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La Tapa (Ver p 5)
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REUNIÓN DE SOCIEDADES DE BIOCIENCIAS 2020

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

LXVIII REUNIÓN ANUAL DE LA
SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

REUNIÓN ANUAL DE LA
SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

10-13 de noviembre de 2020

EDITORES RESPONSABLES
María Cristina Carrillo
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ANNUAL MEETING OF BIOSCIENCE SOCIETIES 2020

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

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SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

ANNUAL MEETING OF
SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

November 10-13, 2020

RESPONSIBLE EDITORS
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Analía Trevani
Maria Cecilia Larocca

LA TAPA

María de Luján Alvarez. Ludueña

Técnica: óleo sobre tela

Medidas: 60 x 40 cm, año 2016

Gentileza de la autora

La obra de tapa refleja un lugar típico rosarino. El arroyo Ludueña nace en los campos de las afueras de Rosario y finaliza en el barrio Arroyito de la ciudad, donde desemboca en el Río Paraná.

María de Luján Alvarez es Bioquímica y Doctora en Ciencias Biológicas. Es investigadora adjunta (CIC-CONICET) en el Instituto de Fisiología Experimental (IFISE-CONICET) y docente en el área Morfología de la Facultad de Ciencias Bioquímicas y Farmacéuticas de la Universidad Nacional de Rosario (UNR). Alumna del taller de arte Tunkeyén, estudió con la pintora rosarina Ana Petrini. Ganó el segundo premio en el 12º Salón de Pintores Nóveles de la Sociedad Argentina de Artistas Plásticos de Rosario (2004), el primer premio en el 2º Salón Pintando Argentina de Rosario (2010), una mención al trabajo realizado en el 2º Encuentro de Pintores de Rosario organizado por la Asociación Cultural Museo Ambrosio Gatti (2018) y el tercer premio en el Concurso de Pinturas 150 años de la Sociedad Filantrópica Suiza (2018). Participa frecuentemente en muestras colectivas de diferentes salones pictóricos rosarinos y sus obras han sido expuestas en espacios de arte organizados por CONICET y la UNR.

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Mensaje de Bienvenida a los Presidentes

Discurso de la Dra. María Cristina Carrillo, Presidenta SAIC

Estimados Miembros de la Comunidad Científica del área Biomédica:

Les doy la bienvenida, en nombre de la Comisión Directiva de la Sociedad Argentina de Investigación Clínica, a la LXV Reunión Anual, que este año se lleva a cabo de manera conjunta con la Sociedad Argentina de Inmunología y la Sociedad Argentina de Fisiología.

En 2020, SAIC cumple 60 años. Durante estas 6 décadas, los trabajadores de la ciencia que han transitado y que transitan la Sociedad han hecho realidad lo que John Ruskin alguna vez citó: “El trabajo de la ciencia es sustituir apariencias por hechos e impresiones por demostraciones”.

Durante 60 años la sociedad funcionó teniendo como meta principal la realización de Reuniones Científicas en donde pudieran confluir las teorías más novedosas, los dilemas más excitantes y las resoluciones científicas más sorprendentes.

Durante su existencia se produjeron acontecimientos políticos y sociales que introdujeron importantes avances, pero también significativos retrocesos en el sistema científico y académico.

En líneas generales, podemos decir que en Argentina, históricamente, no existieron procesos concretos de integración de universidades, instituciones públicas de I+D y empresas privadas promovidos por una red o urdimbre de actores de instrumentación genérica, tal como los que se encuentran en las economías desarrolladas.

La ausencia de esta trama institucional motivó que se fueran incrementando, a lo largo de los años, formas de integración subordinadas, al, por ejemplo, estandarizar la compra de instrumentos al exterior para equipar los laboratorios y al desarrollar una valoración acrítica de los vínculos entre científicos y grupos locales con grupos e instituciones de países avanzados.

Esta situación ha llevado al complejo de Ciencia y Tecnología (CyT) a ser funcional, en parte, a los requerimientos de un sistema internacional de estados muy estratificado debido a la globalización económica que se inició al final de la 2da Guerra Mundial y se profundizó en la década de los ’80. Lo que nos llevó a tener un papel subsidiario en el sistema económico mundial. Podríamos concluir que los vínculos de dependencia en CyT son una exteriorización fragmentaria de la dependencia cultural y económica.

Más allá de esta histórica realidad, tanto la dictadura militar que irrumpió entre 1976 y 1983, como el gobierno de características neoliberales que hegemonizó la década de los ´90, llevaron adelante políticas que desarticularon significativamente la estructura del sistema científico-académico y sus principales proyectos de desarrollo y condujeron al exilio forzado a miles de investigadores, tecnólogos y profesores universitarios con un alto nivel de capacitación.

En un contexto de crisis económica y social sin precedentes, el gobierno que asume en mayo del 2003 se plantea como uno de sus principales objetivos la transformación del modelo económico-social del país, orientándolo hacia un crecimiento centrado en la mejora de las capacidades productivas y la reindustrialización. Esta perspectiva colocó a la educación, la ciencia y la tecnología como sustentos de la reconstrucción de una sociedad con mayor crecimiento e integración social. Como resultado de estas políticas, en el ámbito de CyT se produjo el aumento sostenido de la inversión y la incorporación de un número sin precedentes de investigadores, tecnólogos y becarios al sistema. No menos importante fue el fortalecimiento de una institucionalidad que permitió orientar las prioridades de la investigación a las necesidades del desarrollo productivo del país. La creación del Ministerio de Ciencia, Tecnología e Innovación Productiva en el año 2007 fue uno de los hitos más significativos de esta etapa.

Cabe destacar que, junto con los aspectos virtuosos de este período que, habiendo comenzado en 2003, culminó en 2015, es necesario mencionar un número importante de limitaciones y asignaturas pendientes que no se pudieron afrontar o concluir exitosamente en esta etapa. Muchas de ellas impidieron avanzar en un proceso más profundo de transformación productiva, relacionada con una escasa vinculación entre el sistema científico-tecnológico y el aparato productivo del país y a la pobre integración entre los distintos institutos de investigación, universidades y empresas públicas dedicadas al desarrollo e innovación.

A partir de diciembre de 2015 y hasta 2019, se puso en práctica una concepción política general que tuvo como objetivo el repliegue del papel del Estado como conductor del proceso de crecimiento económico y promotor de la equidad social que implicó el achicamiento del estado en todas sus funciones, y, por supuesto, eso incidió negativamente en el sector científico - académico argentino.

La disminución de recursos aplicada al sector significó no solo que centenares de investigadores formados durante años quedaran fuera del sistema, sino también el fin de las políticas de crecimiento de la carrera científica.

Estas apreciaciones volcadas en este discurso no son nuevas. Repasando los discursos de presidentes y presidentas anteriores, estos comentarios han sido volcados en muchos de ellos.

Aun así, y más allá de los distintos aconteceres de la realidad nacional y de la falta de una interrelación eficiente entre los distintos sectores de I+D, la SAIC siguió funcionando y convirtiéndose para muchos becarios e investigadores jóvenes que se quedaron en el país, en el escenario esperado adonde concurrir y debatir sus resultados, sus hipótesis y sus teorías, de manera tal que el conocimiento en el área biomédica pudo crecer sostenidamente, construido sobre la integración interdisciplinaria. Y esto puede apreciarse en las instituciones que comparten las autorías de muchos de los trabajos presentados: Institutos de investigación, hospitales, bio-empresas. Las ciencias experimentales lograron crear la urdimbre necesaria de manera empírica, y en base a las necesidades reales de cada institución.

Muchos de nosotros comenzamos el año 2020 con muchas esperanzas. Se vislumbraba un cambio de dirección en las políticas de CyT. La reposición del Ministerio de Ciencia Tecnología e Innovación Productiva era un símbolo que indicaba el comienzo de un largo período de reconstrucción.

En lo personal, como Presidenta de SAIC, mi intención era realizar, tal vez siendo muy ambiciosa, una reunión Científica inmensamente rica y constructiva. Pero en marzo todo nuestro universo cambió.

La pandemia fue (y lo sigue siendo) la gran protagonista de este año y ha superado en popularidad a otras amenazas mundiales que parecían ser igual de peligrosas. En los primeros ocho meses de 2020 el coronavirus contagió a más de 24 millones de personas, produjo cerca de 1 millón de muertes alrededor del mundo y hasta hoy no da tregua, con rebrotes en zonas donde ya había bajado su circulación. Sabemos que si hay algo que le puede devolver la normalidad a nuestras vidas es el hallazgo de un medio de prevención, y es en esa dirección en la que científicas y científicos de todo el mundo, incluido nuestro país, trabajan para llegar en tiempo record.

En este contexto, la SAIC no se detuvo. Junto con la Comisión Directiva, decidimos seguir trabajando, tratando de adaptarnos a la dura realidad, inesperada y dramática. En junio realizamos el Simposio de COVID19, nuestra primera experiencia virtual, y en la que pudimos poner de manifiesto las últimas novedades que nuestros científicos estaban produciendo en el combate contra el virus. El éxito alcanzado nos dio la fuerza para continuar y organizar la Reunión Anual con la misma metodología.

A principios de septiembre nos vimos impulsados a publicar un Llamado a la Responsabilidad Ciudadana, debido a la intensidad que había adquirido la pandemia en todo el país. La situación era de una relevancia dramática, y así lo expresamos. La nota repercutió y fue repicada en redes y en muchos medios audiovisuales y gráficos de todo el país. La notoriedad que adquirió esta carta a la ciudadanía, publicada en nuestra página web, puso de relieve la importancia y el nivel que tiene SAIC dentro de la comunidad nacional.

La pandemia ha cambiado, a mi criterio, la concepción que tenía la ciudadanía sobre el sistema científico nacional, sobre todo luego que se pusiera en duda, en los últimos años, la capacidad del mismo. La pregunta que recorrió muchos portales durante el último gobierno liberal era: ¿Para qué sirve la ciencia? La reacción del sistema científico ante la pandemia ha dado la respuesta. Posiblemente haya un cambio esperanzador en el paradigma de lo que significa el desarrollo científico en la sociedad. Y se termine asociando el concepto de Soberanía con el de Desarrollo de Ciencia Nacional.

Agradezco inmensamente a la extraordinaria Comisión Directiva con la que me tocó trabajar. El entusiasmo, las ideas, la solidaridad de todos y todas permitieron un trabajo intenso y profundamente agradable. Agradezco especialmente al Dr. Alejandro Curino, vicepresidente de la SAIC, por su presencia y su soporte y por coincidir en que la forma de pensar la sociedad y el trabajo se pueden planificar por más de un período; al Dr. Enrique Sanchez Pozzi, nuestro tesorero y a la Dra. María Laura Ruiz, nuestra secretaria, por el enorme e invaluable trabajo que han realizado. Les doy también mi agradecimiento al grupo G2, que trabajó codo a codo con todos nosotros, organizando esta tarea absolutamente nueva.

Agradezco a los y las participantes, simposistas, coordinadores, evaluadores, a todas y todos los que presentaron trabajos, y en general, a los y las que confiaron en nosotros y se arriesgaron a esta aventura virtual.

Agradezco, además, a las otras sociedades que nos acompañaron en esta experiencia. Entre todos hemos hecho posible este evento.

Dra. María Cristina Carrillo
Presidenta de SAIC

Discurso de la Dra. Analía Trevani, Presidenta SAI

Estimadas y estimados colegas, amigas y amigos

En nombre de la Comisión Directiva de la Sociedad Argentina de inmunología les doy una cordial bienvenida a una nueva reunión de Sociedades de Biociencias organizada de manera conjunta por la Sociedad Argentina de Investigación Clínica, la Sociedad Argentina de Fisiología y las Sociedad Argentina de Inmunología.

Como es de público conocimiento, este año ha sido particularmente especial en todos los aspectos que incumben a nuestras vidas, y nuestra actividad profesional no ha resultado ajena, como tampoco lo vinculado a la organización de esta actividad científica. Luego de muchos meses de trabajo organizando el tercer congreso Franco-Argentino de Inmunología, con 25 disertantes extranjeros que habían comprometido su participación abonándose sus propios pasajes aéreos, un simposio conjunto con la "Society of Leukocyte Biology" de Estados Unidos proyectado y sponsors internacionales que habían decidido acompañarnos, debimos reprogramar la actividad, la cual, si la pandemia lo permite tendrá lugar en 2021. A pesar de la decepción que esta situación representó para esta comisión directiva, supimos aprovechar alguna de las ventajas que ofrece la virtualidad y logramos confeccionar para esta reunión, un programa que involucra la participación de científicos destacados internacionalmente, que abordarán temáticas diversas de gran interés para los miembros de nuestras sociedades. A todos ellos les agradecemos su gentileza y generosidad por participar de este evento. Frente a la incertidumbre que representaba la realización de una reunión científica virtual, en un año en qué la mayor parte de las líneas de investigación debió ser suspendida, nos sorprendimos gratamente una vez más con la respuesta de los inmunólogos, quienes presentaron 124 trabajos de investigación 4 de los cuales serán defendidos para optar al premio Satz. Ello representa sin duda una muestra más de la fortaleza de nuestro sistema científico al cual nos sentimos orgullosos de pertenecer.

Quiero aprovechar esta oportunidad para agradecer al CONICET que nos ha dado su apoyo mediante la línea de financiamiento para reuniones científicas y a las empresas privadas que nos han acompañado una vez más. Quiero agradecer también a toda la comisión directiva por su apoyo constante y su activa participación en las acciones que mancomunadamente llevamos a cabo a lo largo de este año. También al comité médico y al comité docente de nuestra sociedad, en especial a María Soledad Gori y Julieta Alcaín por su trabajo en el manejo de las redes sociales de la SAI. Pero muy especialmente quiero agradecer a dos de los integrantes de nuestra comisión directiva; a la Dra. Carolina Jancic, la secretaria de nuestra sociedad, con quién diariamente compartimos todas las tareas que involucró esta gestión, y sin cuyo su enorme trabajo generoso, responsable y comprometido habría sido imposible alcanzar los objetivos que nos propusimos; y al Dr. Matías Ostrowski, por su activa labor a cargo de la tesorería y por su ayuda en la organización de lo que iba a ser FAIC2020 y su compromiso con las actividades científicas realizadas este año. A todos ellos quiero decirles gracias con mayúsculas. También quiero agradecer a las y los integrantes de mi laboratorio por comprender mi menor interacción debido al tiempo que me demandó llevar a cabo las actividades de la sociedad.

Nuestro principal objetivo de gestión fue potenciar la participación de los miembros de nuestra sociedad en las distintas actividades a realizar; fomentar la participación y visibilización de los investigadores jóvenes; incrementar la diversidad de miembros involucrados en la más amplia variedad de tareas y federalizar nuestra sociedad. Con este fin, para cada una de las actividades llevadas a cabo, convocamos a miembros de distintos lugares geográficos y de diferentes escalas de formación tanto para actuar como disertantes como en su rol de coordinadores de actividades científicas. En lo que respecta a la presente reunión, seleccionamos a disertantes nacionales que no hubiesen participado en años recientes en nuestras reuniones científicas, con el objetivo de ampliar la difusión del trabajo de distintos miembros de nuestra sociedad. Aun cuando la pandemia significó un impedimento para la ejecución de algunas actividades que teníamos proyectadas, logramos tomar ventaja de la situación y perseguir y alcanzar otra de las metas que nos habíamos impuesto, como la de extender el alcance de nuestras actividades para que pudieran ser aprovechadas por todos aquellos interesados en la adquisición de conocimiento. Por ello, realizamos actividades de gran calidad académica, abiertas y sin costo alguno, las cuales fueron aprovechadas no sólo por colegas de nuestro país sino también de otros países latinoamericanos miembros de ALACI, gracias a la intensa campaña de difusión que emprendimos. Ninguna de estas actividades habría sido posible sin la generosidad y el compromiso de todos aquellos que invirtieron su tiempo y esfuerzo para realizar las presentaciones que nos permitieron actualizarnos en las diversas temáticas que se abordaron. A todas y todos ellos, muchísimas gracias! También a los disertantes internacionales que generosamente, y a pesar de las diferencias horarias y en algunos casos, en medio de sus vacaciones, aceptaron ser parte de un proyecto abierto de difusión del conocimiento científico.

Nos deja un sabor amargo en esta gestión, el haber tomado conocimiento de que algunos miembros del área clínica consideren necesaria la creación de una asociación que los nuclee por no sentir representados sus intereses en esta sociedad. Aun cuando entendemos que en muchos casos el curso natural de la diversificación del conocimiento conduce a la necesidad de generar espacios de discusión propios, para la mayor parte de los miembros de esta comisión directiva sería más enriquecedor que los mismos se gestaran en el marco de la sociedad existente. Las sociedades

científicas deberían ser espacios dinámicos en dónde la discusión constructiva permitiese canalizar cambios orgánicos en acuerdo con el avance del conocimiento. Tenemos claro, sin embargo, que ninguno de esos cambios puede ser llevado a cabo sin el trabajo comprometido de todos los interesados. A la luz del desarrollo de esta nueva asociación, esperamos que en el futuro ambas sociedades se comprometan a realizar actividades conjuntas periódicas, porque ello sin duda representaría un aporte mutuo.

Quiero dedicar los últimos minutos de este espacio que se me ha brindado por mi papel dentro de la Sociedad Argentina de Inmunología, para referirme al contexto en el cual transitamos esta gestión y su impacto. En este tiempo, hemos presenciado con enorme satisfacción la respuesta colectiva del sistema científico mundial para entender la fisiopatología de la COVID-19 y desarrollar estrategias terapéuticas para enfrentarla. Sin embargo, debido a la pandemia, también hemos sido arrasados por una vorágine de información con y sin sustento científico, divulgación de resultados de investigaciones científicas no evaluadas por pares y de información de estudios clínicos carentes de un diseño adecuado. Dado que ello puede conducir a la toma de decisiones clínicas sin la evidencia suficiente y con consecuencias que pueden ser impredecibles para los pacientes y la sociedad, considero que como integrantes del sistema científico, esto merece que reflexionemos al respecto.

Por otra parte, en este tiempo, también asistimos a la aparición en las redes y medios de comunicación, de individuos que amparados en sus títulos académicos, divulgan información pseudocientífica tergiversada a una sociedad que está ansiosa de certezas y esperanza, ocasionando un gran daño a la sociedad ante esta situación tan compleja que nos ha tocado vivir. Por ello, la SAI, así como muchos de sus miembros de forma individual o colectiva, ha hecho un gran esfuerzo por divulgar información certera a través de sus seminarios virtuales y su comunicación a través de las redes sociales.

