rAd26/mRNA-1273 followed by a dose of mRNA-1273 elicited significantly higher responses in LTR. Our results show that the most potent vaccine regime for LTR is the heterologous rAd26/mRNA-1273 regime, followed by mRNA-1273 as a third dose. However, it would be necessary to analyze a larger amount of samples to deliver a general recommendation for this population.

835. (782) **A DISORDERED REGION RETAINS THE FULL PROTEASE INHIBITOR ACTIVITY AND THE CAPACITY TO INDUCE CD8+ T CELLS IN VIVO OF THE ORAL VACCINE ADJUVANT U-OMP19**

M. Laura Darriba[1], Celeste Pueblas Castro[1], Lorena M. Co-

ria[1], Laura Bruno[1], Lucía B. Chemes[1], Rodolfo M. Rasia[2], Sebastián Klinke[3], Juliana Cassataro[1], Karina A. Pasquevich[1,*].
[1] Instituto de Investigaciones Biotecnológicas (UNSAM-CONICET), Universidad Nacional de San Martín, Buenos Aires, Argentina. [2] Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Santa Fe, Argentina and Plataforma Argentina de Biología Estructural y Metabolómica PLABEM, Buenos Aires, Argentina. [3] Fundación Instituto Leloir, IIBBA-CONICET, and Plataforma Argentina de Biología Estructural y Metabolómica PLABEM, Buenos Aires, Argentina-
·E-mail address of presenting author: kpasquevich@iib.unsam.edu.ar.

U-Omp19 is a bacterial protease inhibitor from *Brucella abortus* that inhibits gastrointestinal and lysosomal proteases, enhancing the half-life and immunogenicity of co-delivered antigens. U-Omp19 is a novel adjuvant that is in preclinical development with various vaccine candidates. However, the molecular mechanisms by which U-Omp19 exerts these functions and the structural elements responsible for these activities remain unknown. To elucidate the molecular basis of the U-Omp19 protease inhibitor and adjuvant activities we performed a structural, biochemical, and functional characterization of U-Omp19. Protein crystallography and NMR studies revealed that the protein consists of a compact C-terminal beta-barrel domain and a flexible N-terminal domain. To evaluate the contribution of each domain to U-Omp19´s activities several truncated protein constructs were designed and obtained. Our results demonstrate that the N-terminal domain behaves as an intrinsically disordered protein and that it retains the full protease inhibitor and adjuvant activities of U-Omp19. Collectively, these results may contribute to fulfill the need for vaccine adjuvant development by offering insights into the U-Omp19 biological mechanisms of action and by enabling the design of novel engineered structures that may have enhanced activity, stability, or be conjugated with other bioactive molecules or vaccine antigens.

836. (830) **TLR9 ACTIVATION IS REQUIRED FOR CYTOTOXIC RESPONSE ELICITED BY BACULOVIRUS CAPSID DISPLAY**

María Inés Crespo[1], Paula Molinari[2], Guido N. Molina[2], Belkys Maletto[1], Oscar Taboga[2], Ignacio Cebrian[3], Gabriel Morón[1].
[1] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET. Dpto. de Bioquímica Clínica - Facultad de Ciencias Químicas – UNC. Córdoba, Argentina.
[2] Instituto de Agrobiotecnología y Biología Molecular (IABIMO), INTA, CONICET. Buenos Aires, Argentina.
[3] Instituto de Histología y Embriología de Mendoza (IHEM) - Universidad Nacional de Cuyo - CONICET, Mendoza, Argentina.