La aparición de la COVID-19 también puso de relieve como nunca antes la capacidad de nuestros científicos, los que a pesar de estar inmersos en un sistema que en la gestión gubernamental anterior sufrió un enorme deterioro, con un Ministerio de ciencia degradado a la categoría de secretaría, con una inversión en insumos, equipamiento y salarios absolutamente devaluados, fue capaz de responder de manera colectiva para enfrentar esta pandemia. Fue gracias a la calidad académica de sus integrantes que se pudo dar respuesta en tiempo récord al desarrollo de kits diagnósticos, de protocolos de investigación básica y clínica, al de herramientas terapéuticas y vacunas, y al asesoramiento al sistema de salud. Esta capacidad de respuesta no se logra de manera repentina. Se sustenta en años de tiempo y esfuerzo para la formación de recursos humanos altamente calificados e inversión económica; se sustenta en la capacidad de acceso a tecnologías de vanguardia y en una planificación estratégica pensando en un modelo de país soberano. La construcción de un sistema científico sólido es un camino lento y sembrado de numerosos obstáculos, pero su destrucción puede ocurrir de forma acelerada, como lo han demostrado los años recientes, en que las políticas aplicadas condujeron a la pérdida de profesionales que costaron dinero y dedicación en formar, e incrementaron la brecha tecnológica debida a una desinversión sostenida. En lo personal, celebro que la actual gestión gubernamental haya devuelto el rango ministerial a la ciencia, dado que esto no implica sólo un cambio de nombre sino de políticas y planificación. También celebro que sean nuestros colegas científicos quienes estén a cargo de dichas decisiones y su esfuerzo por retener los recursos humanos ya formados y por realizar una gestión previsible y transparente. Pero soy consciente de que esto no es suficiente; necesitamos incrementar la inversión económica en ciencia dado que los insumos y equipos se encuentran dolarizados y nuestros subsidios pesificados. Entiendo las dificultades de llevarlo a la práctica en el contexto de una crisis económica mundial producto de la pandemia. Pero como en todos los órdenes de la vida, resulta necesario establecer prioridades, y la pandemia de COVID-19 ha puesto de manifiesto que el sostenimiento del sistema científico debería ser una de ellas. Por ello, espero que los legisladores de las distintas fuerzas políticas finalmente aprueben en el parlamento una Ley de Ciencia que garantice un incremento sostenido del porcentaje de la función ciencia y técnica sobre el PBI, para llevarlo en los próximos diez años al 1,5%.

Para concluir, quiero expresar un profundo y sentido agradecimiento a todos los miembros de las sociedades científicas participantes que han contribuido con su trabajo y esfuerzo al diagnóstico y atención de los pacientes con COVID-19, a la investigación sobre esta temática y a la difusión de información a la población.

También quiero expresar el deseo de la Comisión Directiva de la SAI de que esta primera reunión científica virtual sea fructífera y que a pesar de la falta de presencialidad nos permita conectarnos y que sea el cimiento de cooperaciones futuras.

Muchas gracias, un saludo afectuoso,

Analía Trevani
Presidenta SAI

Discurso de la Dra. María Cecilia Larocca, Presidenta SAFIS

Queridos colegas, compañeros y amigos,

Es un placer y un honor para mí darles la bienvenida a la Reunión anual conjunta 2020, en representación de la Comisión directiva de la Sociedad Argentina de Fisiología (SAFIS).

De más está describir el difícil momento que estamos viviendo como sociedad, en el que el sentimiento de angustia se mezcla con la tristeza y la impotencia, pero también con la esperanza y la solidaridad. Analizando cómo impacta este momento a la comunidad científica surge mi convicción de que el mismo nos ofrece una oportunidad de gran empoderamiento. No creo que sea necesario en este ámbito remarcar la importancia que ha tenido la investigación científica en la búsqueda de respuestas de fondo en este momento. Pero sí me parece importante observar cómo cada uno de nosotros ha logrado cosas previamente inimaginables: continuar con nuestra tarea como investigadores respetando los protocolos de aislamiento a la vez que damos clases online, desarrollamos estrategias de evaluación virtual, hacemos la escuela de nuestros niños, trapeamos y preparamos la masa madre. Y todo esto en medio de una situación emocional muy dura. Por lo tanto, creo que este momento también nos puede hacer repensar la posibilidad de potenciarnos realmente como científicos. La posibilidad de lograr un sistema científico con mayor representatividad, en el que todos nos sintamos protagonistas y demos lo mejor de nosotros. Un sistema donde exijamos ser escuchados, donde podamos defender nuestros proyectos, y también donde escuchemos a nuestros pares independientemente de su trayectoria. Un sistema que permita a los investigadores más jóvenes irse en búsqueda de capacitación extra y nuevas perspectivas, pero que también les genere expectativas para querer volver. Y, por supuesto, un sistema que no deje de trabajar por una ley que garantice un crecimiento sostenido de la inversión en ciencia. Es el sistema que nos garantizará ser más fuertes y estar mejor preparados para dar respuestas a las demandas de estos contextos.

Respecto a las actividades específicas del congreso, los invitamos a participar de los distintos espacios generados para compartir los resultados de nuestras investigaciones, incluyendo la plataforma de videoposters y sus respectivas salas de discusión y el simposio donde presentarán sus resultados los investigadores jóvenes preseleccionados para al Premio SAFIS. También a participar de la Conferencia SAFIS (a cargo del Dr. Guillermo Lehmann, Weill Cornell Medicine, Regeneron Pharmaceuticals, Inc.) enfocada en la discusión de la genómica de células individuales aplicada a la biología ocular y al desarrollo de nuevas estrategias terapéuticas y del Simposio de Nutrición y Fitoterapia, en el que prestigiosas investigadoras de nuestro país y del exterior presentarán y discutirán sus estudios. Asimismo, la comisión de Educación de SAFIS organizó un simposio donde analizar con especialistas cómo educar en tiempos de aislamiento social.

Los alentamos a generar nuevos vínculos, a consultar, a contestar y a escuchar. Aguardando que en la próxima oportunidad podamos encontrarnos presencialmente, y brindar por ello, los saludo afectuosamente.

M. Cecilia Larocca
Presidenta SAFIS

SAIC CONFERENCE 'ALFREDO LANARI'

MATERNAL-FETAL INTERACTION: WHY VIP IS A VERY IMPORTANT PEPTIDE

Claudia Pérez Leirós

Laboratorio de Inmunofarmacología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN, CONICET-UBA.

Normal pregnancy and fetal growth entail a successful maternal-placental cooperation. A network of cell-cell communication through contact and soluble factors supports the interaction of trophoblast cells with maternal vascular cells and leukocytes recruited to the decidua to sustain the varying demands of gestation. Deficient regulation of this interaction at early stages is associated with pregnancy complications with high rates of maternal and neonatal morbidity and mortality. We propose that the vasoactive intestinal peptide (VIP) synthesized by trophoblast cells has a central role in homeostasis maintenance at the maternal-fetal interface. VIP regulates trophoblast cell function and their interaction with maternal vascular and immune cells through autocrine and paracrine loops. It increases migration, invasiveness and glucose and amino acid uptake by human cytotrophoblast cells. Interplay between VIP and mTOR in nutrient sensing and transport is observed. Invasive trophoblast cells express VIP in human first trimester placental explants where it stimulates trophoblast outgrowth and modulates cytokine production by extravillous trophoblast cells, decidual macrophages and NK cells contributing to a tolerogenic microenvironment. Moreover, trophoblast cells and VIP inhibit neutrophil extracellular trap formation and favor a proangiogenic functional profile. A VIP-deficient pregnancy model in mice presents abnormal placental structure and lower fetal weight that is overcome by VIP treatment at early stages. In this model, VIP-deficient trophoblast cells exhibit reduced migration and invasiveness along with a lower expression of proangiogenic and antiinflammatory markers at the implantation sites. VIP treatment also improves pregnancy outcome in two other murine models, the CBAxDBA and the non-obese diabetic mice. Our results point to the role of VIP in normal pregnancy and provide new clues for pharmacological targeting in pregnancy complications associated with the disruption of placental homeostasis.

SAIC CONFERENCE 'ALBERTO TAQUINI'

TRANSLATIONAL RESEARCH FOR HEPATOCELLULAR CARCINOMA THERAPY, MANY FAILURES IN THE PAST, NEW CHALLENGES AND OPPORTUNITIES.

Guillermo D. Mazzolini

Instituto De Investigaciones en Medicina Traslacional (IIMT) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Hepatocellular carcinoma (HCC) is a complex and heterogeneous malignancy, and is ranked as the 2nd leading cause of cancer-related death worldwide. Unfortunately, its incidence and mortality are steadily increasing. HCC is a deadly disease, which is the consequence of 2 dramatic facts: i) in 2018, 841,080 new cases were diagnosed, and 781,631 patients died, and ii) the 5-year survival of patients with HCC is less than 18%. Despite advances in surgical and ablative techniques, the majority of patients are diagnosed at advanced stages. Palliative treatments, namely multi-kinase inhibitors or, more recently, immune checkpoint inhibitors, increase patient overall survival although only in a subset of patients. Therefore, it is of great urgency to develop new and more effective agents for HCC. Tumor heterogeneity in HCC, such as that found in second primary tumors after curative treatment, synchronous multifocal tumors of different clonality, or intratumor heterogeneity, poses a great therapeutic challenge for clinicians. Contrarily to other tumors such as breast cancer (e.g. HER-2 / neu) or colorectal carcinoma (e.g. K- ras) there are no "driver" mutations that can be exploited therapeutically in HCC. On the other hand, HCC is a unique malignancy, as the majority of patients have underlying cirrhosis. As such, the treatment consideration should not only look at the oncological perspective but also the functional status of the liver. Over the lasts 15 years, our laboratory was focused on developing a platform for testing molecular and cellular therapeutic strategies, using a translational approach. We have generated HCC models for translational drug development using *in vitro* and *in vivo* experiments. Using our models, we have studied different therapeutic strategies for HCC such as gene therapy with immunostimulatory cytokines, remodeling of the

immunosuppressive tumor microenvironment, and the use of new inhibitors for novel "druggable" targets such as epigenetic modulators, and Rho GTPase. Importantly, we have incorporated bioinformatic analysis of HCC patients transcriptional and genomic data to corroborate the role of the targets tested in our lab models, and to generate a new multigene signature associated with patient survival. Finally, we have identified and developed a number of innovative treatments with potential for a future clinical development.

SAIC HONOR LECTURE TO DR. ROBERTO COCCO

EXPERIENCE WITH 1000 PROSPECTIVE CASES OF NON-INVASIVE PRENATAL TESTING FOR ANEUPLOIDY SCREENING AND THE REPORTING OF RARE TRISOMIES, MONOSOMY X AND MICRODELETIONS.

I Canonero; G Méjico; C Rohr; B Brun; D Llarrull; AL Ruggieri; E Nazzi; D Pereyra; F Fay; MP Vázquez.
HERITAS, Ocampo 210 bis, Rosario, Argentina

Introduction: Non-invasive prenatal testing (NIPT) for aneuploidy screening has become first line of testing in many countries for pregnant women independent of the risk and age, mainly due to its high sensitivity and specificity for chromosomes 21, 18 and 13. However, many laboratories around the world report other chromosomes aneuplodies, monosomy X and even microdeletions / microduplications, not following ACMG and other international guidance. We developed the first non-invasive prenatal test in Argentina and hereby we report our experience with 1000 cases. Results: We used a cut-off of 3% fetal fractions. Prospective analysis of 1000 samples detected a total of 88 aneuploidies distributed as following: trisomy 21 (49 cases), trisomy 18 (17 cases), trisomy 13 (4 cases), Monosomy X (8 cases) and other rare trisomies in chromosomes 6, 7, 9, 14, 15, 22 (10 cases). All the detected cases presented ultrasound findings or altered biochemical markers and were confirmed by invasive testing. There was a single false negative, which resulted in a triploidy, a known limitation of NIPT. There was a single false positive related to monosomy X. Three abnormalities were confined to the placenta, 2 suspected cases were confirmed as placental mosaics. The non-result rate due to low fetal fraction was 2,53 %. To obtain better accuracy while reporting monosomy X, we introduced several changes in our pipeline that yielded a correct result during the re-analysis of the single false positive in this prospective cohort. We also developed an improved version of our pipeline of circular binary segmentation to report putative microdeletions/microduplications. However, we observed several issues during the reporting of these anomalies related to interpretations and confirmation of the results by invasive testing. On the other hand, we did not observe accuracy problems in reporting rare trisomies since all of them were confirmed by invasive testing.

SAIC HONOR LECTURE TO DR. ELVIRA ARRIZURIETA

BEHAVIOR OF THE RENAL KALLIKREIN KININ SYSTEM (RKKS). INFLUENCE OF SEXUAL HORMONES AND ALDOSTERONE SENSITIVE DISTAL NEPHRON ION CHANNELS.

Elisabet Oddo
Laboratorio de Nefrología Experimental y Bioquímica Molecular. Instituto de Investigaciones Médicas A. Lanari, Facultad de Medicina, Universidad de Buenos Aires.

The renal kallikrein-kinin system (RKKS) has long been related with blood pressure control and sodium and water balance but how the gene expression, tissue kallikrein activity and urinary kallikrein release are regulated still remain to be established.

Previous results obtained in our laboratory, have shown that female rats (F) have a high urinary kallikrein activity (UKa) and a little lower blood pressure (BP) than male (M) spontaneously hypertensive rats (SHR).

Through studies in adult Wistar rats of either sex, with and without gonadectomy (Gx), we showed that ovariectomy (oVx) stimulates urinary kallikrein activity (UKa) and diminishes BP. Also, RKKS blockade increased BP exclusively in the oVx group. The renal kallikrein content (RK) and UKa were higher in F than in M rats. After Gx, RK increased and UKa showed a similar pattern. Renal klk1 mRNA levels did not show gender difference in non-Gx rats, but an increase after oVx was observed.

Under normal salt intake, renal Na^+ excretion in oVx rats was equal to that in intact female (IF) while BP was slightly lower but, when IF and oVx were subjected to high salt intake the pattern of response to salt loading differed in both groups. oVx rats have a lower Na^+ excretion and a higher BP than IF.

Additionally, we also showed that high K^+ intake and Gx diminish BP with a concomitant increase in UKa and plasma aldosterone levels in SHR, revealing a link between those systems. Since kallikrein co-localize in the same distal nephron segments of aldosterone effectors, we explored the blockage of aldosterone, epithelial Na^+ (ENaC) and the rectifying outer medulla K^+ (ROMK) channels upon the RKKS behavior in different

gonad contexts.
Spironolactone increased RK in all groups. Likewise, inhibition of mRNA synthesis and UK release were observed, except in intact males. ENaC inhibition increased RK without changes in klk1 mRNA and UKa levels. UKa correlated with urinary K^+ and a decrease in BP was observed without changes in urine Na^+/K^+. ROMK blockage only shows effects by increasing the mRNA levels and RK in M while orchidectomized, but not oVx, tends to increase UKa and showed the lowest urine Na^+/K^+.
We concluded that RKKS regulation showed a sexual dimorphism and seems to be modulated by sex hormones throughout a process that involved aldosterone and its sensitive-ion channels.

SAI CONFERENCES

IMAGING IMMUNITY: DEVELOPING A DEEP SPATIO-TEMPORAL UNDERSTANDING OF HOST DEFENSE AND TISSUE HOMEOSTASIS

Germain, Ronald N.[1], Zhao, Chen[1,2], Uderhardt, Stefan[1,3], Yu, Weiming[1], Hassan, Raffit[2], Thakur, Nishant[1], Grant, Spencer[1], Beuschel, Rebecca[1], Chu, Colin[1], Speranza, Emily[1], Park, Kyemyung[1], Tsang, John[1], Radtke, Andrea[1], Wong, Harikesh[1]
[1]Laboratory of Immune System Biology, NIAID, NIH; [2]CCR, National Cancer Institute, NIH; [3]Dept. Medicine, Univ. Hospital, Erlangen, Germany

Background: Immune responses involve cell-cell interactions within lymphoid tissues, trafficking of activated cells to sites of effector function, and the migration of effector cells within peripheral tissues including tumors. To gain insight into the relationships among cell movement, organ architecture, immune function, and the local tissue environment, we have used intravital multiphoton microscopy and novel multiplex immunohistochemical methods we have developed called Histo-cytometry, IBEX, and Ce3D.
Observations: The role of cell localization in both innate and adaptive immunity has been addressed using Histo-cytometry in combination with a new clarification method called Ce3D. These techniques allow the use of 8-12 different antibodies not only to surface markers but to phospho-proteins and cytokines in each cycle of imaging. Together with new methods for rapid iteration of staining and analysis developed in our laboratory (IBEX), these methods permit imaging of >60 target proteins in a single tissue slice in a quantitative manner. Recent advances include combining RNA FISH with antibody-based staining to take advantage of RNA-seq data. These multiplex imaging technologies in conjunction with new computational tools facilitate analysis of the phenotype, number, location, signaling state, and function of immune cells and stromal elements in infected, inflamed, or tumor sites.
Conclusion: This talk will illustrate the power of in situ imaging for the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses and cancer, and the use of new deep learning methods for evaluating the high content data these imaging tools provide.
This work was supported by the Intramural Research Program of the NIH, NIAID.

THE HUMAN ANTIBODY RESPONSE

Michel Nussenzweig
The Rockefeller University; USA

Dr. Nussenzweig will speak about the development of antibody responses focusing on neutralizing antibody responses to SARS-CoViD-2. Over a decade ago, the Nussenzweig laboratory developed methods for rapid antibody cloning from humans in order to understand humoral immune responses to pathogens beginning with HIV-1. These methods have been widely adapted by others facilitating antibody cloning for multiple human pathogens and their clinical development. At the start of the CoViD-19 pandemic a group of 148 individuals that had recovered from SARS-CoViD-2 infection were recruited to Rockefeller University to give blood for analysis. The lecture will summarize features of their humoral immune response in these individuals and their neutralizing properties in vitro and in animal models.

MULTIPLE ROLES OF PHOSPHOINOSITIDES IN PHAGOCYTOSIS AND MACROPINOCYTOSIS

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Cells of the innate immune system actively survey their environment for signs of infection or damage. To this end, they sample their surrounding milieu by macropinocytosis and engulf foreign particles and apoptotic cells by phagocytosis. Phosphoinositides play critical roles at different stages of these processes. The conference will consist of two parts: 1) an analysis of the dynamic cytoskeletal changes that drive phagosome formation and 2) the maturation of macropinosomes, with emphasis on the disposition of their contents and the generation of tubular structures. The detection, metabolism and signaling roles of PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and PtdIns(3,5)P_2 in these processes will be highlighted.

SAI HONOR LECTURE TO DR. LEONARDO SATZ

TARGETING TREGS IN DISEASE: A TRANSLATIONAL APPROACH

Eliane Piaggio
Translational Immunotherapy Team, U932, Institut Curie, Paris, Francia

CD25+ CD4+ Foxp3+ regulatory T cells (Tregs) maintain self-tolerance and homeostasis of the immune system, but also dampen anti-tumor immunity. So, understanding their biology and function represent the holly grail for immunologists. In mouse models, manipulation of Tregs has given impressive results, however, their therapeutic application in medicine is still at the level of experimental research. What has precluded translation into the clinic? i) the difficulty in identifying unique Treg markers; ii) the fact that Tregs from different tissues represent a highly heterogeneous population shaped by microenvironmental cues; and iii) the inflammatory milieu associated to different pathologies can distinctly affect the Treg molecular profile and associated functions. Consequently, to efficiently manipulate Tregs for therapeutic aims, it is mandatory to understand the unique Treg traits associated to each pathology.

Information on cancer-associated Treg biology in humans is limited. Few RNAseq analyses of Tregs purified from human cancers have been performed, and helped to define a tumor-Treg-specific signature and correlation with patient's survival. Notwithstanding, immune modulation of an immune response occurs not only during the effector T cell phase in the tumor bed, but also, at the level of T-cell priming in the tumor-draining lymph nodes (LNs). Thus, Tregs present in the LNs will largely shape the quality of the anti-tumoral T-cell response. The properties of the Tregs residing in human LNs remain largely undefined. During the last years, our team has been studying the function and molecular characteristics of human Tregs present in the blood, LNs and tumors of breast and lung cancer patients. We have carried out multiparametric high-dimensional analyses of Tregs from the three locations, including coupled single-cell transcriptome/TCR RNAseq analysis. We have observed an unprecedented heterogeneity and functional diversity among Treg cells, and identified a unique molecular signature of clonally expanded tumor-enriched Tregs, including known and novel molecular targets. Next step is the translation of our results to novel approaches specifically targeting tumor-associated Tregs, and sparing all body Tregs, avoiding generalized immunosuppression.

SAFIS CONFERENCE

SINGLE-CELL TRANSCRIPTOMICS IN THE STUDY OF OCULAR BIOLOGY AND DISEASE.

Guillermo Lehmann
Margaret Dyson Vision Research Institute, Weill Cornell Medicine, New York, NY. Regeneron Pharmaceuticals, Inc., Tarrytown, NY.

The choroid is a highly vascularized layer of the eye localized between the sclera and the outermost retinal layer, the retinal pigment epithelium (RPE). Blood supplied by choroidal circulation is the main source of oxygen and nutrients for the RPE and photoreceptors (rods and cones), as well as the main evacuation route for retinal waste. On the other hand, the RPE provides essential support functions for photoreceptor homeostasis and visual function. Thus, it is not surprising that RPE/choroid alterations are associated with secondary photoreceptor death in a number of blinding ophthalmic diseases, including age-related macular degeneration (AMD). Although chronic inflammation of RPE/choroid tissue is believed to be central to the disease, AMD etiology remains unknown due to lack of information on RPE/choroid cell diversity and intercellular crosstalk mechanisms. Hence, there is urgent scientific and medical need for systematic RPE/choroid studies that characterize in molecular de-

tail the different cell types and crosstalk signaling mechanisms that regulate choroid and retinal homeostasis. As a first step, we produced a single cell RNAseq atlas of rodent RPE/choroid tissue and defined the molecular signature of 13 cell types in the RPE/choroid, and identified transcriptionally-distinct cell subtypes within stromal, hematopoietic and endothelial cells (ECs). By combining our scRNAseq data with the analysis of tissue-specific ECs by RNAseq, we provide for the first time a list of choroid EC '*signature genes*' and their relative expression within choroid EC subtypes. Compared to ECs from other tissues, we found that Ihh expression was highly enriched in choroid ECs, in particular in the ECs that constitute the choriocapillaris, a highly fenestrated microvascular bed located in close apposition to the RPE. This exciting observation lead us to further explore the potential role of Ihh in the adult eye. By using a reporter mouse line, we identified the target of choroidal Hedgehog signaling as a large population of stromal, GLI1+ perivascular cells with mesenchymal stem cell properties. By using two distinct transgenic adult mouse lines we observed that Ihh deletion reduced choroidal expression levels of several mast cell genes consistent with the loss of choroidal mast cells. We also found that EC-specific Ihh deletion significantly reduced molecular markers of alternatively-activated M2 macrophages. Finally, we found that in a context of tissue damage, EC-specific *Ihh* deletion results in an exacerbated inflammatory response in the RPE/choroid and neural retina that correlates with a more severe visual function impairment. In summary, our findings increase our understanding of the eye choroid at the molecular and cellular level and decipher key choroidal intercellular crosstalk mechanisms that immunomodulate eye inflammatory processes.

SAIC SYMPOSIUM: MULTIDRUG RESISTANCE TRANSPORTERS. INVOLVEMENT IN PHYSIOPATHOLOGICAL PROCESSES.

MULTIDRUG TRANSPORTER MRP4/ABCC4 AS A KEY DETERMINANT OF PANCREATIC CANCER AGGRESSIVENESS

Carlos A. Davio

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies, due to its late diagnosis, inherent resistance to treatment and early dissemination. This type of tumor is expected to become the second leading cause of cancer mortality by the year 2030 and has limited therapeutic options. Even after the development of new targeted agents and the use of multiple therapeutic combinations, there is no clear benefit for this disease. Recent findings from our laboratory show that MRP4 is critical for PDAC cell proliferation. Nevertheless, the significance of MRP4 protein levels and function in PDAC progression is still unclear. Bioinformatic studies revealed that PDAC samples show higher MRP4 transcript levels compared to normal adjacent pancreatic tissue and circulating tumor cells express higher levels of MRP4 than primary tumors. Also, high levels of MRP4 are typical of high-grade PDAC cell lines and associate with an epithelial-mesenchymal phenotype. Moreover, PDAC patients with high levels of MRP4 depict dysregulation of pathways associated with migration, chemotaxis and cell adhesion. Silencing MRP4 in PANC1 cells reduced tumorigenicity and tumor growth and impaired cell migration. Transcriptomic analysis revealed that MRP4 silencing alters PANC1 gene expression, mainly dysregulating pathways related to cell-to-cell interactions and focal adhesion. Contrarily, overexpression of MRP4 in BxPC-3 cells produced a switch in the expression of EMT markers, significantly increased tumor growth, and enhanced experimental metastatic incidence. Overall, our findings indicate that MRP4 upregulation could represent an adaptive advantage associated with poor prognosis, evidenced by the co-expression of mesenchymal markers, higher cell proliferation, tumorigenicity and invasiveness in PDAC models. Thus, we provide theoretical and experimental support for targeted treatment of pancreatic cancer by making an important contribution to the understanding of pancreatic tumor cell biology.

MOLECULAR MECHANISM OF THIRD-GENERATION P-GLYCOPROTEIN INHIBITORS

Guillermo A. Altenberg

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The multidrug resistance protein P-glycoprotein is an ATP-binding cassette (ABC) exporter pump that has two nucleotide-binding domains that bind and hydrolyze ATP, and two transmembrane domains that contain a polyspecific drug-binding pocket. Transport of drugs by P-glycoprotein makes this protein an important pharmacological target. ATP-driven association of the nucleotide-binding domains into tight dimers and their dissociation that follows ATP hydrolysis control the accessibility of the binding pocket and lead to substrate transport (alternating access model). Binding of substrates and inhibitors affects P-glycoprotein ATPase activity, but the nature of the long-distance conformational changes that occur on the nucleotide-binding domains side in response to drug binding to the transmembrane domains has not been established. We used luminescence (or lanthanide-based) resonance energy transfer (LRET) to study P-glycoprotein reconstituted in nanodiscs and determined the effects of the substrates verapamil, valinomycin and taxol, and the third-generation inhibitors tariquidar and zosuquidar. LRET is a highly sensitive spectroscopic technique that allows the study of conformational changes with angstrom resolution of functional membrane proteins in a lipid bilayer membrane environment at physiological temperature. We identified distinct nucleotide-binding domains conformational changes that can explain the stimulation of ATPase activity by substrates and the inhibition by tariquidar and zosuquidar. We propose that these inhibitors act by preventing the formation of a tight nucleotide-binding domain dimer and that their inhibition requires P-glycoprotein to go through at least one hydrolysis event.

MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 4 (MRP4) EXPRESSION AND IT RELATIONSHIP TO OBESITY AND DIABETES

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Multidrug resistance protein 4 (Mrp4) is an efflux transporter known to transport several xenobiotics and endogenous molecules. We recently identified that the lack of Mrp4 increases adipose tissue and body weights in mice. However, the role of Mrp4 in adipose tissue physiology is unknown. Here we present recent studies aimed at characterizing these roles of Mrp4 using wildtype (WT) and knockout (Mrp4-/-) mice. Our investigations showed that Mrp4 is expressed in mouse adipose tissue and that the lack of Mrp4 expression is associated with adipocyte hypertrophy. Furthermore, absence of Mrp4 increases blood glucose and leptin levels, and impairs glucose tolerance. Additionally, in 3T3-L1 cells and human pre-adipocytes, pharmacological inhibition of Mrp4 increased adipogenesis in association with altered expression of adipogenic genes. Lack of Mrp4 activity in both of in vivo and in vitro experiments leads to increased activation of adipose tissue cAMP response element-binding protein (Creb) and decreased plasma prostaglandin E (PGE) metabolite levels. The combined effect of Creb activation and reduced PGE levels promoted the observed metabolic phenotype in Mrp4-/- mice. Our collective findings led us to the conclusion that Mrp4 is as a novel genetic factor that contributes to the pathogenesis of metabolic diseases, such as obesity and diabetes.

SAIC SYMPOSIUM: MITOCHONDRIA, STRESS AND METABOLIC DISORDERS

FROM INSULIN RESISTANCE TO NON-ALCOHOLIC FATTY LIVER DISEASE: NEW EXPERIMENTAL APPROACHES

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The liver is a major contributor in the regulation of glucose homeostasis by maintaining blood glucose levels within the normal range. In obesity, excessive caloric intake, adipose tissue inflammation and elevated hepatic oxidative stress expose the liver to hyper-afflux of free fatty acids and excessive accumulation of lipid metabolites, ultimately increasing the risk of insulin resistance-associated conditions including type 2 diabetes mellitus (T2DM) and Non-Alcoholic Fatty Liver Disease (NAFLD). As such, these diseases belong to the termed referred as non-communicable diseases. As a vicious circle, once NAFLD is established it boots the hepatic insulin resistance, which, in turn, triggers evolution of the disease towards non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, liver failure and, ultimately, hepatocellular carcinoma (HCC). NAFLD also promotes systemic low-grade inflammation and impairs insulin sensitivity in other tissues and relevant organs for metabolic control. NAFLD increases the risk of T2DM and diabetes' complications and is fully considered as an independent risk factor for cardiovascular morbidity and mortality. In the last decade, NAFLD has become the most common chronic liver disease worldwide. At present, there are no approved pharmacological treatments for this pathological condition, therefore identifying molecular pathways where the hits leading to NAFLD converge represents the first step for designing an effective targeted treatment. In this conference, an overview of the molecular mechanisms leading from insulin resistance to NASH, an emerging role of oval (progenitor) liver cells in lipotoxicity during NAFLD and new therapeutic approaches based on dual agonism of glucagon-like peptide-1 (GLP-1) and glucagon receptors will be presented.

MITOCHONDRIA-DEPENDENT MECHANISMS IN TISSUE DAMAGE TRIGGERED BY AIR POLLUTION

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According to the World Health Organization, 9 out of 10 people worldwide breathe low-quality air. Consequently, more than 9 million premature deaths occur every year due to the effects of ambient air pollution exposure. Recently, it has been estimated that the exposure to polluted air in urban environments reduces life expectancy by almost 3 years globally. Increased incidence of respiratory diseases, such as pneumonia, chronic obstructive pul-

monary disease (COPD), and lung cancer, is frequently associated with air pollution exposure. However, cardiovascular diseases largely account for most of the increase in morbidity and mortality rates. In fact, according to the Global Burden of Disease study, air pollution is responsible for one-fourth of the total death count from ischemic heart disease and stroke. Besides the well demonstrated toxicity of air pollution gaseous components, epidemiological studies indicate that particulate matter (PM) is the main responsible for pollution exposure adverse effects. PM derived from anthropogenic emissions is a complex mixture of particles of variable sizes and chemical composition. Motor vehicle emissions and fossil fuel combustion during industrial processes and power generation are the main sources of PM in urban areas, as a result from incomplete oxidation of carbonaceous materials.

Following PM inhalation, the activation of oxidative stress and inflammatory pathways largely account for PM biological effects, both locally as well as systemically and in secondary organs, such as the heart and brain. In the lung, increased levels of pro-inflammatory cytokines, including interleukin (IL) -1β, tumor necrosis factor-α, IL-6, and monocyte chemoattractant protein-1, are a frequent finding after PM exposure. Therefore, lung inflammatory cell recruitment is usually observed following PM exposure, both in humans and in different animal models. Increased plasma levels of these inflammatory mediators are a common finding, indicating that PM exposure triggers an inflammatory response that is not only confined to the lung, but is also systemic. As a result, metabolism is impaired in distant organs.

We and others have studied the role of the exposure to air pollution PM over lung and heart redox metabolism, in which altered mitochondrial respiration together with enhanced NADPH oxidase 2 (NOX2) activity plays a central role as shown. Interestingly, NOX2 seems to account for increased reactive oxygen species (ROS) production in the lung following PM exposure, while mitochondrial mild uncoupling, characterized as increased oxygen consumption rate and decreased inner membrane potential, together with decreased ATP production rate and lower efficiency of the oxidative phosphorylation process (lower P/O ratio), may prevent further ROS release from this organelle. When increasing electron transport rate at the respiratory chain complexes, mitochondrial ROS production is attenuated by different mechanisms: First, mitochondria can significantly reduce $O_2^{\cdot-}$ production by decreasing oxygen tension in the mitochondrial microenvironment; Second, by favoring more oxidized levels of respiratory chain intermediates; Third, by lowering NADH levels that could be used by mitochondrial matrix flavoenzymes; Forth, by preventing reverse electron transfer due to lower membrane potential.

Alveolar macrophages play a central role in maintaining lung homeostasis through the removal of exogenous materials and microorganisms from the respiratory surface by phagocytosis, including PM. However, PM usually overwhelms cell capacity for foreign material removal, leading to uncontrolled cell activation and ROS production, as well as an exaggerated inflammatory response and pro-inflammatory cytokine release. Activation of the NLRP3 inflammasome following PM uptake seems to represent a central step in the cellular inflammatory response to PM in alveolar macrophages. Interestingly, PM has been also shown to accumulate inside mitochondria, suggesting a specific direct effect of PM over this organelle. Accordingly, PM exposure induces altered mitochondrial ultrastructure in alveolar macrophages, including swelling, cristae disorder, and organelle fragmentation at high doses, as well as modulation of mitochondrial fission/fusion gene expression. In an experimental model tested in our laboratory, an increased mitochondrial production of $O_2^{\cdot-}$ in intact cells exposed to PM was measured, which may be a consequence of impaired mitochondrial function. These findings suggest that mitochondria are important mediators in the events that follow PM exposure in macrophages. Interestingly, recently published evidence suggests that mitochondria are not only a source of ROS, but also a target of oxidative damage in the context of PM exposure. In this sense, the relevance of NOX2 as a source of $O_2^{\cdot-}$ in inflammatory macrophages is suggested, which encouraged the search for such contribution in this model. Indeed, an increase in NOX activity was found, which may contribute to the development of a crosstalk between the observed oxidative response and mitochondrial dysfunction. Considering these findings, it is proposed that mitochondria and NOX are the main sources of $O_2^{\cdot-}$, which is converted into H_2O_2 by superoxide dismutase, and ultimately acts as an effector molecule in redox signaling and oxidative damage to macromolecules, following PM exposure in macrophages.

Impaired cardiac mitochondrial function also arises as a central feature of air pollution PM toxicology. Mechanistically, an acute exposure to PM induces a decrease in active, but not rest, state oxygen consumption rate, together with inner membrane depolarization and reduced mitochondrial ATP production. Consequently, deficient contractile and lusitropic reserve is observed in PM-exposed mice, as the heart fails to properly increase cardiac contractility after a β-adrenergic stimulus with isoproterenol. Blunted mitochondrial ATP supply in mice breathing PM may account for this effect, as decreased ATP levels are a frequent finding in the failing heart. Interestingly, this cardiac mitochondrial bioenergetic dysfunction seems to be partially mediated by an inflammatory response triggered by PM exposure, since impaired mitochondrial respiration and cardiac contractility is attenuated by pretreatment with a chimeric anti-TNF-α antibody (Infliximab) in PM-exposed mice.

The central nervous system is also a target of air pollution, causing tissue damage and functional alterations, with oxidative stress and neuroinflammation as possible mechanisms mediating these effects. Glutathione levels, assessed as GSH/GSSG ratio, were found to be decreased in cerebral cortex after exposure to urban air, and at later time points in the olfactory bulb (OB). Activation of NOX was also observed. Increased GFAP

expression levels showed reactive astrocytes in OB, probably associated with the altered olfactory function observed by a behavioral test. Interestingly, impaired mitochondrial function, due to reduction in O_2 consumption in active state, a decrease in ATP production rate and an increase of H_2O_2 production was found, accompanied by decreased activities of the respiratory complexes I-III and II-III.

Taken together, impaired mitochondrial respiration, enhanced ROS release, and deficient ATP supply, play a central role in the adverse health effects reported after air pollution PM exposure in the lungs and distant organs, such as the heart and brain. In this context, the modulation of mitochondrial function (e.g. by mitochondrial targeted antioxidants) arises as a potential therapeutic target to prevent excessive lung inflammatory response, as well as the alterations observed in cardiac and brain tissues in PM-exposed individuals at particular high risk.

ROLE OF CYCLOOXYGENASE 2 IN LIVER PATHOPHYSIOLOGY.

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Cyclooxygenase (COX) is the enzyme that catalyzes the limiting step in prostanoid synthesis. Prostaglandins play an important role in numerous biological processes such as platelet aggregation, maintenance of the gastric mucosa, reproduction, etc. and also in pathological processes such as inflammation and cancer. COX-2 is expressed and induced by different stimuli in various tissues and cell types, but in the hepatocyte its expression is limited to those pathophysiological situations that lead to cell dedifferentiation, regeneration or proliferation. Through the use of cell models and transgenic mice, our group has shown that COX-2 expression in hepatocytes protects against hyperglycemia-induced liver damage, insulin resistance and obesity, as well as steatohepatitis and fibrosis suggesting an improvement in mitochondrial function and oxidative phosphorylation. Furthermore, COX-2-dependent prostaglandins protect against damage induced by liver ischemia/reperfusion due to attenuation in inflammation, oxidative stress and liver apoptosis, an increase in autophagic flow, and a decrease in endoplasmic reticulum stress. Measurement of plasma PGE2 levels from patients undergoing liver transplantation revealed a significantly positive correlation of PGE2 levels and graft function, and an inverse correlation with ischemia time. Taking all these data into account, we hypothesized that COX-2 induction plays a protective role as a physiological response against liver injury. We believe that PGs are important actors in the observed processes, and that local relative concentrations are decisive for the hepatoprotective effect.

THE AMAZING WORLD OF RNA BINDING PROTEINS REGULATING THE MITOCHONDRIAL FUNCTION

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Translational control is essential for proper eukaryotic protein expression and so mRNA metabolism and function must be finely regulated at different levels. The modulation of mRNA nuclear export, stability, rate of translation, and localization is regulated by RNA binding proteins (RBPs) that therefore determine the final amount of synthesized proteins. Some RBPs are essential for the function of all cellular mRNAs, while others control a subset of transcripts coding for proteins implicated in distinctive processes. RBPs make up assemblies denominated membrane-less organelles (MLOs) which concentrate space and temporarily the mRNA molecules necessary to perform a specific function.

Mitochondria are dynamic and plastic organelles, which flexibly adapt morphology, ATP production, and metabolic function to meet extrinsic challenges and demands. In the last decade, the posttranscriptional regulation of the expression of nuclear-encoded mitochondrial proteins has emerged as a fast, flexible, and powerful mechanism to shape mitochondrial function and coordinate it with other cellular processes. In fact, several recent studies have highlighted that the fate of mRNAs encoding mitochondrial proteins is dictated by RBPs that orchestrates mitochondrial function during physiological and pathological conditions.