The baculovirus (BV) infects lepidopteran as natural hosts and represents an efficient vector for vaccine development. We showed that BV expressing OVA in its capsid (BVOVA) improved the immune response by eliciting $CD8^+$ T cell activation. BVs enter intact into subcellular compartments of dendritic cells (DC) where both viral surface proteins and capsid follow separate intracellular routes, allowing the viral capsid containing the foreign Ag to reach the cytosol. In this study, we analyzed the intracellular mechanisms involved in $CD8^+$ T cell activation by BVOVA. We first evaluated if the BV GP64-fusogenic protein is required for OVA cross-presentation by pretreating BVOVA with an anti-GP64 neutralizing antibody. In this case, infected splenic DCs failed to activate the B3Z $CD8^+$ T cell hibridoma, specific for the MHCI(K^b)-$OVA_{257-264}$. Moreover, T cell activation was inhibited when DCs were treated with lactacystin, indicating that proteasome is required for efficiently cross-presentation. We then analyzed the dependence of TLR9 and STING signaling by infecting DCs with BVOVA in the presence of H151, a STING inhibitor, or IRS869, a TLR9 signaling inhibitor. Only the incubation with IRS869 blocked B3Z activation by DCs. We also evaluated whether TLR9 is required to induce proliferation of *naïve* $CD8^+$ T cells using BVOVA-infected DCs from WT and $TLR9^{-/-}$ mice. We found that $TLR9^{-/-}$ DCs cannot activate *naïve* $CD8^+$ T cells efficiently. Also, $TLR9^{-/-}$ mice fail to generate an OVA-specific cytotoxic response after BVOVA injection. Finally, using $IFNAR^{-/-}$ mice we observed type I IFN are not required for cytotoxic response induction. In this work, we found that the viral envelope fuses with the endosomal membrane allowing the viral capsid exits to cytosol for proteasomal degradation. During this BVOVA intracellular trafficking, endosomal TLR9 signaling in DCs is triggered, thereby stimulating the presentation of the MHCI/$OVA_{257-264}$ complex to $CD8^+$ T cells, which activate into CTL.

837. (843) **PRECLINICAL IMMUNOGENICITY STUDIES OF A RECOMBINANT VACCINE FORMULATION AGAINST COVID-19**

Agostina Demaría[1], Lorena Coria[1], Celeste Pueblas Castro[1], Laura Bruno[1], Eliana Castro[1], Lucas Saposnik[1], Mayra Ríos Medrano[1], Valeria Krum[2], Ignacio Drehe[2], Mariana Li Causi[2], Johanna Sidabra[2], Jonathan Baqué[2], Cristian Payes[2], Brenda Heinrich[2], Analía De Nichilo[3], Francisco Zurvarra[3], Diego Alvarez[1], Karina Pasquevich[1], Juliana Cassataro[1].
1 Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde", Universidad Nacional de San Martín (IIB-UNSAM, IIBIO-CONICET), Buenos Aires, Argentina.
2 I+D Biofármacos, Laboratorio Pablo Cassará SRL. Sitio 2. Unidad de Biológicos. Saladillo 2452 C1440FFX, CABA, Argentina.
3 I+D Biofármacos, Fundación Pablo Cassará Saladillo 2452 C1440FFX, CABA, Argentina.

In this work, we studied the immunogenicity of vaccine formulations based on the receptor binding domain (RBD) of the spike protein from SARS-CoV-2. BALB/c mice were immunized by intramuscular route with two vaccine formulations that contained different antigen (Ag) doses. Induction of Ag-specific IgG antibody titers were determined in sera of immunized animals. Results showed that both formulations induced high but similar anti-RBD IgG titers. Evaluation of Ag-specific IgG subclasses showed that both vaccine formulations induced higher titers of IgG1 than IgG2a. Interestingly, independently of the Ag dose specific IgG antibody titers against RBD remained elevated until 253 days post prime vaccination. Virus neutralization capacity of antibodies induced by these vaccines was evaluated in a live virus neutralization assay. Results indicate that both vaccine formulations elicited antibodies that have neutralizing activity against ancestral, gamma, alpha, lambda and delta SARS-CoV-2 variants. Finally, to determine T-cell-mediated immune responses, splenocytes from immunized mice were stimulated *in vitro* with complete medium or the Ag and cytokine levels were measured in supernatants by ELISA. Splenocytes from all vaccinated mice induced significant levels of IFN-γ and IL-5 upon antigen stimulation ($p<0.05$). Moreover, intracellular flow cytometry analysis revealed that stimulated splenocytes from both groups of vaccinated mice induced Ag-specific IFN-γ and TNF-α producing $CD4^+$ T cells ($p<0.05$) while only the high dose of Ag could induce $CD8^+$ T cells that produce IFN-γ and TNF-α ($p<0.05$). These results indicate that the vaccine formulation with the higher dose of Ag has a better performance: induces high levels of specific antibodies with broad neutralizing activity against different SARS-CoV-2 variants and specific $CD4^+$ and $CD8^+$ T cell responses. Based on these results we selected the high dose Ag formulation to continue our actual preclinical studies.