In our laboratory we have implicated human RBP Smaug in the regulation of mitochondrial function. Smaug is a conserved translational repressor that recognizes specific RNA motifs present in a large number of mRNAs, including nuclear transcripts that encode mitochondrial enzymes and forms cytosolic MLOs in several organisms and cell types. We demonstrated that the loss of Smaug function affects mitochondrial activity and mitochondrial network morphology. Single molecule FISH reveal that transcripts that encode SDHB and Uqcrc1 associate with Smaug MLOs. In addition, defective Smaug MLO formation affects mitochondrial activity. Finally, rotenone and metformin but not the uncoupler CCCP rapidly induce Smaug MLO dissolution and the release of bounded mRNAs. We propose that Smaug MLOs respond to changes in the energetic metabolism to coordinate the expression of mRNAs that encode key mitochondrial proteins.

SAIC HONOR SYPOSIUM TO DR HORACIO A. REPETTO: PEDIATRIC INFECTION: CONTRIBUTIONS OF SCIENTIFIC RESEARCH TO THE DIAGNOSIS, TREATMENT AND CONTROL OF PEDIATRIC INFECTION DISEASES IN ARGENTINA.

PROTECTING INFANTS AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV)

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Respiratory syncytial virus (RSV), responsible for more than three million yearly hospitalizations and up to 118 000 deaths in children under 5 years, is the leading pulmonary cause of death for this age group that lacks a licensed vaccine. Ninety-nine percent of deaths due to the virus occur in developing countries. Community deaths affect low-income children from socially vulnerable families and appear to be as frequent as inpatient fatalities. In industrialized countries, RSV deaths occur almost exclusively in children with premorbid conditions. In a sense, RSV is an «opportunistic» killer. It needs a synergistic premorbid, medical practice-related, infectious, or social co-factor to cause a fatal outcome.

CONTRIBUTIONS OF SCIENTIFIC RESEARCH TO THE DIAGNOSIS, TREATMENT AND CONTROL OF HEMOLYTIC UREMIC SYNDROME IN ARGENTINA

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Argentina has the sad privilege to have the highest incidence of Hemolytic Uremic Syndrome (HUS) in the world. HUS is an endemic disease, characterized by the triad microangiopathic hemolytic anemia, thrombocytopenia and renal compromise, with an annual incidence of 6.23 per 100,000 children under 5 years of age.

Most cases are associated with diarrheal prodromal and Shiga toxin-producing *Escherichia coli* (STEC) infections detected in more than 70 % of the patients.

HUS is the leading cause of acute renal failure, the second cause of chronic kidney disease and 3 % of the patients die every year.

Despite the huge experience in the diagnosis, treatment and control of this disease there are questions without a definitive answer:

1)Why do we have the highest incidence in the world?

The fact that Argentina is an agricultural and livestock country and cattle is the main reservoir for STEC, children start eating meat at an early age, the high meat consumption per person could explain in part the high incidence of HUS but the most important issue is the high prevalence of a hypervirulent strain.

2) Which patients with STEC-infection have more risk to develop HUS?

The earlier the detection of STEC, the earlier the treatment**.** Only 10-15% patients with STEC-diarrhea evolve to HUS. Antibiotics, anticholinergic drugs, high leucocytes counts and dehydration increase the risk.

3) Which patients with STEC-HUS have more risk to develop a severe disease requiring dialysis, with severe neurological involvement and even death?

Identify clinical and laboratory predictors of severity at onset like high leucocytes counts, hemoconcentration, low C3 levels and hyponatremia could help to improve the treatment.

4) Which patients with STEC-HUS have more risk to have a poor long-term renal prognosis?

Recognize clinical and laboratory predictors of poor-long term renal prognosis allows intensifying the importance of the follow-up to prevent or to slow down the evolution to chronic kidney disease.

ADVANCES IN THE TREATMENT OF CHAGAS DISEASE. HOSPITAL DE NIÑOS “RICARDO GUTIÉRREZ” IS A COORDINATING CENTER FOR STUDIES IN THE DEVELOPMENT OF PEDIATRIC FORMULATIONS.

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A challenge in the treatment of Chagas disease, which is caused by the parasite Trypanosoma cruzi, is the establishment of feasible cure criteria. Currently, immunological and parasitological methods have been reported with applicability for post-treatment cure assessment. For conventional serology methods, one of the most relevant limitations is the long time required for conversion of serological responses to negative. Furthermore, no test currently in use (e.g. ELISA, IHA, PCR, etc) has been validated for long term follow up of patients, as they were initially developed for diagnostic purposes.

The aim of pharmacological treatment for patients with Chagas disease is to avoid disease progression into the chronic symptomatic phase, preventing cardiac involvement.

Two drugs are available for the treatment of CD: benzni-

dazole and nifurtimox. The effectiveness of these drugs, especially in the chronic stage of infection, is still a topic of debate due to inconsistent results and a lack of early biomarkers of treatment response.
These drugs have demonstrated efficacy in pediatric patients, but the difficulty was in the formulations, which only existed for adults.
After several studies conducted by our team, pediatric formulations of both drugs were achieved, being approved by the FDA. This guarantees the correct treatment of pediatric patients, increasing safety and decreasing adverse events.

SAIC SYMPOSIUM: IMMUNOLOGICAL THERAPY AND CARCINOGENESIS

STRESS MANAGEMENT IN INTRATUMORAL IMMUNE CELLS

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Malignant cells utilize diverse strategies that enable them to thrive under adverse conditions while simultaneously inhibiting the development of anti-tumor immune responses. Hostile microenvironmental conditions within tumor masses, such as nutrient deprivation, oxygen limitation, high metabolic demand and oxidative stress disturb the protein folding capacity of the Endoplasmic Reticulum (ER), thereby provoking a cellular state of "ER stress". Sustained activation of ER stress sensors endows malignant cells with greater tumorigenic, metastatic and drug resistant capacity. Additionally, recent studies have uncovered that ER stress responses further impede the development of anti-cancer immunity by manipulating the function of immune cells in the tumor microenvironment. This seminar will describe the major immunoregulatory effects of ER stress in cancer and will highlight the significant immunotherapeutic potential of targeting ER stress response pathways to eliminate tumors.

CHRONIC LYMPHOCYTIC LEUKEMIA: THE EFFECT OF NOVEL THERAPEUTIC AGENTS ON THE LEUKEMIC CLONE AND NON-MALIGNANT CELLS.

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in western countries. It is characterized by the presence of malignant B cells in peripheral blood and lymphoid tissues. The approval of multiple new targeted agents has allowed important advances in therapeutic management of CLL patients, improving clinical outcomes and quality of life. However, CLL is still an incurable disease. Lymphoid tissues act as survival niches, where leukemic cells receive signals through the B cell receptor (BCR) and signals from non-malignant cells that favor leukemic cell accumulation. Our group has been working with different novel therapeutic agents for CLL patients: inhibitors of the BCR-associated kinases (BCR-KIs), and the selective BCL-2 inhibitor, venetoclax. We ~~first~~ confirmed that peripheral blood leukemic cells from CLL patients are very sensitive to venetoclax *in vitro* in a dose-dependent manner, while T cells, NK cells, and monocytes are less sensitive to the drug. We also found that CLL cells that have received survival signals from the tumor microenvironment, such as those triggered by autologous activated T cells, are less responsive to venetoclax due to the upregulation of anti-apoptotic proteins MCL-1 or BCL-XL, which are not targeted by the drug. Venetoclax-resistant leukemic cells are characterized by high levels of activation and proliferation markers, increased PD-1 expression and show resistance to a second treatment with the drug. Finally, we found that BCR-KIs entospletinib and idelalisib by impairing T cell activation, prevent the generation of CLL cells with an aggressive phenotype and, more importantly, completely restore the sensitivity to venetoclax. Altogether, these *in vitro* evidences highlight the relevance of the tumor microenvironment in the generation of venetoclax-resistant CLL cells and provide an interesting rationale for combining venetoclax with entospletinib or idelalisib to reduce the emergence of drug resistance *in vivo*.

PRACTICAL BIOINFORMATICS IN ONCO/IMMUNOGENOMICS STUDIES

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Advances in cancer genomics during the last decade have been driven by the development of NGS technologies and the generation of huge omics data. These NGS-based technologies have inspired new computational techniques enabling interrogation of biological and clinical aspects in an unprecedented manner. In this presentation, several state-of-the-art computational approaches are discussed, providing a comprehensive insight into the field, including the identification of mutational process, cell signaling, immune cell profiling, and prediction of the immunotherapy response.

EPIGENETICS BASIS OF B-CELL LYMPHOMAS

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A majority of B-cell lymphomas –diffuse large B cell lymphomas (DLBCLs) and follicular lymphomas (FLs)– originate from germinal center (GC) B cells. GCs are transient structures within which B cells undergo massive proliferation and somatic hypermutation of their immunoglobulin loci in order to generate high affinity antibodies. Proliferation of GC B cells is strictly dependent on interactions with T follicular helper cells. More than 70% of the genes mutated in DLBCL and FL are epigenetic modifiers, such as the H3K4 methyltransferase KMT2D, the H3 acetyltransferases CREBBP and EP300, and the H3K27 methyltransferase EZH2. These genes are required to either establish the GC phenotype or to facilitate exit from the GC reaction. Mutations in these genes lead to disruption of the immune synapse plasticity. All are acquired early during pathogenesis and are considered founder mutations. Unlike DLBCLs, FLs are slow-growing, indolent tumors containing extensive follicular dendritic cell (FDC) networks. Heterozygous somatic mutation of EZH2 occurs in approximately 30% of FL patients and primarily affects the EZH2 SET domain, conferring more efficient H3K27 trimethylation activity. We show that EZH2 gain-of-function mutations initiate FL by attenuating GC B cell requirement for T cell help and driving slow expansion of GC centrocytes that become enmeshed with and dependent on FDCs. By impairing T cell help, mutant EZH2 prevents induction of proliferative MYC programs. Thus, EZH2 mutation fosters malignant transformation by epigenetically reprograming B cells to form an aberrant immunological niche that reflects characteristic features of human FLs, explaining how indolent tumors arise from GC B cells.

SAIC SYMPOSIUM: NEUROSCIENCES: TARGETING NEUROINFLAMMATION IN CENTRAL NERVOUS SYSTEM DISORDERS AND NEURODEGENERATIVE DISEASES

DUAL ROLE OF ANGIOTENSIN RECEPTORS IN INFLAMMATION: TARGETING A WOLF IN SHEEP´S CLOTHING.

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IHEM-CONICET (FCM-UNCuyo-UNViME)

Abnormal activation of the local renin-angiotensin system (RAS) in brain tissue has been associated with the pathophysiology of neonatal encephalopathy. This is a heterogeneous condition characterized by decreased responsiveness, seizures, hypotonia, abnormal primitive reflexes, apnea, feeding disturbance, and abnormal cry. Perinatal insults activate the immune system and trigger central responses that involve glial activation and release of cytokines and chemokines, reactive oxygen species (ROS), reactive nitrosative species, excitotoxicity, mitochondrial and vascular impairment. Changes in blood–brain barrier (BBB) permeability and infiltration of peripheral immune cells can further contribute to inflammation in the brain. We observed inflammatory responses to hypoxia on the neonatal brain and cerebellum. In addition to the ‘‘classical’’ circulating RAS, a second local or tissue RAS has been observed in all nervous tissues. Angiotensin II (Ang II), via its type 1 receptor (AT1R) exerts pro-oxidative and pro-inflammatory effects. However, these effects are counteracted by a RAS opposite arm constituted by Ang II/AT2R and Ang 1-7/Mas receptor (MasR) signaling. We studied the role of the AT2R microvasculature responses to hypoxia in the neonatal brain. Other studies of our laboratory explored how cutaneous inflammation affected the expression of AT1R and AT2R in subpopulations of rat dorsal root ganglion neurons. We found that AT2R was selectively and differentially modulated throughout the inflammation process. Recently another member of the RAS family, ACE2 has been identified as a functional receptor for SARS-CoV-2, which causes Covid-19. ACE2 and AT2R seem to counteract the detrimental actions of AT1R. Regulation of mutually antagonistic AT1R and ACE2/AT2R is essential for maintaining control of inflammation and an imbalance between these two receptors is potentially pathological. The complexity of these mechanisms remains under study.

IDH1 MUTATION IN GLIOMA REPROGRAMS EARLY MYELOID DIFFERENTIATION LEADING TO A NON-IMMUNESUPPRESSIVE BRAIN TUMOR MICROENVIRONMENT

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Gliomas are the most common primary brain tumors; patients exhibit a poor prognosis. Mutations in isocitrate dehydrogenase (mIDH) are present in most patients with lower grade gliomas (LGG), and are correlated with better prognosis and survival. We hypothesized that mIDH1 via the production of 2HG, induces epigenetic reprogramming leading to alterations in brain tumor infiltrating immune cells' phenotype and function. To examine the role of mIDH1 in the brain tumor immune microenvironment (TME), we generated LGG glioma models using the sleeping beauty system (Koschmann et al., 2016; Nunez et al., 2019). Our data indicate that mIDH1 gliomas exhibit increased levels of $CD11b^+$ $Ly6G^+$ cells in the TME, BM, circulation and spleen of mice. Interestingly, the TME-derived $CD11b^+Ly6G^+$ myeloid cells from mIDH1 tumors didn't inhibit tumor antigen specific T-cells' expansion. Further analysis using single cell RNA sequencing coupled with Mass cytometry revealed that the majority of $CD11b^+Ly6G^+$ in the TME-mIDH1 are composed of non-immune suppressive neutrophils and pre-neutrophils. In contrast, $CD11b^+Ly6G^+$ from the TME-wtIDH1 are composed of immune suppressive PMN-MDSCs. This effect of reprogramming granulocytes in the TME-mIDH1 is mediated by epigenetic upregulation of the granulocyte-colony stimulating factor (G-CSF). Blocking G-CSF restored the inhibitory function of $CD11b^+$ $Ly6G^+$ in mIDH1, and shortened the median survival of mIDH1 to the same level of the wtIDH1 tumor bearing animals. Consistent with that, LGG is the sole tumor cohort within all TCGA data sets where high G-CSF is correlated with favorable patient outcome. Our results provide insights into novel epigenetic mechanisms triggered by mIDH1 which regulate myeloid cells' heterogeneity and immunosuppression within the brain TME; a feature that can be harnessed to develop novel immunotherapeutic strategies.

IGF-1 GENE THERAPY MODULATES GLIAL CELL IN NEURODEGENERATIVE PROCESS

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The natural process of aging and Parkinson´s disease (PD) are both neurodegenerative disorders with glial changes and progressive neuronal loss with a variety of motor and non-motor dysfunctions. It has been reported that the progression of many neurodegenerative diseases depends on the activation of microglia and their polarization towards a proinflammatory phenotype and establish an exacerbated immune response. Many neurotrophic factors produced by glial cells, such as Insulin like growth factor 1 (IGF1), can modulate glial cells phenotype, promoting neuronal survival. IGF-1 could be neuroprotective in neurodegenerative models by improving changes in neuronal and glial activity.

Our research group has set the goal to fight the deleterious effects of aging in senile rats and 6OH Dopamine animal model to understand plasticity processes associated with behavior decline, analyze modifications on glial cells through different brain areas involved in the proposed circuit and to carry out therapeutic approaches with IGF-1 gene therapy.

We showed that IGF1 gene therapy in senile rats increased the number of microglial cells, specifically in the Striatum. In addition, these cells presented higher reactivity and were polarized towards an M2 anti-inflammatory phenotype and those treated with RAd-IGF1 presented higher phagocytic activity and greater synaptic remodeling. Moreover, in PD animal model, we found an early behavioral cognitive decline that was partially modified with IGF1 overexpression on ongoing experiments. We observed astrocytes changes in different dorsal hippocampus areas and changes of TH reactivity that correlates with IGF-I overexpression. Conclusion: Further understanding of glial cells and their functions could allow modulation of the microenvironment resulting in potential therapeutic strategies, such as IGF-1 gene therapy, that improve the course and progression of the neurodegenerative process.

ASTROCYTES ARE CENTRAL PLAYERS IN THE BRAIN NEUROINFLAMMATION AFTER INJURY

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Damage Associated Molecular Patterns (DAMP) are intracellular molecules released to the extracellular milieu after tissue damage. In the Central Nervous System (CNS), acute brain injury releases DAMP and these molecules activate astrocytes and microglia which are the innate immunity effectors in the CNS. Astrocytes rapidly respond to damage with reactive astrogliosis that is characterized by prominent phenotypical alterations that have been classically described for almost a century. More recently, it was shown that subsequent astroglial polarization to the A1 phenotype can induce neurodegeneration; while conversion to the A2 phenotype usually facilitates neuronal survival. Using a combination of *in vivo* models of brain damage (traumatic brain injury, brain ischemia and epileptic seizures); *in vitro* glial and neuronal cell cultures and *in silico* mathematical modeling; we have been able to demonstrate that astrocytes require microglia to become reactive and to polarize to the A1 proinflammatory-neurodegenerative phenotype after DAMP stimulation. Conversely, microglial cells also increase their response to DAMP when they are co-cultured with astrocytes. Moreover, disruption of DAMP-mediated signaling pathways shows beneficial effects by reducing neuroinflammation and neurodegeneration in the injured brain. We conclude that bidirectional microglial-astroglial cooperation is required for astrocytes to respond to DAMP and to induce neuroinflammation in the injured brain. Thus, early treatment strategies aimed at blocking downstream DAMP-activated signaling pathways are likely to have a significant beneficial effect in neuroinflammation and neurodegeneration.

SAIC SYMPOSIUM: LIPIDS IN CONTEXT: FROM HEALTH TO DISEASE

ARYLACETAMIDE DEACETYLASE (AADAC), A NEW PLAYER IN HEPATIC LIPID HOMEOSTASIS

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The liver plays a central role in maintaining whole-body lipid and energy homeostasis. Hepatic triacylglycerol (TG) and cholesteryl ester (CE) synthesis, lipolysis and transport are regulated by nutritional status. Fasting increases hepatic TG/CE storage and this storage is rapidly depleted during refeeding. The lipolytic processes responsible for TG/CE turnover during refeeding have not yet been characterized. We have identified a novel liver lipase arylacetamide deacetylase (AADAC) that is localized in the endoplasmic reticulum and exhibits hydrolytic activity toward diacylglycerol, TG and CE. We have found that AADAC is responsible for the hepatic TG/CE turnover during the fasting/refeeding transition. TG/CE depletion was abolished during the refeeding period in AADAC deficient mice. AADAC knockout mice accumulated significantly more hepatic neutral lipids than wild type mice when challenged with western-type diet, and exhibited accelerated progression of hepatic steatosis in the long-term western-type diet feeding study. Decreased lipolysis, altered lipid droplet dynamics, and increased *de novo* lipogenesis in the fasting/refeeding cycle could contribute to the blunted lipid turnover in AADAC deficient hepatocytes. These findings indicate that AADAC plays an important role in hepatic lipid metabolism adapted to changing nutritional conditions and that dysregulation of AADAC activity to interrupt this process leads to fatty liver.

CONSEQUENCES OF ENHANCED CHOLESTEROL HYDROXYLATION IN THE OLD BRAIN

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Cholesterol is essential for the function of all the animal cells. As the major component of the plasma membrane, it plays a key role in membrane integrity and fluidity and ion permeability. In addition, through its close interaction with membrane receptors, cholesterol plays essential roles in intracellular signaling and processes of en-

do-exocytosis.
Due to the incapacity of cholesterol to cross the blood-brain barrier, the brain cholesterol homeostasis is strictly controlled through synthesis *de novo* mainly carried out by glial cells and its elimination. Cholesterol elimination from the brain takes place mainly by hydroxylation, a step catalyzed by a brain specific cytochrome P450 containing enzyme called Cholesterol 24-hydroxylase or CYP46.
We have shown increased expression of CYP46 in old neurons, where membrane cholesterol reduction has a number of consequences as altered function, mobility and endocytosis of synaptic receptors. Cholesterol decreases in the old also impairs the proper activation of neuronal signaling pathways with a negative impact on learning and memory.
Since abnormal increase of CYP46 has been found in glial cells either in neurologic diseases or after traumatic brain injury, we investigated the specific brain regions and cell types in which CYP46 expression is increased during aging. Finally, we analyzed if aging is able to predispose for abnormal CYP46 expression after brain injury, where cholesterol hydroxylation would mediate inflammatory processes.

THE USE OF FAT-SOLUBLE VITAMINSAS SUPPORTIVE THERAPY DURING THE TREATMENT OF LIVER DISEASES WITH IFN-A-2B

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Vitamins are essential components of the diet that do not provide calories, and that are necessary for the maintenance of metabolic and growth functions. The fat-soluble vitamins (A, D, E and K) are absorbed in the gastrointestinal tract and usually stored in the liver, adipose tissue and muscle, and eliminated with feces. Generally, vitamins are used as reinforcement therapy in patients with complex diseases, such as cancer, whose course and/or treatment puts them in a state of general discomfort. Many experts worldwide advise patients against the use of supplements and promote obtaining nutrients from foods whenever possible.
IFN-α has been used clinically for deferring the progression of liver damage or for the prevention of hepatocellular carcinoma progression in patients with chronic hepatitis B or C. Interestingly, many IFN-α-treated patients undergo a flu-like state, which makes them eager to consume supportive vitamins as nutritional supplements. However, the benefits of such combinations are not fully studied.
It has been demonstrated that simultaneous treatment of mouse cells with interferon and vitamin A (retinoic acid) resulted in an inhibition of interferon action. On the other hand, it has been reported that vitamin D exerts very interesting outcomes when combined with interferon to treat hepatitis and other hepatic malignances. The number one fat-soluble vitamin consumed worldwide, vitamin E, has been demonstrated to be not entirely safe, if safe at all, when used combined with drugs that act as pro-oxidant, as interferon. And finally, vitamin K has been demonstrated to be effective when combined with antitumor drugs to enhance their antiproliferative effects; but also, it has been demonstrated to block the apoptotic effects of IFN-α in a rat model of hepatocarcinogenesis.
The aim of this presentation is to summarize the effects of different fat soluble vitamins when consumed as supportive therapy during the treatment of liver diseases with IFN-α-2b.
Findings from our own and other authors reveal that supportive therapies with vitamins are not always safe, as they could put patients' lives at risk. It is necessary to start paying attention to the overall treatment layout, since vitamins might have a negative role on the final outcome.

POTENTIAL ATHEROGENICITY OF TRIGLYCERIDES RICH LIPOPROTEINS

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In the past decade, several studies and investigator´s opinions questioned the role of elevated triglycerides (TG) as a cardiovascular disease risk factor. These uncertainties are mainly based on meta-analysis that, although they found direct association between TG and adverse outcomes, this association becomes nonsignificant after multivariate statistical analysis.
It is difficult, then, to explain the relationship between the isolated and direct effect of elevated TG on cardiovascular disease, in part because TG levels are commonly associated with concomitant alterations such as decreased HDL-C or predominance of small dense LDL.
When high TG concentrations are measured, it means that there is an accumulation of circulating triglyceride rich lipoproteins (TRL), that is a set of VLDLs, Chylomicrons and their remnants, potentially atherogenic
Certain points will be explained for the understanding of the underlying atherogenic mechanisms of TRL, through some stages of the TRL metabolism: TRL production rate, Lipoprotein alterations in chemical composition and

size, their lipolytic degradation and the effect of TRL on endothelium. These points will be addressed through insulin-resistance (IR) models and functional studies

Some years ago, we implemented an IR animal model to assess the rate of VLDL secretion. By means of Triton injection the clearance of circulating VLDL was inhibited for 2 h, and an increase in plasma TG accumulation was observed in IR rats, allowing the rate of VLDL secretion to be calculated. We could confirm that hyperinsulinemia induces an over-secretion of VLDL particles

In metabolic syndrome patients, we also evidenced an overproduction of VLDL through the total mass and VLDL-apoB levels, as particle number indicators. Most of these patients have fatty liver and then our results indicate that hepatic steatosis could have a role in the overproduction. Regarding chemical composition and size, isolated VLDL showed over enrichment in TG, and analyzing the HPLC results of the VLDL profile, we found that the area of picks belonging to IR patients were higher than controls, showing a predominance of larger TRL. These results are in line with the experiments based on in vivo kinetic studies in humans, where they observed large VLDLs, being liver fat a driven force for their production.

As part of the mechanisms, the aforementioned results can be attributed to differences in the regulators of the level of liver fatty acids. We found increased SREBP1c and reduced PPARα, which impacted on the synthesis of fatty acids in IR rats, and were significantly related to the production of large VLDL particles, with a high content of TG. At this point the question that may arise is whether these large VLDLs are atherogenic.

Another alteration among the circulating TRL consist of smaller VLDL particles, cholesterol enriched. The cholesterol ester transfer protein (CETP), that facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins, probably has a role on the circulating VLDL characteristics of individuals suffer from several conditions. In metabolic syndrome patients, CETP maintained increased activity and also showed a significant correlation with VLDL cholesterol content, as product of its activity. Cholesterol-rich VLDL are like IDL or remnants particles (RLP). We think that secreted VLDL in IR are over-enriched in TG, and in circulation become rich in cholesterol. Moreover, another consequence that is likely occurring when CETP activity is increased is the production of small and dense LDL, that also contributes to the atherogenic profile

Continuing with the lipolytic degradation stage of the lipoproteins, there are many issues to comment because not only the enzyme activities are relevant, but also their regulators, such as Apo CII and Apo CIII, GPIHDLBP1, Apo AV, among other factors, being lipoprotein-lipase (LPL) activity a resultant of the action of its regulators. Two points were scarcely studied. 1- the controversial activity of LPL in IR states, and 2- the quality or behavior of TRL as substrate of the lipolytic enzymes

On the one hand, we observed in metabolic syndrome and obese subjects a decreased LPL activity, and increased HL as well. The finding of LPL reduced activity contribute to elucidate debated and paradoxical mechanisms regarding lipolysis of TRL in IR states.

Following with the affinity of TRL and LPL, in normal conditions this goes on well. When VLDL is TG overloaded, it would seem to be a bad substrate for LPL, and it would not produce LDL. However, several years ago, we have design an *in vitro* assay incubating different types of VLDL with commercial (standard) LPL, in adequate conditions, obtained the kinetic curve and the Km, being the inverse of Km a marker of enzyme-substrate affinity. Then we observed that actually LPL has a high affinity with VLDL overloaded in TG. Thus, lipolysis occurs, size of VLDL become smaller and then it surely can pass through the arterial wall

Another interesting point is the role of Adiponectin not only in the production but also in degradation of TRL. Adiponectin, is reduced in IR, we observed this in the present or absent of fatty liver, and its reduction was associated with the increase in the number of large VLDL particles. Moreover, we found a positive correlation between LPL and Adiponectin, even significant after adjusting by HOMA and other IR factors, suggesting that Adiponectin may favor lipolysis in normal conditions, and when it is reduced, contributes to a delay in lipoprotein degradation.

With the aim to assess the effect on endothelium of altered TRL, we applied a known system to measure endothelial function dependent on nitric oxide (NO), working with isolated aorta rings (endothelium tissue) incubated with VLDLs isolated from patients with IR, and controls. We have induced 100 % contraction with nor-adrenaline and then allowed relaxation with increasing concentration of acetylcholine. Then dose-responses curves were plotted, and comparing with typical VLDL from controls, VLDL from IR patients showed an inhibition in the vasorelaxation, consequence to endothelial dysfunction. Thus, beyond high plasma TG levels, alterations in TRL can induced endothelial dysfunction, that represent one of the first step of atherogenic mechanism.

However, in the same system, the presence of HDL, allows the inhibition of relaxation to be lower, because HDL neutralizes or protects the effect of endothelial dysfunction that exert the altered VLDL

In Summary, the accumulation of TRL in circulation promotes influx into the arterial wall and their retention. Large particles cannot enter but LPL has high affinity and can quickly hydrolyzed these particles, reducing their size and can easily enter being taken by macrophages without previous modification, because TRL are usually oxidized in plasma. Moreover, these particles exert endothelial dysfunction and promote an inflammatory context that could be evidenced by an increase in circulating high sensitivity reactive “C” protein.

SAIC SYMPOSIUM: IMMUNONUTRITION

MACRO AND MICRONUTRIENTS AND IMMUNE SYSTEM

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The first studies related to the interaction between nutrition and immunity were carried out in children with caloric-protein malnutrition; changes in lymphoid tissues, decrease in size and weight of the thymus with loss in corticomedullary differentiation, deformed Hassal corpuscles and occasionally calcified were observed.
Omega3 fatty acid family induces an increase in the production of IgA, IgM and IgG, with a decrease in the synthesis of IgE. In the last 2 decades, it´s interesting to note the increase of the prevalence of atopic diseases in industrialized countries. This phenomenon is linked to changes in eating habits. These diseases are characterized by an increase in IL-4 production and a decrease in IFN- γ .The deficiency of essential fatty acids impairs the immune response in different ways. Many of the immunological changes associated with essential fatty acids and arising from in vitro and animal model studies have been attributed to modifications in endogenous eicosanoids production and subsequent control of cytokines and immunoglobulin synthesis. Arachidonic acid derivatives (omega6) - *Prostaglandin PGE2 and Leukotriene LTB4*- are mediators of inflammation; on the contrary, those from the omega3 polyunsaturated fatty acids from fish oils seem to exert an antitumor effect through their immune-modulatory and anti-inflammatory action and the direct inhibition of tumor cell proliferation, through the alteration in the pathway of the production of the PGE2.
Moreover, the function of many cells of the immune system depends on metabolic steps that need various nutrients - *minerals and vitamins* - as critical cofactors, among them we can mention some minerals (Zn, Fe, Cu, Se) and vitamins: fat soluble Vitamins (A, D, E) and water soluble vitamins(B6, B12, C, Folic Acid)
It is important to note that all considerations related to the effects of nutritional imbalances on the immune system must be based on the complexity and heterogeneity of immunecompetent cells, their subpopulations and products such as interleukins and interferon and other inducing and / or regulatory systems.

MICROBIOTA AND LIFESTYLE

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There is wide scientific evidence about the necessity to maintain a good balance and functionality of the composition of the microbiota to regulate homeostasis and preserve health. However, the characteristics of the microbiota are still controversial and there is a lack of definition considering a healthy gut microbiota. This fact along with the multifactorial nature intrinsic features of humans complicates to make general recommendations on appropriate dietary patterns and diverse lifestyle habits to improve our microbiota. Indeed, not only food consumption, but also, eating behaviour, physical activity, sleep quality and quantity, as well as stress situations and socioeconomic conditions are very important factors to bear in mind, since they can affect the microbiota and hence, the immune system function.
Furthermore, the variable response of healthy adults to the intake of probiotics and prebiotics suggest the existence of potential intra-individual factors affecting the initial interaction between commensal microorganisms and dietary ingredients and thus accounting for gut microbiota changes in a greater extent than diet. The changing dietary habits worldwide with a large offer of processed and ultra-processed foods containing artificial ingredients such as sweeteners, the coincident rise in emotional disorders, and the worsening of other lifestyle habits such as smoking, alcohol and drugs consumption, sedentary lifestyle and sleep, can together contribute to gut dysbiosis and thus, to health impairment and non-communicable chronic diseases development.
Therefore, our main concern is to have the skills to build a healthy microbiota, capable to defend the organism together with the immune system against any adverse effects produced by pathogens or strange substances (toxins, carcinogenic and environmental molecules). This is the reason why the study of the effects of specific dietary ingredients (probiotics, prebiotics, alcohol, sweeteners and fats) in the gut microbiota of healthy adults acquires a great interest as well as the potential intra-individual factors involved, and the influence of other potential lifestyle factors.

ROLE OF PROBIOTIC BACTERIA IN IMMUNITY AND INFLAMMATION

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Scientific advances in the signals induced for the microbiota allowed to understand how the probiotics influence the mucosal immune system (MIS). Probiotics induce a network of signals to the gut immune cells to activate the MIS. These bacteria interact with the intestinal immune cells (IECs) inducing Goblet and Paneth cells to produce mucus and antimicrobial peptides (AMPs). The interaction between IECs and probiotics is mediated by cytokines release without eliciting inflammatory mechanisms in the host, in contrast with pathogens. How probiotics achieve a balance between stimulation and anti-inflammatory mechanisms? Probiotic strains stimulate the MIS increasing IgA+ cells in the intestine, bronchus and breast and induce immunomodulation through IL-10. We also demonstrated that probiotics stimulate innate immune cells: dendritic cells and macrophages without tissue damage. Probiotics induce an activation of proteins regulatory genes that modulate the NFkB and MAPK via. These genes are not activated by pathogens. The biological mechanisms observed open the door for the study of anti-inflammatory mechanisms, from probiotic, probiotic fermented milk (PFM) and Yogurt. In an acute inflammatory model induced by *Salmonella,* probiotics lactobacilli oral administration was able to reduce the inflammation through the production of AMPs that minimize the internalization of the pathogen to the intestine. In a respiratory allergy model the PFM induced a balance to Th1 with production of IgG instead of IgE. These previous results led us to study the anti-inflammatory mechanisms mediated by yogurt in a model of ulcerative colitis. We demonstrated that the main effect of yogurt is the decrease of IL-17 and the increase of IL-10. In a model of chronic inflammation as obesity we observed a diminution in markers of metabolic syndrome. Probiotics, PFM and yogurt have an important role in the mechanisms of the immune surveillance, playing a role in the immunoregulation of the MIS.

VITAMIN D AND IMMUNITY

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Vitamin D is a fat-soluble vitamin, found in very few foods, such as egg yolk, oily fish and organ meats, as well as in fortified dairy products. Its main source is through the conversion of 7-dehydrocholesterol to pro-vitamin D_2 in skin when exposed to UV light from sun. Vitamins D2 and D3 are further metabolized in liver and kidney to render $1,25(OH)_2D$ (calcitriol).

Its traditionally known functions are related to bone mineralization and skeletal health. However, in the last decades, other functions have been described. All nucleated cells have vitamin D receptors (VDR) on their surface, indicating a role not only in calcium absorption, but in many other metabolic processes. With respect to the immune system, VDRs are present in T and B cells, monocytes and antigen presenting cells.

It increases chemotaxis and phagocytosis by macrophages and monocytes, inducing antimicrobial peptide production. It has been observed that calcitriol promotes differentiation of dendritic cells, while inducing tolerance as a protective effect from autoimmunity. With respect to T-cells, it reduces the production of Th17 and the secretion of pro-inflammatory cytokines, such as IL-2 and IL-17. B-cell homeostasis is also modulated by vitamin D, as it inhibits the development of plasma and memory cells, promoting apoptosis and autophagy after an infection or stressor.

Vitamin D is also necessary to maintain normal immune system in the gastrointestinal tract and its subclinical deficiency may be associated with dysbiosis of the gut microbiota, leading to low-grade inflammation through metabolic endotoxemia.

While clinical deficiency is associated with rickets and osteoporosis, subclinical deficiency has been found in several upper respiratory tract infections, obstructive pulmonary disease and allergic asthma among others.

We can conclude that vitamin D is indispensable for the adequate function of the innate and acquired immune system, as well as the gastrointestinal local immunity.

SAIC -SAI SYMPOSIUM

AUTOINFLAMMATORY INTERFERONOPATHIES: AN EXPANDING DISEASE SPECTRUM AND UPDATES ON TREATMENTS

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Genetic causes of autoinflammatory diseases unraveled cytoplasmic innate immune pathways that constitute an innate surveillance system and provided mechanistic insight into inflammatory pathways that drive sterile

inflammation. Recently, disease-causing gain-of-function (GOF) mutations in viral sensors or loss-of-function (LOF) mutations in the ubiquitin proteasome system cause autoinflammatory diseases amplified by Type-I IFN dysregulation, that can be identified and monitored by a chronically elevated IFN response gene (IRG) signature in blood.

Monogenic defects that cause autoinflammatory interferonopathies can be linked to increased transcription of *IFNA*s and *IFNB1* and to the production of IRGs that have become the "read out" for IFN signaling in these patients. While GOF mutations in the viral sensor and adaptor molecule *STING1* lead to STING ligand independent activation of downstream pathways and predominantly *IFNB1* transcription, mutations in genes encoding proteasome components lead to viral sensor independent *IFNA*s and *IFNB1* transcription in hematopoietic and non-hematopoietic cells that can be modeled by progressive downregulation of proteasome genes *in vitro*. Lastly LOF mutations in genes that encode nucleases and DNA and RNA editing enzymes, result in accumulation of intracellular self-nucleic acids that activate the IFN pathways though engagement with viral intracellular sensors. These only partially overlapping mechanisms that drive the IRG signature are associated with different clinical phenotypes and provide insights into mechanisms that drive systemic autoimmunity.

Recent discoveries of specific mutations in *IKBKG, OTULIN* and *SAMD9L* that activate the IRG signature and additional inflammatory pathways, which is reflected by the only partial response to JAK inhibitors, coupled with murine data implying NFKB activation downstream of STING point to crosstalk between IFN regulating and other inflammatory pathways that remain elusive.

As the currently available treatments for presumed interferonopathies target IFN signaling, mechanistic insights into the pathways that cause CANDLE, SAVI and AGS and their clinical mimics, may reveal novel targets for treatment of these and other conditions with phenotypic overlap.

DIAGNOSIS OF IL-1-ASSOCIATED AUTOINFLAMMATORY DISEASES IN ARGENTINA

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Monogenic autoinflammatory diseases triggered by a dysregulated activation of the inflammasome that is usually accompanied by an hyperactivation of the IL-1 pathway can be exemplified by Familial Mediterranean Fever (FMF). This pathology, which was the first hereditary autoinflammatory disease described (1945), is also one of the most prevalent and one of the first in which the genetic etiology could be identified. Indeed, since 1997, when two groups identified the MEFV gene by positional cloning, we know that FMF is due to mutations in this gene, which codes for the pyrin protein. Since then, in our laboratory of the Immunology and Rheumatology service of the Garrahan hospital, we have studied the presence of mutations in patients with suspected FMF by Sanger sequencing. At the beginning of the 21st century, the discovery of a caspase activating complex called the Inflammasome, laid the foundations for unraveling the precise molecular mechanisms in which pyrin participates in the pathophysiological process of FMF development. These findings opened the doors for the identification of different components of the inflammasome, in particular caspase-1 and interleukin-1ß. Inflammasomopathies have since been expanded to include those arising from "gain-of-function" mutations in other components of the inflammasome (such as NLRP3) or in upstream molecules that regulate inflammation of the inflammasome (such as MVK). Furthermore, inflammasomopathies include "loss of function" mutations in molecules that decrease antagonism to IL-1. This scenario in which mutations in different genes may be responsible for a specific clinical phenotype opened the doors to broaden our methodological approach when establishing molecular diagnoses for these pathologies.