838. (880) **ASSESSING THE IMMUNOPROPHYLACTIC PROPERTIES OF A NOVEL CHIMERIC ANTIGEN CANDIDATE TO CONTROL *TRYPANOSOMA CRUZI* INFECTION**

María Elisa Vázquez[1], Brenda Zabala[1], Andrea C. Mesías[1], Cecilia Parodi[1], Natalia Corbalan[2], Bladimiro Lenis[3], Lucía Biscari[4], Andrés Alloatti[4], Cecilia Pérez Brandan[1], Leonardo Acuña[1]
[1]Instituto de Patología Experimental, CONICET, Salta, Argentina, [2]Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina, [3]Unidad de Conocimiento Traslacional, Hospital Dr. Arturo Oñativia, Salta, Argentina, [4]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER), CONICET, Universidad Nacional de Rosario, San-

ta Fe, Argentina.

Chagas disease (CD) is a neglected tropical disease, affecting more than 8 million people worldwide. Patients with CD develop myocarditis and/or digestive clinical manifestations. The agent responsible for this disease is *Trypanosoma cruzi,* a parasite that can persist in its mammalian host because it has evolved multiple strategies to evade the immune response. This persistence is considered the main factor contributing to the late symptoms of CD. The aim of the present work is to depict the immunological outcome of a new antigen candidate in a prime/boost/challenge mouse model of infection. For this, animals were given three doses, in conjunction with a saponin type adjuvant, of a chimeric antigen composed of fragments of two immunogenic defined proteins of the parasites. Around day 20 post boost serum samples were taken to measure specific antibodies response and half of them were later sacrificed for spleen removal. Cytokines production after *in vitro* splenocytes re-stimulation and memory T cells subsets were analyzed. The other half of animals were challenged with virulent *T. cruzi* parasites. During infection period parasite load in blood was recorded twice a week to test vaccine efficiency. After that animals were sacrificed and heart, colon and skeletal muscle were taken to analyze parasite burden and histological damage. Briefly, mice vaccinated with the chimera were capable to produce specific antibodies. Regarding memory T-cells, no significative differences were found among experimental groups; however, IL-10 levels were lower in vaccinated animals than in control groups. Interestingly, mice inoculated with our chimera were able to control parasite load in blood during the first days of infection and dimmish parasite burden in targeted tissues exhibiting minor inflammation and preventing parasite nesting. This study reveals the immunological potential of this chimeric protein as new immunogen to use in vaccine formulations to defeat *T. cruzi* infection.

839. (898) **FOOT AND MOUTH DISEASE VIRUS PEPTIDES PRESENT ON SMALL EXTRACELLULAR VESICLES DERIVED FROM ANTIGEN PRESENTING CELLS PULSED WITH VACCINE ANTIGENS ARE ACCESSIBLE TO THE BCR RECEPTOR AND INDUCE B AND T CELL-SPECIFIC

IMMUNE RESPONSE

Florencia Menay[1], Federico Cocozza[2], Maria José Gravisaco[3], Analía Elisei[4], Javier Re[5], Alejandra Ferella[6], Claudia Waldner[7] and Claudia Mongini[1].

1. Instituto de Virología e Innovaciones Tecnológicas (IVIT), INTA-CONICET, Buenos Aires, Argentina.

2. Institut Curie Centre de Recherche (Curret address), Paris, Francia.

3. Instituto de Agrobiotecnología y Biología Molecular (IABIMO), INTA-CONICET, Buenos Aires, Argentina.

4. SENASA (Curret address), Buenos Aires, Argentina.

5. Laboratorio ELEA (Curret address), Buenos Aires, Argentina.

6. Laboratorio Biogénesis Bagó, Buenos Aires, Argentina.

7. Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), Buenos Aires, Argentina