SAI SYMPOSIUM I

CELL MIGRATION PROMOTES THE FUNCTIONAL DIVERSIFICATION OF GUT DENDRITIC CELLS

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Dendritic cells (DCs) patrol peripheral tissues and transport antigens to lymph nodes to initiate and regulate adaptive immune responses. Within tissues, DCs constitute a complex cell population made of numerous DC subsets that express different surface markers and eventually display distinct functions. How cell-intrinsic programs and tissue-specific cues drive this functional diversification of DCs is only partially understood. We here show that in the small intestine, transmigration of lamina propria $CD103^+CD11b^+$ conventional DC2s (cDC2s) into the epithelium deeply modifies their transcriptional profile, promoting the acquisition of an immature DC phenotype with low expression of pro-inflammatory genes, co-stimulatory molecules and CCR7. Accordingly, we found that intraepithelial cDC2s efficiently internalize antigens but lead to T cell anergy. We further identify Myosin II-dependent migration as a critical cell-intrinsic mechanism essential for the formation of the intraepithelial DC pop-

ulation, and retinoic acid as an extracellular cue driving this process. As a consequence of this, intraepithelial DCs predominantly exist in the upper regions of the gut wherein this metabolite concentrates. Thus, the fine-tuning of DC migration by both cell-intrinsic and environmental cues controls their compartmentalization and functional diversification within tissues.

CRUZIPAIN AND ITS PHYSIOLOGICAL INHIBITOR, CHAGASIN, AS A DNA-BASED THERAPEUTIC VACCINE AGAINST *TRYPANOSOMA CRUZI*

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Instituto de estudios de la inmunidad humoral Prof. Ricardo A. Margni (IDEHU), Universidad de Buenos Aires (UBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Chagas disease caused by the protozoan parasite *Trypanosoma cruzi* is endemic in 21 Latin America countries and the southern United States of America, and now is spreading into several other countries due to migration. Despite the efforts to control the vector throughout the Americas, currently there are almost 7 million infected people worldwide, causing approximately 10,000 deaths per year, and 70 million people at risk to acquire the infection. Chagas disease treatment is restricted only to two parasiticidal drugs, benznidazole and nifurtimox which are effective during the acute and early infections but have not been found to be as effective in chronic infection. No prophylactic or therapeutic vaccine for human use has been communicated at this moment. Here we evaluate in a mouse model, a therapeutic DNA vaccine combining Cruzipain (Cz), a *T. cruzi* cysteine protease that proved to be protective in several settings, and Chagasin (Chg), which is the natural Cz inhibitor. The DNA of both antigens, as well as a plasmid encoding GM-CSF as adjuvant, were orally administrated and delivered by an attenuated *Salmonella* strain to treat mice during the acute phase of *T. cruzi* infection. The bi-component vaccine based on *Salmonella* carrying Cz and Chg (SChg+SCz) was able to improve the protection obtained by each antigen as mono-component therapeutic vaccine and significantly increased the titers of antigen- and parasite-specific antibodies. More importantly, the bi-component vaccine triggered a robust cellular response with IFN-γ secretion that rapidly reduced the parasitemia during the acute phase and decreased the tissue damage in the chronic stage of the infection, suggesting it could be an effective tool to ameliorate the pathology associated to Chagas disease.

SAI SYMPOSIUM II

DEFINING THE MECHANISMS THAT UNDERLIE THE INITIATION AND RESOLUTION OF INFLAMMATORY BOWEL DISEASES (IBD).

Eduardo Villablanca

Karolinska Institute, Sweden.

The focus of my research program is to understand the mechanisms that underlie the **initiation** and **resolution** of Inflammatory bowel disease (IBD), an immune-mediated inflammatory disease that affects more than three million people in Europe and for which no cure currently exists. To achieve these goals, we have developed a research program that integrates cellular immunology, bioinformatics and the creation of novel *in vivo* models to ultimately interrogate the function of IBD-associated polymorphisms, to study the crosstalk between the host and the environment, and to identify novel genes and pathways involved in mucosal healing. In this talk, I will discuss our new experimental models to visualize the initiation of IBD. Finally, I will discuss the use of data mining, single cell and spatial transcriptomics to identify new pathways promoting mucosal healing upon intestinal injury.

CANDIDA ALBICANS AND INNATE IMMUNITY: FROM COMMENSALISM TO INFECTION PATHOGENESIS

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Candida albicans is a pleomorphic fungus commensal of different mucosa surfaces. As part of the vaginal microbiome coexists with bacterial communities in a harmonious balance and under strict control of local immune response. The disruption of this condition such as dysbiosis and an abnormal host mucosal immune response to *Candida,* favored the fungal overgrowth, morphogenetic changes, expression of hyphae-associated virulence factors and tissue invasion. Vaginal infection by *Candida* species, known as vulvovaginal candidiasis (VVC), is considered an acute inflammatory disease that affects more than 75% of healthy women at least once

in their lifetime. 5-10% of women suffer from recurrent VVC (RVVC), defined as ≥4 episodes per year. Clinical and experimental evidences revealed the critical role of local innate immunity in both defense and pathogenesis of vaginal infection by *Candida spp.* In the vaginal tract, epithelial cells provide the first barrier against the fungus and are actively involved both, in the recognition of pathogenic phenotype of fungus and in the initiation of local response through the release of antimicrobial peptides (AMP), alarmins, chemokines and some cytokines. Through integration of immunological studies in animal models of VVC, patients with acute and recurrent form of mycosis and proteomic approaches we identified the contribution of AMPs and Interferon Type I (IFN-I) in the pathogenesis of *Candida* vaginitis. Ours results provide evidences about the ability of fungal virulence factors to down-regulate the expression of Beta Defensin, AMPs with relevant candidacidal and immunomodulatory properties in vaginal tract. Our findings also reveal new data about the activation of IFN-I pathway on epithelial cells after fungal immune recognition and its protective role during VVC. Identification of cellular processes and molecular pathways contributes to a better understanding of this mycosis and open new therapeutic avenues.

SIMPOSIO SAFIS. "NUTRICIÓN Y FITOTERAPIA"

PRENATAL AND EARLY POSNATAL NUTRITION: A CARDIOVASCULAR AND METABOLIC RISK FACTOR?

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Numerous epidemiological and experimental studies demonstrate a correlation between an adverse intrauterine environment and increased risk of cardiovascular and metabolic diseases in adulthood. People exposed to famine in utero show a more atherogenic lipid profile, impaired glucose tolerance and higher prevalence of hypertension and diabetes. The fetal programming hypothesis, suggests that an injury during fetal life, which leads to restricted intrauterine growth, not only results in low birth weight but also adaptive responses that can lead to the loss of structural units (nephrons, cardiomyocytes, β pancreatic cells, skeletal muscle cells) in order to maintain the development of other organs, such as the brain. These adaptive changes can bring immediate advantages by increasing perinatal survival in a poor nutritional environment, but program a lower morphological and functional capacity for their life and the increased risk of developing chronic diseases, such as diabetes type II, high blood pressure, renal disease, obesity, insulin resistance and metabolic syndrome. In addition, it is known that vitamins and minerals are essential for human health and development since they participate in numerous biochemical functions and are involved in intermediate and energy metabolism and have antioxidant properties. Inadequate intake of micronutrients, also known as hidden malnutrition, during critical periods of growth has become also a major health problem in developed and developing countries, particularly in pregnant women, infants and children who have an unbalanced diet. In summary, numerous epidemiological and experimental studies have shown that metabolic and nutritional imbalances during a critical time window in development have persistent effects on the health of the offspring and may be responsible for *in utero* programming of diseases such as obesity, diabetes and high blood pressure in adulthood and highlights the critical importance of perinatal care optimization for better management and prevention of adult diseases.

EFFECTS OF CANNABIS ON MYOCARDIAL DAMAGE INDUCED BY ISCHEMIA-REPERFUSION

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There is growing evidence that the endocannabinoid system is implicated both in cardiovascular physiology and the pathogenesis of hypertension, heart disease and atherosclerosis. Our aim was to determine the effects of the acute treatment of a cannabinoid extract (CBE), obtained from the *Cannabis sativa sp,* on myocardial ischemia-reperfusion injury. The extract was characterized and quantified by gas chromatography coupled to mass spectrometry (GC-MS). The profile was: Δ9-tetrahydrocannabinol (Δ9-THC): 1.35 mg/ml; cannabidiol (CBD): 0.51 mg/ml and cannabinol (CBN); 0.34 mg/ml. Isolated rat hearts perfused by Langendorff system were assigned to the following experimental groups: Non ischemic control (NIC): 110 min of perfusion: Ischemic control (IC): 30 min of global ischemia and 60 min of reperfusion (R) and CBE: 0.1 μg/ml of the extract was administered during the first 10 min of R. Infarct size (IS) was determined by TTC staining. Systolic and diastolic function was assessed by the left ventricular developed pressure (LVDP) and the end diastolic ventricular pressure (LVEDP), respectively. The expression of phosphorylated forms of Akt, PKCε and e-NOS, and the content of CB1 and CB2 receptors were determined by western blot. CBE significantly decreased IS (2.3 ± 0.5 % vs 31 ± 2 % in IC) and improved the post-ischemic recovery of myocardial function. At the end of R, LVDP was 70 ± 10 % vs 17 ± 3 % and LVEDP 15 ± 3 vs 57 ± 5 mmHg with respect to IC. The expression of P-Akt, P-eNOS and P-PKCε decreased ~ 40% in IC and increased ~ 150 %

in CBE treated hearts, both compared to NIC. The content of CB1 increased ~ 140 % and CB2 decreased ~ 40 % in IC hearts. Opposite changes were observed in CBE treated hearts: CB1 decreased ~ 60 % y CB2 increase up to 150 %. These data demonstrate that CBE reduces cell death and myocardial post-ischemic contractile dysfunction. These beneficial effects appear mediated by Akt/PKCε/eNOS-dependent pathways activated through CB2 receptors.

FLAVONOIDS AT THE GASTROINTESTINAL TRACT: IMPACT ON OBESITY-RELATED METABOLIC DISORDERS

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The gastrointestinal (GI) tract plays a central role in the absorption, distribution, metabolism, and excretion of flavonoids. Flavonoids and/or their metabolites can modulate events at the GI tract that can have both local and systemic impact. At the GI tract, they can modulate nutrient absorption, GI barrier permeability, the activity of luminal enzymes, neutralize luminal toxins and oxidant species, and mitigate dysbiosis, tumorigenesis and intestinal inflammation. Such local effects have systemic extra-intestinal consequences, e.g. on inflammation, glucose homeostasis, lipid and energy metabolism. Overnutrition and the associated obesity, negatively impact GI functions. This contributes to the development of insulin resistance and type 2 diabetes (T2D), steatosis, non-alcoholic liver disease (NAFLD), and several other co-morbidities. Taking the flavan-3-ol (-)-epicatechin (EC) as an example of flavonoid, we observed in rodent models of high fat- or high sugar-induced obesity and dysmetabolism, that EC exerts beneficial effects at the GI tract mitigating also the development of T2D and NAFLD. At the GI tract, EC and/or its oligomers, the procyanidins were able to : i) regulate lipid absorption; ii) maintain the intestinal barrier integrity and prevent endotoxemia; iii) regulate the synthesis/secretion of gut hormones that have GI trophic actions and modulate glucose/lipid metabolism, iv) inhibit inflammation and oxidative stress; v) exert anti-colorectal cancer activity. These effects were associated with decreased systemic inflammation improved insulin sensitivity and mitigation of steatosis. NADPH oxidase and redox-regulated signaling cascades (NF-κB, JNK1/2, ERK1/2) emerge as central targets of EC's actions. This can in part explain EC capacity to mitigate GI barrier permeabilization, liver/adipose tissue endoplasmic reticulum stress, inflammation and inhibition of the insulin pathway. Some of the described effects/mechanisms are also exerted by other structurally-related flavonoids. In summary, further understanding of the mechanisms mediating the effects of flavonoids at the GI tract is of critical importance given the relevance of the GI tract in sustaining overall health and of the widespread recommendations of increasing the intake of plant bioactives. *Supported by NIFA-USDA*.

BURNING FAT: ROLE OF POLYPHENOLS IN WHITE ADIPOSE TISSUE BROWNING

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Obesity is one of the main public health concerns worldwide. White adipose tissue (WAT) is an endocrine organ that stores energy excess as triglycerides. Increased adiposity, mainly visceral WAT, is strongly associated with insulin resistance, type 2 diabetes and cardiovascular disease, among others. In contrast to WAT, brown adipose tissue (BAT) has the ability to dissipate energy in the form of heat due to the presence of the uncoupling protein 1 (UCP-1), a key determinant of mitochondrial thermogenesis. Interestingly, "brown-like" adipocytes can be observed in WAT and are characterized by the presence of multilocular lipid droplets and high number of mitochondria which are associated with a reduction of total adiposity and improvement of metabolic alterations. WAT browning can be triggered by different stimuli such as cold, exercise or pharmacological treatment, such as β-adrenergic stimulation or peroxisome proliferator activated receptor-γ (PPARγ) agonists. In addition, bioactive compounds such as polyphenols had been recently implicated in the emergence of brown-like cells in WAT. Polyphenols are bioactive compounds widely distributed in fruits and vegetables with an important role in preventing and managing increased adiposity and its comorbidities. We observed in rodent models of high fat-induced increased adiposity and metabolic alterations that supplementation with grape pomace extract (GPE), rich in polyphenols, stimulate the expression of the main transcriptional regulators of brown-like cell development, i.e., PPARγ-coactivator-1α (PGC-1α), PPARγ, PR domain containing 16 (PRDM16), and UCP-1 reducing adipose hypertrophy and inflammation in WAT and insulin resistance. GPE and two of the major GPE flavonoids, quercetin and (-)- epicatechin, enhanced the expression of transcriptional regulators of browning and UCP-1 through the up-regulation of the β-adrenergic receptor downstream cascade in 3T3-L1 adipocytes treated with palmitate. Overall, this finding highlights the potential utilization of bioactive grape-derived compounds to prevent/attenuate adiposity associated pathologies.

SAIC - LEÓN CHERNY AWARD

ESSENTIAL OILS FOR THE DISCOVERY OF NEW ANTHELMINTIC COMPOUNDS TESTED ON THE NEMATODE CAENORHABDITIS ELEGANS

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Parasitic nematodes of humans and animals cause diseases of major socio-economic importance globally. Control of infections in both human and veterinary medicine currently relies mainly on chemotherapy, but resistance is an increasing problem, so there is an urgent need for discovery of novel drugs. As parasitic nematodes are not ideal laboratory animals, the free-living nematode C. elegans was demonstrated to be an excellent model system for the discovery of new anthelmintics and for characterizing their mechanisms of action and resistance. Essential oils (EOs) are natural products produced by aromatic plants. EOs are complex mixtures that contain 2 or 3 major phytochemicals, which can be terpenes or aromatic compounds. We used paralysis assays of wild-type and mutant C. elegans strain to identify EOs with potential anthelmintic activities, reveal the active components, the target sites and the mechanisms of action. We found that EOs belonging to six different orders produced rapid paralysis of C. elegans and we established the half maximal effective concentration values between 0.02-1.2 percent of EOs. All EOs tested also inhibited egg hatching, a property related to anthelmintic ability. Thus, EOs mediate both rapid and long-term anthelmintic effects. We determined that trans-cinnamaldehyde (TC), a major component of C. verum EO, produces both paralysis and egg-hatching inhibition. By testing mutant worms, we identified the muscle L-AChR and GABA receptors as EOs and TC targets in vivo. Thus, by modulating two receptors with key roles in worm motility, these EOs emerge as novel sources of anthelmintic compounds. Likewise, the N-AChR mutant strain is slightly resistant to TC, thus revealing a third target receptor for terpenes. Due to the potential of EOs as sources of novel antiparasitic compounds, additional studies will be carried out to determine in more detail the molecular mechanisms of action and structure-activity relationships of their active compounds.

MAGEC2: A NOVEL MEMBER OF THE RAS/B-RAF ONCOGENIC PATHWAY TO COUNTERACT THE P53 RESPONSE IN HUMAN MELANOMA

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Ras proteins (H-, N- and K-Ras) transduce proliferation signals from growth-factor activated receptor tyrosine kinases (RTKs) through the mitogen activated protein kinase (MAPK: Raf/MEK/ERK) pathway. Activating mutations in Ras or B-Raf proto-oncogenes (i.e, RasV12 or B-RafV600E) are frequent in human melanoma. In normal cells oncogenic Ras or B-Raf activates p53 and/or pRb tumor suppressor response. We aimed to study the MAGE-I (Melanoma Antigen Genes-I) proteins involvement in wt-p53 regulation in melanoma (p53 regulators highly expressed in melanoma).

Here, we identified MageC2 protein as a p53 regulator in an oncogene-activated MAPKs context. First, we observed that MageC2 protein levels respond to serum deprivation in cultured cells. Since growth factors activate RTK/Ras, we studied Ras role, and we observed that RasV12 enhanced MageC2 levels depending on a functional MEK/ERK pathway by PD184352 MEK inhibitor treatment. MageC2 raising did not involve MageC2 expression changes and required active proteasome as indicated by MG132 treatment, and accumulated MageC2 was phosphorylated only in threonine as assessed by anti-phospho aminoacids. To study the MageC2 role in p53 regulation activity in oncogene-activated MEK/ERK condition, we generated CRISPR/CAS9 mediated MageC2 KO in A375 melanoma cells and we regulated MAPK hyperactivity with PD184352 to study p53 activity by its targets (p21, Mdm2 and PUMA) quantification, and we observed that MageC2 plays a key role as a downstream target of the B-Raf/MAPK oncogenic pathway by controlling the p53 response. Finally, gene expression analysis of TCGA skin cutaneous melanoma (n=448) through Cbioportal showed a significant inverse correlation between high MageC2 expression (RSEM>1000) and p53 targets expression (p21, BAX and PUMA) only in a Ras/B-Raf hyperactivated context (n=263). In conclusion, we propose that Ras or B-Raf downregulates wt-p53 activity by increasing MageC2 protein levels through the MAPK pathway.

DISRUPTION OF SKIN HOMEOSTASIS ACTIVATES A LECTIN-DRIVEN CIRCUITRY THAT CONTROLS IMMUNOPATHOLOGY AT THE CROSS-ROADS OF CANCER AND INFLAMMATORY PSORIATIC DISEASE

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Disruption of skin homeostasis by environmental insults eventually activates pathologic circuitries leading to inflammation and carcinogenesis. Here we report essential roles of Galectin-7 (Gal-7), a β-galactoside-binding protein preferentially expressed in keratinocytes (KCs), in skin carcinogenesis and inflammatory disorders. In a chemically induced carcinogenesis model, mice constitutively over-expressing Gal-7 in KCs (*Tg46 mice*) showed higher number ($p<0.001$) and earlier occurrence ($p<0.001$) of papillomas compared with *WT* and *Lgals7*$^{-/-}$ animals. In line with transcriptomic data which showed higher expression of myeloid derived suppressor cells (MDSCs) recruiting chemokines in *Tg46* lesions ($p<0.01$), a higher frequency of MDSCs in draining lymph nodes from *Tg46* mice ($p<0.01$), was observed. Analysis of RNAseq datasets from human non-melanoma skin cancer lesions supported these findings. Moreover, through binding to glycosylated receptors on MDSCs, Gal-7 enhanced their immunosuppressive capacities ($p<0.01$). Two different *in vivo* settings confirmed the role of M-MDSCs as critical mediators of Gal-7-driven skin carcinogenesis. In a different scenario -a skin inflammatory model induced by topical administration of Imiquimod (TLR agonist)- *Lgals7*$^{-/-}$ mice showed more severe psoriasis-like clinical signs compared with *Tg46* and *WT* animals ($p<0.01$). When delving into mechanistic, Gal-7, was found to endow Langerhans Cells (LCs) with tolerogenic potential, inducing an increased secretion of TGF-b1 and IL-10 ($p<0.05$). The resulting microenvironment favoured the expansion of regulatory T cells ($p<0.05$) thus ameliorating the signs of skin inflammation. Our findings identify Gal-7 as a multifunctional checkpoint which controlled expression regulates skin homeostasis and commands skin fate. Whilst skin insults leading to enhanced Gal-7 expression may promote skin carcinogenesis, in the opposite context its down-regulation could potentiate skin inflammatory disorders.

NEW THERAPEUTIC STRATEGY FOR HEPATOCELLULAR CARCINOMA: BIOINFORMATIC IDENTIFICATION AND PHARMACOLOGICAL INHIBITION OF RAC1 GTPASA.

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Introduction: Tumors take advantage of the deregulation of RHO GTPases to acquire several cancer hallmarks. We aimed to identify and target deregulated RHO family members in human hepatocellular carcinoma (HCC).

Methodology: Expression deregulation, clinical prognosis, and transcription programs relevant to HCC was studied employing 3 public datasets (TCGA, ICGC and GSE14520). The therapeutic potential of RAC1 inhibitors was study in vitro by MTT, apoptosis (Propidium iodide) and cell cycle (Annexin-V) in HCC cell line; and in vivo in a subcutaneous (sc) HuH7 or orthotopic Hepa129 tumor mice models. RNA-Seq analysis of RAC1 inhibition on HuH7 cells was assessed and data correlated with the HCC datasets to characterize the underlying mechanism. The therapeutic effect of RAC1 inhibition on liver fibrosis was evaluated on Thioacetamide experimental model (600 mg/Kg/week).

Results: Among RHO family, only RAC1 is upregulated (paired t-test, $p<0.05$), correlates with poor patient survival (Kaplan-Meier, $p<0.05$), and is strongly linked with a pro-oncogenic transcriptional program. From a panel of novel RAC1 inhibitors studied, 1D-142 was able to induce apoptosis and cell cycle arrest in HCC cells (2-way ANOVA, ***$p<0.001$), displaying a stronger effect in highly proliferative cells. Partial rescue of the RAC1-related oncogenic transcriptional program was obtained upon RAC1 inhibition by 1D-142 in HCC. Strikingly, 1D-142 treatment not only reduce tumor growth and survival in sc HuH7 tumor model (***$p<0.01$, 2-way ANOVA), but also tumor volume and intrahepatic metastasis in orthotopic Hepa129 model (**$p<0.01$, t-test). Additionally, 1D-142 decreases hepatic stellate cell activation and exerts an anti-fibrotic effect in vivo (***$p<0.001$, t-test).

Conclusion: By bioinformatics analysis we identify RAC1 as a new therapeutic target for HCC. The pharmacological inhibition of RAC1 by 1D-142 induced a potent antitumoral effect in highly proliferative HCC established in fibrotic livers.

NEUTROPHIL AUTOPHAGY IS MODULATED BY SLAMF1 DURING HUMAN ACTIVE TUBERCULOSIS

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Neutrophils infected with *Mycobacterium tuberculosis* (*Mtb*) predominate in tuberculosis (TB) patients' lungs and phagocytose the pathogen but the mechanism of microbial elimination is controversial. Autophagy, a crucial mechanism for several neutrophil functions, can be modulated by immunological mediators. Besides, the costimulatory molecule SLAMF1 can function as a microbial sensor and also interact with autophagy-related proteins in macrophages. Therefore, we investigated whether SLAMF1 participates in neutrophil autophagy against *Mtb*. Neutrophils isolated from heparinized blood from TB patients and healthy donors ($2x10^6$cells/ml) were infected with *Mtb*H37Rv strain (MOI:1) during 2h or stimulated with *Mtb*-Antigen (*Mtb*-Ag,10μg/ml) or different mycobacterial compounds. In several experiments, SLAMF1 mAb (10μg/ml) was added to cell cultures to activate this receptor. Additionally, experiments including NADPH-oxidase, ERK or p38 inhibitors were performed as well. SLAMF1 and autophagy levels were analyzed by flow cytometry and confocal microscopy. Interestingly, in concordance with bioinformatics analyses, we demonstrated for the first time that *Mtb* induces surface expression of SLAMF1 in human neutrophils. Moreover, we observed that diverse mycobacterial components might be recognized by these cells during *Mtb* infection and induce SLAMF1 surface expression through ROS and MAPK activation ($p<0,05$). Furthermore, *Mtb*-Ag stimulation promoted autophagy flux in human neutrophils, which was further increased by activation of SLAMF1 ($p<0,01$). Remarkably, TB patients' neutrophils displayed reduced levels of SLAMF1 ($p<0,01$) and autophagy ($p<0,05$) against *Mtb*-Ag as compared to healthy controls. Altogether, we identified SLAMF1 as an innate receptor in human neutrophils that participates in the autophagy of these cells during active TB. Therefore, either inducing autophagy in myeloid cells or increasing Th1 responses, SLAMF1 would be a key receptor in human immunity against *Mtb.*

SAIC – YOUNG INVESTIGATORS BIGAND AWARD

INTERTUMORAL HETEROGENEITY IN GLIOBLASTOMA DICTATES DIFFERENT CELLULAR REDOX STATUS AND SUSCEPTIBILITY TO PHOTODYNAMIC THERAPY WITH DOPED CONJUGATED POLYMER NANOPARTICLES

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Glioblastoma (GBM) is the most aggressive malignant tumor of the CNS. It presents high recurrence and resistance to treatments. This leads to the search for new therapies that improve the prognosis of GBM. Conjugated polymer nanoparticles (CPNs) have been shown to be excellent photosensitizers (PS) in Photodynamic therapy (PDT). We have developed metallated porphyrin-doped CPNs for PDT. GBM presents inter and intratumoral cellular and genetic heterogeneity which include the adaptation to reactive oxygen species (ROS) and could be expressed as different basal levels of oxidative stress and antioxidant enzymes. The tumor heterogeneity and their associated difference in sensitivity to ROS-producing therapeutic agents must be taken into account in designing PDT protocols. Catalase (CAT), glutathione reductase (GSR) and superoxide dismutase 2 (SOD2) mRNA expressions in GBM samples were remarkably higher than that in normal tissues (GEPIA database). These expression patterns were used as biomarkers to predict the performance of CPN-PDT. CPN-PDT efficacy was compared in different GBM cell lines with different initial redox status. Basal mRNA expression levels of GSR, CAT and SOD2 were similar between U-87 MG and MO59K. However, T98G showed the highest levels for the 3 antioxidant enzymes. The inherent ROS level, quantified by DCFDA probe, was the lowest in T98G compared to the other two GBM cell lines. Cells were incubated with CPNs at various concentrations and subsequently irradiated with a MultiLED system. Cells not incubated with CPN were viable at all light doses, while a significant toxic effect was found in all cell lines after PDT. MO59K and U-87 MG cells died at a CPN concentration and light

dose-dependent manner with very similar IC50 values. However, T98G cells were significantly more resistant to CNP-PDT. The superior resistance of T98G cells could be attributed to their higher antioxidant enzyme genes expression levels before and immediately after PDT.

METABOLIC LIPOTOXICITY-INDUCED NEUROINFLAMMATION: POTENTIAL ROLE OF GLIAL CELLS INTERACTION VIA EXTRACELLULAR VESICLES

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Obesity and related metabolic disorders are important risk factors for brain aging, promoting alterations in the plasticity of limbic structures such as the hippocampus. Among the underlying mechanisms, chronic inflammation and insulin resistance are crucial factors, also associated with alterations of sphingolipid metabolism. Taking this into account, we intend to study mechanisms associated with the impact of metabolic disturbances on the brain. We aim to assess the inflammatory response induced by a lipotoxic context and the communication between glial cells mediated by the release of extracellular vesicles (EVs) as vehicles of damage propagation.

We previously found that C57BL/6 mice exposed to a high fat diet (HFD) presented neuroinflammation, decreased neurogenesis and structural synaptic alterations, together with spatial memory impairment. To assess potential mechanisms involved, we used an *in vitro* approach emulating the lipotoxic context with the saturated fatty acid palmitate (PA). Microglial cultures exposed to PA showed a pro-inflammatory profile and, after purification of EVs from the conditioned media (CM), we found that exosomes altered dendritic spine morphology of hippocampal neurons. Here, we show that in the presence of ceramide synthesis inhibitor Cambinol, the induced expression of IL1β in PA-exposed BV2 microglial cells was diminished ($p<0.05$). In the same line, Cambinol seemed to prevent the decreased phagocytic capacity of BV2 cells exposed to PA. Finally, regarding the interaction between glial cells, preliminary results showed that CM from PA-stimulated BV2 cells was able to induce the expression of IL1β in C6 astrocytic cell line. Interestingly, after EVs isolation, exosomes derived from PA-microglia exerted the same effect.

Our results suggest a role of ceramide pathway in the inflammatory context induced by PA and the potential involvement of exosome-like EVs in the propagation of the damage response between glial cells.

THE ONCOGENIC ROLE OF P63 AND ITS CONTRIBUTION TO THE MOLECULAR CLASSIFICATION OF BLADDER TUMORS

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A new classification of muscle-invasive bladder cancer (MIBCs) has been proposed. The over-expression or the activating mutations in the Fibroblast Growth Factor Receptor 3 (FGFR3) are associated with the luminal subtype while basal MIBCs are characterized by p63 activation. Even so, the gene regulatory pathway of an altered-FGFR3 and a functional validation of p63 in bladder cancer (BC) remains poorly characterized and it is therefore our main aim. Loss of p63 led to a decreased 2D and 3D growth in cell lines bearing FGFR3 mutations ($p<0.001$). Gelatin degradation and migration ability in p63 depleted cells are significantly lower compared CRL ($p<0,01$). Later on, tumor volume was significantly lower in p63 depleted tumors compared CRL ($p<0.01$) indicating that p63 is an essential protein through which the cell mediates its proliferative and migratory mechanism. A p63 decreased levels together with a blunted cell migration was observed following the FGFR3 pharmacological inhibition while no variations in protein levels or cell migration have been observed in FGFR3 WT cells. Overall, these results suggested that p63 mediates cell migration in FGFR3-dependent cells. Supporting the regulation of p63 by FGFR3, analysis of mRNA levels in human bladder tumors showed that p63 expression was significantly higher in tumors mutated for FGFR3. Moreover, higher p63 levels in patients with mutated FGFR3 is associated with worse prognosis compared to ones with lower p63 levels. In order to identify p63-involved molecular targets in cells bearing FGFR3 mutations, several molecular pathways inhibition downstream of FGFR3 were carried out and p63 expression and cell proliferation in 2D and 3D were analyzed. Inhibition of PI3K pathway showed the best and most sustained effect over time ($p<0.001$). Here we demonstrated for the first time that p63 mediates tumor growth, cell proliferation and migration of FGFR3-dependent BC cells which could be block by targeting PI3K molecular pathway.

SAIC – REPETTO AWARD

DURATION OF FECAL SHEDDING OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN PATIENTS WITH HEMOLYTIC UREMIC SYNDROME

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Hig Penna

Introduction: Knowing the duration of fecal shedding of Shiga toxin-producing Escherichia coli (STEC) in Hemolytic Uremic Syndrome (HUS) patients would be useful to prevent secondary cases. Objectives: 1) To describe and analyze STEC fecal shedding duration. 2) To evaluate the association between STEC excretion duration and sex, age, dialysis requirement, antibiotics and STEC serotype. Population and Methods: Prospective, observational, longitudinal and analytic study. Period 2013-2019. Stool cultures were performed at admission and every 5 to 7 days until 2 negative samples were obtained. The duration of STEC shedding was defined as the interval between the diarrhea onset and the first of two consecutive negative stool cultures. STEC infection was confirmed by screening of stx1, stx2 y rfbO157 genes by polymerase chain reaction assays.
Patients without stool culture or with a negative result were excluded and those who did not deliver stool samples were eliminated. Mean (95% CI) and percentiles of STEC fecal shedding duration were calculated and compared between the different variables studied by T-test. Results: Out a total of 88 patients, 37 were excluded, 8 were eliminated and 43 were included. Mean fecal shedding was 10.2 days (CI 95% 8.92-11.59). Ninety percent of the patients had negative cultures after 15 days. No relationship was found between fecal shedding duration and sex (p=0,419), age (p=0,937), dialysis requirement (p=0,917), antibiotics (p=0,147), nor serotypes (p=0,231). Conclusion: Ninety percent of the patients had negative cultures after 15 days and all after the day 22th. No relationship was found between fecal shedding duration and sex, age, dialysis, antibiotic, or STEC serotype.

SAI – LEONARDO SATZ AWARD

RAPID DETECTION OF PATHOLOGICAL CD1A^{+}CD207^{+} MYELOID CIRCULATING CELLS IN BLOOD AND THEIR IMPLICATION IN LANGERHANS CELL HISTIOCYTOSIS.

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Langerhans Cell Histiocytosis (LCH) is an inflammatory neoplasm characterized by an abnormal accumulation of CD207^{+}CD1a^{+} myeloid cells in almost any tissue. Disease etiology is under debate, and it is not clear if LCH results from malignant transformation or unbalanced immune response. Previously, we found CD207^{+}CD1a^{+} circulating cells in patients with active LCH with an important prognostic potential. We aimed to validate a rapid detection method of blood circulating CD1a^{+}CD207^{+} cells to define a cellular disease activity in pediatric LCH patients and correlate with organ location and gene expression. CD207 and CD1a levels were measured in 7 blood myeloid compartments by flow cytometry. 188 pediatric LCH blood samples and 22 controls were analyzed. Survival and migration genes were evaluated in sorted circulating myeloid cells by RT-qPCR. We found CD207^{+} and/or CD1a^{+} circulating cells in all active LCH patients, while controls and non-active LCH patients were negative. We established a cellular activity score adding the percentage of CD207^{+}/CD1a^{+} cells in each of the 7 myeloid compartments, which was validated by ROC curve analysis (AUC:0.81, Sen.:0.79, Spe.:0.77, Y.Index:29.5). LCH patients with a score higher than 29 were categorized as cellular active disease. Multivariate analysis showed specific CD207^{+}/CD1a^{+} cell populations correlate with organ localization. Bone LCH was defined by CD1a^{+} in CD11b^{H}CD14^{L}/CD11c^{-}CD1c^{+}. Skin LCH showed CD1a^{+}CD207^{+} cells in CD11b^{H}CD11c^{H}/CD11b^{+}CD11c^{L} populations. Multisystem patients presented CD207^{+} cells in CD11b^{H}CD14^{H}/CD11b^{H}CD14^{L}/CD11c^{-}CD1c^{+}. We also observed a differential gene expression of IL7R, SNAIL, NCAD and MMP1/9 dependent on organ compromise. Summarizing, we propose a rapid method to detect pathological CD207^{+}CD1a^{+} cells in LCH patient's peripheral blood and define a cellular activity score as an instrument to follow-up the activity disease, even before clinical manifestations, and potentially predict organ compromise.

EXACERBATED METABOLISM AND MITOCHONDRIAL REACTIVE OXYGEN SPECIES CONTRIBUTE TO MITOCHONDRIAL ALTERATIONS AND APOPTOSIS IN CD4 T CELLS DURING ACUTE PHASE OF TRYPANOSOMA CRUZI INFECTION

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Chagas disease is characterized by inefficient host immune response during acute phase enabling the establishment of chronic disease. Imbalances in T cells´s metabolism can be detrimental. The aim of our work was to evaluate metabolic and mitochondrial parameters in CD4 T cells during T. cruzi infection. To achieve this, CD4 T cells were isolated from spleen of non-infected (NI), acute (AP) and chronic (CP) infected BALB/c mice, with 500 trypomastigotes. Mitochondrial parameters were measured by FACS combining potential-dependent and independent probes while mitochondrial ROS (mROS) was measured using MitoSOX. Seahorse XF24 was employed for bioenergetics analysis. We found a raised basal glycolysis and high oxidative metabolism in CD4 T cells from the AP. Besides, these cells showed increased proton leak and uncoupling protein 3 (UCP3) expression that correlates with our previous results that demonstrated mROS accumulation, mitochondrial membrane potential depolarization, PD1 expression and less IL-2 released (analyzed by ELISA) after stimulation during AP of infection. Furthermore, CD4 T cells with mitochondrial alterations (MA) displayed an activated phenotype, and were more prone to apoptosis. This phenotype was dependent on TCR signalling, since MA in CD4 T cells from AP were significantly reduced in OTII mice. Mn-Superoxide Dismutase expression, involved in mROS detoxification, was increase during the AP and CP of infection. Apoptosis observed in CD4 T cells with depolarized mitochondria, was prevented by incubation with N-acetyl cysteine (NAC). It is probably that antioxidant availability may not be sufficient to avoid MA rendering these cells more susceptible to apoptosis. Thus, our results showed that acute infection triggers an exacerbated metabolism together with mROS production in CD4 T cells. Taken together, this evidence stablishes association between disturbed metabolism and impaired CD4 T cell response during acute T. cruzi infection.

GLYCOSYLATION REGULATES PLASMA CELLS AND SECRETORY IgA FUNCTION IN GUT INFLAMMATION

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Inflammatory bowel diseases (IBD) are characterized by inflammation of the digestive tract resulting in severe damage to the tissues involved. Secretory IgA (SIgA) is a heavily glycosylated protein complex that plays a key role in maintaining gut immune homeostasis. However, functional consequences of an altered SIgA glycosylation during gut inflammation have not been addressed yet. Our goal was to evaluate a potentially aberrant glycosylation of plasma cells (PCs) and SIgA, focusing into its pathological relevance. Analysis from single cell RNA seq databases on PCs from IBD patients showed an altered N-glycosylation machinery. Considering that DSS-induced colitis showed decreased α2,6 sialylation (α2,6sia) specifically on IgA$^+$ PCs ($p < 0,05$) and SIgA *in vivo*, we investigated the functionality of B cells deficient in ST6Gal1, an enzyme that adds α2,6sia to N-glycans. ST6Gal1$^{-/-}$ B cells are impaired in their ability to suppress T cell driven colitis in Rag2$^{-/-}$ mice, resulting in greater histologic score than their wild type counterparts ($p < 0,05$). To unravel the mechanisms involved in the defective regulation of inflammation, we focused in analyzing the functionality of α2,6sia-deficient SIgA. Notably, desialylated SIgA showed higher binding to fecal bacteria ($p < 0,05$) and human monocytes ($p < 0,05$) *in vitro* and potentiates the activation of LPS-stimulated monocytes, upregulating HLA-DR and IL-1β ($p < 0,05$). Moreover, binding of desialylated SIgA to THP-1 monocytic cells is inhibited by EGTA and Lewis X ($p < 0,05$), suggesting that these interactions are mediated by a C-type lectin. The evidence provided here postulates a novel immune circuit where gut inflammation may promote transcriptional changes in PCs resulting in decreased α2,6sia. In turn, desialylated SIgA promotes colitis through a newly gained proinflammatory activity. This work contributes to the construction of a new paradigm where aberrant glycosylation modulates the immunoregulatory activity of SIgA.