Foot and mouth disease (FMD) is an infection of global economic importance caused by foot and mouth disease virus (FMDV). Extracellular vesicles (EVs) secreted by antigen presenting cells (APCs) participate on activation of adaptive immune cells. In our previous work we have demonstrated that murine APCs can internalize inactivated FMDV O1 Campos (FMDVi) vaccine antigens and release sEVs expressing APC markers and a high level of viral peptides (sEVs-FMDV) that stimulate FMDV-specific immune response. The aim of the present work was to characterize the specific immune response generated by sEVs-FMDV, we focused the study on B cell subpopulations, mainly marginal zone (MZ) and follicular B cells (FoB). The ability of sEVs-FMDV to induce a specific primary and secondary immune response was evaluated. Specific lymphocyte proliferation was tested using splenocytes obtained from naïve (NAÏVE) or immunized mice (IMMUNE). Our results shown that sEV-FMDV stimulated *in vitro* B cell proliferation in antigen-sensitized and naive mice. Similar and significant response was induced by FMDVi in MZ and FoB cells ($p<0.001$*** for both NAÏVE and IMMUNE compared to the respective unstimulated controls). This stimulation was greater in IMMUNE splenocytes ($p<0.05$*). The B cells activation would be triggered by specific recognition of viral epitopes on the membrane of sEVs-FMDV. In contrast, sEVs-FMDV only stimulated T cells previously sensitized *in vivo* with FMDVi. We suggest that this stimulation was antigen-specific. However, we cannot rule out the presence of immunomodulatory molecules present in sEVs. We demonstrated that sEVs act as antigen carriers, being the platform where intact or semi-processed proteins can be recognized as membrane-associated antigen. This work extends the current knowledge of the role of APCs in the antiviral immune response, proposing sEVs derived from these cells as a new player in the regulation of adaptive immunity against FMDV.

840. (913) **ALPHAVIRUS REPLICON DNA ENCODING TRASPAIN SHOWED IMMUNOGENICITY AND EFFICACY AS VACCINE CANDIDATE AGAINST *T. CRUZI* INFECTION**

Delfino MA[1], Trinitario SN[1], Dzvonyk P[1] Cerny N[1], Bivona AE[1],, Cardoso A[1], Tarleton RL[2], Malchiodi EL[1], Sánchez Alberti A[1]

[1] Cátedra de Inmunología. IDEHU-CONICET. Facultad de Farmacia y Bioquímica. IMPAM-CONICET. Facultad de Medicina. Universidad de Buenos Aires. Buenos Aires, Argentina; [2] Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, USA.

Chagas disease, caused by *Trypanosoma cruzi*, is parasitic disease that affect 6-7 million people worldwide. Treatment is limited to the acute phase and there is not approved vaccine. Nucleic acid-based vaccines are strong type I response inducers, effective to control intracellular pathogens infection. Previously, we developed a DNA-launched RNA replicon encoding Traspain, a chimeric *T. cruzi* antigen (DREP-Tp). Here, we determined humoral and cellular immune response, and efficacy in a murine model. Semliki Forest virus based DREP was constructed employing a quality by design approach, applying DNA assembly tools. Its identity was confirmed by sequencing and restriction analysis. Antigen expression was detected by Western blot in transfected cells. To evaluate its immunogenicity, groups of C3H female mice were vaccinated by the intramuscular route with 3 doses of either 10 μg, 100 μg or 250 μg of naked DREP-Tp. Placebo group received PBS and a reference group was immunized with 3 doses of 10 μg of recombinant Traspain combined with 50 μg of cyclic-di-AMP adjuvant (Tp-CDA). Higher specific antibody titers were detected in Tp-CDA vs DREP-Tp groups (IgG titers: 64834 vs <400). The latter, conversely, showed an increased expansion of epitope-specific CD8+ T cells at endpoint (%$CD8^{+}CD44^{high}$TEWETGQI^{+} 1.16 vs 2.37, Tp-CDA and DREP-Tp 250 μg respectively). Memory phenotype of this subset at early time-point showed a predominance of effector vs central and effector memory T cells in DREP-Tp groups compared to Tp-CDA. To evaluate vaccine efficacy, immunized mice were challenged with the RA strain of *T. cruzi* (DTU VI). All DREP-Tp groups showed a significant parasitemia reduction vs placebo [(AUC: 25.7**, 18.7****, 26.2** vs 44.2, DREP-Tp 10 μg, 100 μg and 250 μg vs placebo (**$p<0.005$ ****$p<0.0001$)]. In conclusion, these results strongly suggest the utility of SFV-DREP encoding Traspain as potential vaccine against *T. cruzi*.