SULFATED HIALURONAN: ANTITUMOR AND ANTIANGIOGENIC EFFECT ON TUMOR CELLS AND MONOCITES/MACROPHAGES IN BREAST CANCER CONTEXT

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Breast carcinoma is one the most frequent types of cancer. The potentiation of immune action again this tumor will be a promising therapeutic option. Monocytes/macrophages (Mo/MØ) are critical modulators of the tumor microenvironment. Chemically modified hyaluronan (HA) like sulfated hyaluronan (sHA) are biomaterials that can work as a possible adjuvant therapy. It demonstrated that sHA can inhibit hyaluronidases and have a potent antitumor and antiangiogenic action in prostate and bladder cancer cells. In this sense, the aim is to evaluate sHA effect on breast tumor cells and on Mo/MØ in tumor context.

M&M. sHA (sHA1 and sHA3) were synthesized from the tetrabutyl ammonium salt of HA in Fidia Farmaceutici s.p.a. Cells: i)human breast cancer cell line (MDA-MB-231), ii)human microvascular endothelium (HMEC-1) and iii)Mo/MØ (obtain from human peripheric blood with Ficoll/Percoll). MTS assay was used to measure cell viability. LDH assay was used to measure cytotoxicity. TGFβ1 and VEGF levels were measured by ELISA. VEGF, FGF-2 and IL8 levels were measured by qPCR. Cell migration was evaluated using a modified Boyden chamber. Mammospheres were obtained through hanging drop. In vivo experiments: Mo/MØ pulsed with sHA were inoculated in MDA-MB-231 xenograft mice model. Tumors were fixed and stained with: i)Lectin GSLI-FITC for vasculature detection and ii)HA and TSG-6.

Results. sHA treatments in MDA-MB-231: enhanced cytotoxicity and decreased cell viability, mammosphere size, VEGF levels and HMEC-1 migration. Whereas, sHA treatments in Mo/MØ in breast tumor context increased Mo/MØ viability, decreased TGFβ1 total levels, modulated TSG-6 and decreased the expression of angiogenic factors and HMEC-1 migration. Even more, Mo/MØ primed with sHA and inoculated breast tumor model decreased blood vessel formation, TSG-6 and HA levels.

Conclusion. sHA showed an antitumor and antiangiogenic role in breast tumor cells, as well as in Mo/MØ in breast cancer context.

SAFIS AWARD

AEROBIC TRAINING-INDUCED MITOCHONDRIAL ADAPTATIONS IN HYPERTENSIVE MYOCARDIUM

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Pathological cardiac remodeling occurs in essential hypertension and consists of ultrastructural alterations and mitochondrial dysfunction. Physiological cardiac adaptations occur in response to aerobic training to satisfy the work-load during exercise. Despite this, its beneficial effects on the mitochondria have not been completely elucidated in the hypertensive myocardium.

Objective. To determine whether sustained swimming training improves myocardial mitochondrial phenotype in spontaneously hypertensive rats (SHR).

Methods. 3-month old SHR were randomized to sedentary (Sed) and trained (Swim) groups. After the swimming protocol (8 weeks, 5 days/week) the hearts were destined for transmission electron microscopy (TEM) imaging, RT-PCR analysis, or mitochondrial isolation. Results are expressed as mean±SEM (TEM as median-IQR) and are statistically different ($p<0.05$), otherwise, the p-value was stated.

Results. TEM images showed increased morphological values in Swim group: cross-sectional area (μm², Swim: 0.79-0.74, Sed: 0.72-0.71) and aspect ratio (Swim: 1.90-1.13, Sed: 1.60-0.77). The myocardial ultrastructural disarray in Sed trended to be restored by training (clusters/photo, Swim: 4.63±1.07, Sed: 8.45 ± 0.84, $p=0.057$). Training modified mitochondrial dynamics (% vs Sed): mtDNA/nDNA: 153.9±21.6 ($p=0.082$); PGC1-α: 149.19±19; DRP-1: 309.4±77.5; MFN1: 59.0±8.2; PINK1: 209.9±49.4 ($p=0.063$). Moreover, training improved: ΔΨm (mV, Swim: -175.2±5.8, Sed: -148.6±9.2), mitochondrial calcium content (nmol/mg, Swim: 151.4±21.6, Sed: 87.6±17.8), and citrate synthase activity (μmol/min*mg, Swim: 0.87±0.03, Sed: 0.64±0.05).

Conclusion. These results suggest that aerobic training: 1) improved the mitochondrial-sarcomere array; 2) promoted a dysfunctional mitochondrial clearance process: reduced fusion and enhanced biogenesis, fission, and mitophagy; and 3) improved mitochondrial phenotype through less depolarized ΔΨm, increased mitochondrial Ca^{+2} and citrate synthase activity.

INHIBITION OF THE TRANSLOCON IS SUFFICIENT TO ALLEVIATE ENDOPLASMIC RETICULUM STRESS AND IMPROVE THE POST-ISCHEMIC RECOVERY OF THE STUNNED MYOCARDIUM.

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Myocardial stunning is a temporary contractile dysfunction after the relief of a discrete ischemia. Oxidative stress and intracellular Ca2+ overload are proposed for the pathogenesis of this phenomenon. The endoplasmic reticulum (ER), involved in folding and sorting of proteins, is also a Ca2+ storage compartment sensitive to alterations in intracellular homeostasis. The perturbation in ER function leads to accumulation of misfolded proteins, ER stress, activation of the unfolded protein response (UPR) and frequently ER Ca2+ loss. In stunned hearts, the triggered ER stress impairs the post-ischemic myocardial performance. Since ER Ca2+ release could participate in the altered Ca2+ homeostasis, we explored whether the translocon, an ER Ca2+ leak channel, contributes to the contractile dysfunction.

Mechanical performance, UPR markers and oxidative damage of perfused rat hearts subjected to 20 min of ischemia followed by 30 min of reperfusion in absence (I/R) or presence of the translocon inhibitor, Emetine (I/R+E,1 mM) (n=4-7), were evaluated.

Emetine precluded the I/R-induced increase in relative mRNA levels of two UPR signaling markers: GRP78 1.79±0.09 (I/R) vs 1.16±0.19 (I/R+E) and sXBP1 2.14±0.21 (I/R) vs 1.12±0.15 (I/R+E), $P< 0.05$. The contractile recovery significantly improved together with a remarkable attenuation in myocardial stiffness. At the end of reperfusion, left ventricular developed pressure values (% of pre-ischemia) were 45.63±3.16 (I/R) and 105.68±6.34 (I/R+E) while left ventricular end diastolic pressure values (mmHg) were 48.40±1.87 (I/R) and 14.89±4.81 (I/R+E). This occurred despite more severe oxidative stress conditions than that elicited by non-treated I/R hearts [TBARS (nmol/mgprot) 0.71±0.06 (I/R) vs 0.97±0.09 (I/R+E)].

In conclusion, blocking ER Ca2+depletion via translocon suppressed ER stress and produced a beneficial effect on the mechanical performance of stunned myocardium even in the absence of any attenuation of oxidative stress.

SR-MITOCHONDRIA COMMUNICATION PROMOTES MITOCHONDRIAL DAMAGE AND TISSUE DISARRANGEMENT IN PREDIABETIC HEARTS

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SR-mitochondria communication plays an essential role in pathophysiological heart conditions. Prediabetic hearts present Ca2+ mishandling by CaMKII (Ca2+-Calmodulin kinase II) hyperactivity. Mitochondrial Ca2+ overload can open mitochondrial permeability transition pore (MPTP) and trigger cell death. We hypothesize that SR Ca2+ leak through RyR2 and the decreased distance between organelles in a CaMKII-dependent pathway, induce a decreased mitochondrial Ca2+ retention capacity (CRC) inducing mitochondrial damage.

We measured spontaneous Ca2+ release events (SCaRE), RyR2 activity by [3H]Ryanodine binding assay, mitochondrial CRC, O2 consumption and ATP and H2O2 production, mitochondrial morphology and fission/fusion processes in a prediabetic model induced by fructose-rich diet (FRD) in WT and AC3I mice (express a CaMKII-inhibitor at heart level).

We found significantly increased SCaRE and RyR2 activity in WT FRD vs CD, without changes in AC3I hearts. Increased expression of Mfn2, VDAC and Grp75, tether proteins that could explain the decreased distance between organelles, was found in WT FRD vs CD.

In isolated mitochondria, O2 consumption in state 4 was increased with a decreased respiratory control ratio and without changes in ATP production. Moreover, H2O2 production rate was increased. Transmission electron microscopy photographs showed decreased mitochondria size and number, plus a clear disarrangement in the tissue of WT FRD vs CD. DRP1 was found increased and without changes in Opa1 in WT FRD vs CD.

We conclude that CaMKII hyperactivity induces SR Ca2+ leak by RyR2 hyperactivity, which in turn increases mitochondrial Ca2+ content and decreases CRC due to, at least in part, the nearness between the two organelles. Besides, the ETC is partially uncoupled and H2O2 production increases in agreement with mitochondrial fragmentation and tissue disarrangement. These events are prevented in AC3I mice, where myocardial CaMKII is genetically inhibited.

POLYUNSATURATED N-3 FATTY ACIDS (PUFAS) PREVENT THE CARDIAC HYPERTROPHY IN HYPERTENSIVE RATS

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It has been demonstrated that supplementation with the two main polyunsaturated n-3 fatty acids (PUFAs), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) ,leads to modifications on the cardiac physiology. PUFAs can affect membrane´s lipid composition, as well as proteins´ location and/or function. The Na+/H+ exchanger (NHE1) is an integral membrane protein involved in maintenance of intracellular pH (pHi). Its activity is regulated by allosteric site sensitivity for H+, phosphorylation and by union of ATP, lipids, growth factors in its cytoplasmic tail.

The purpose of this work was to evaluate the effect of early supplementing in diet with EPA and DHA over the modulation of NHE1 activity and cardiac function in normo rats (Wistar, W) and spontaneous hypertensive (SHR).

After weaning, the animals received orally EPA and DHA for three months (200 mg/kg body mass/day). Then we measured systolic pressure (SP) and different echocardiography parameters, which was used to calculated left ventricular mass index (LVMI), ejection fraction (EF%) and fractional shortening (FS%). The rats were sacrificed and obtain ventricular cardiomyocytes for measured the NHE1 activity.

The SP was significantly greater in SHR compared to W. While the treatment with PUFAs did not affect the SP in SHR, we observed a significant reduction in LVMI.

The NHE1 activity was measured as velocity of pHi recovery (dpHi/dt) after intracellular acidification. As is previously described, the NHE1 activity was significantly higher in SHR compared with W. NHE1 activity was not modified by the treatment with PUFA in W. However, NHE1 activity was significant decreased by PUFA in SHR, reaching values comparable with W.

These preliminary results allow us to suggest that diet supplementation with PUFAs since early age in SHR prevents the development of cardiac hypertrophy, perhaps by decreasing NHE1 activity, without altering hemodynamic overload.

SAIC

BIOINFORMÁTICA, GENOMA, PROTEOMA, Y BLANCOS TERAPÉUTICOS

1. (23) **PLASMA AND STOOL METABOLOMIC BIOMARKERS OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN ARGENTINA**

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Non-invasive biomarkers are urgently needed to identify NAFLD patients at risk of progression to non-alcoholic steatohepatitis (NASH), particularly in high prevalence areas such as Latin America. Thus, we aimed to identify potential metabolomic biomarkers related to NAFLD stage in Argentina, and to assess their relationship with clinical and host genetic factors.

Healthy volunteers (n=19) and biopsy-proven simple steatosis (n=12) or non-alcoholic steatohepatitis (n=22) patients with similar food intake data were recruited. Plasma and stool samples, as well as demographic and clinical data were collected. SNP rs738409 (PNPLA3 gene) was determined in all volunteers. HPLC and flow injection analysis with MS/MS in tandem was applied for metabolomic studies using the MxP Quant 500 Kit (Biocrates Life Sciences AG, Austria) and MetaboAnalyst v4.0. Bivariate and multivariate analyses identified variables independently related to NAFLD stage. Forward stepwise logistic regression models were constructed to diagnose NAFLD and NASH. ROC curves were used to evaluate models' accuracy.

The concentration of 33 out of the 424 detected metabolites (25 in plasma and 8 in stool) significantly differed among groups. Levels of triglycerides (TG) were higher among NAFLD patients, whereas levels of phosphatidylcholines (PC) and lysoPC were lower among them. The PNPLA3 risk genotype was related to higher plasma levels of eicosenoic acid FA(20:1) ($p<0.001$). Plasma metabolites showed a higher accuracy for diagnosis of NAFLD and NASH (AUROC=1) than stool metabolites (AUROC=0.79 and 0.90, respectively). Body mass index and plasma levels of PC aa C24:0, FA(20:1) and TG(16:1_34:1) showed higher accuracy for diagnosis of NAFLD (AUROC=1); whereas plasma levels of PC aa C24:0 and PC ae C40:1 showed AUROC=1 for discriminating NAFLD stages.

In conclusion, potential metabolomic biomarkers for diagnosis and progression of NAFLD were identified in Argentina. Further validation studies are needed.

2. (58) **IMPLEMENTATION OF MACHINE LEARNING ALGORITHMS FOR DRUG SCREENING: TREATMENT OF CORONAVIRUS DISEASE**

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Purpose: This study propose the implementation of machine learning algorithms based on a quantitative structure-activity relationship (QSAR) model for drug screening of compounds with the potential to inhibit quinone reductase 2 (QR2) and to replace the anti-inflammatory function of chloroquine and hydroxychloroquine in the treatment of COVID-19 avoiding its adverse effects.

Methods: QSAR modeling was performed to calculate the mathematical correlations between the chemical properties of QR2 inhibitor compounds, from different bioassays, and their biochemical response on QR2 activity. The values of 22 properties were obtained by means of automatic extraction techniques from PubChem?s PUG REST service. The following classification algorithms were applied: Logistic Regression, Random Forest and Multi-Layer Perceptron. To perform the computational screening, 279 drugs were selected and divided into 7 groups: Group I or PubChem-Covid-19, settled for compounds labeled by PubChem as COVID-19 (n=104); Group II, drugs with structure similar to dihydroxyphenylalanine (dopa) (n=110); Group III, ubiquins (n=16); Group IV, used in clinical trials (n=18); Group V, amantadine, pramipexole, dabigatran, rotigotine and naphthoquinone (n=5); Group VI, vitamins B (n=10); and Group VII, vitamins K (n=16). A classification threshold for Active of 0.95 was established.

Results: 54 compounds were identified as Actives. Camostat, relacatib, 5-Aminopyrimidine, clovamide, coenzyme Q4, decylubiquinone, sarilumab, fingolimod, rivaroxabán, prosultiamine and alinamin, for its potential use in COVID-19, were the most significant.

Conclusions: It was presented a series of compounds identified by the QSAR model as QR2 inhibitors and we analyze the main drugs in that series according to their availability and current use.

3. (70) **ROLE OF RHOA-GTPASES ACTIVATOR, GEF-H1, IN THYROID CANCER**

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GEF-H1 is a Rho-GTPases activator whose overexpression has been shown to be associated with tumor development. However, its role in thyroid cancer (TC) progression has not yet been studied. TC has been dramatically rising worldwide in recent decades and represents the most prevalent endocrine malignancy. For this reason, we have begun analyzing GEF-H1 expression in human thyroid biopsies. We observed higher cytoplasmic protein concentration when comparing by immunohistochemistry tumor tissue (TT) with non-malignant tissue (NMT) (n=52; p=0.0003). Similar results were obtained by Western blot in thyroid biopsies and cancer cell lines. Furthermore, clinical-histopathological data showed significant GEF-H1 overexpression in TT than NMT (p=7E-07), which correlates with a less patient survival (p=0.0088). mRNA data analysis from biopsies (Human Protein Atlas and Oncomine platforms) also showed a significant GEF-H1 overexpression in TT compared with NMT. Analyzing Gene Expression Omnibus microarray data with R language, we observed that GEF-H1 is between 2-17% of the most expressed genes in different TC histotypes. We also determined that GEF-H1 expression is significantly higher in papillary and anaplastic carcinomas than NMT ($p<0.05$) and its expression increased in papillary carcinomas with lymph node invasion and/or metastasis ($p<0.001$). Moreover, we looked for those genes whose mRNA expression correlates with GEF-H1 and we evaluated their function through gene ontology analysis (DAVID and STRING platforms) and their participation in signaling pathways (KEGG and Reactome). Genes associated with migration, mechanical signaling, cytoskeleton remodeling and focal adhesions positive correlated with GEF-H1 expression in TC ($p<0.001$). The results suggest that GEF-H1 might be a potential tumor biomarker and/or therapeutic target in TC, since it would be involved in the pro-tumorigenic signaling by coordinating changes in cell morphology, proliferation, migration and invasion.

4. (104) **OSTEOSARCOMA AND MIRNAS: COMBINING IN SILICO MIRNA ANALYSIS AND PROTEOMIC PROFILING IN SEARCH OF POTENTIAL DIAGNOSTIC PANEL**

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Osteosarcoma (OS) is the most frequent bone tumor in pediatrics and presents two critical clinical challenges, metastasis and chemoresistance. Better diagnostic and prognostic tools for OS disease progression are in need. Here we propose the use of micro-RNAs (miRNAs) as alternative diagnostic biomarkers for OS. MiRNAs are small and stable non-coding RNAs that can be obtained from liquid biopsies of different body fluids such as plasma, which in the last years have been proposed as diagnostic and prognostic biomarkers. The aim of this work was to assess an OS miRNAs database and contrast it with our own molecular and functional profiling in an OS model with metastatic behavior, in order to propose possible miRNAs as biomarker candidates. We analyzed circulating miRNAs present in the plasma of 15 healthy donors and 20 OS patients (10 with localized OS and 10 with metastatic OS) using the miRNAs dataset GSE65071. Our analysis revealed that miR-34a-5p, -200a-3p, -582-5p, -624-5p and let-7a-3p were upregulated in OS patients plasma as compared to healthy donors (fold change: 0.43; 0.78; 0.78; 0.95; 0.5 respectively; $p < 0.0001$), while miR-27a-3p and -221-3p were found downregulated in the plasma of OS patients as compared to healthy donors (fold change: -0.73; -2.64 respectively; $p < 0.0001$). There was no difference in expression between localized and metastatic OS for these miRNAs. Bioinformatics analysis of the target genes of these miRNAs revealed that they are implicated in the regulation of different cancer-related biological pathways like ECM- receptor interaction, cell cycle control and EMT, in coincidence with our proteomic approach on metastatic and non-metastatic OS cells. These results strengthen the in-silico search and constitute a proof of concept on the use of this cross-omic approach as a tool for the identification of potential miRNAs as liquid biopsy biomarkers for diseases characterized by scarce extensive population-based data.

5. (130) **REGULATORY CIS-ELEMENTS AND TRANSCRIPTION FACTOR ANALYSIS BEHIND MRP4/ABCC4 EPIGENETIC AND TRANSCRIPTIONAL PROFILE IN PANCREATIC CANCER**

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The multidrug resistance-associated protein 4 MRP4/ABCC4 is a xenobiotic transporter highly expressed in pancreatic ductal adenocarcinoma (PDAC), thas was found linked to increased proliferation and poor prognosis. We queried ChIP-seq and RNA-seq data from PDAC cell lines available at public repositories including the Gene Expression Omnibus (GEO) and the Encyclopedia of DNA Elements (ENCODE), analyzing Abcc4 mRNA levels and the associated epigenetic landscape of histone marks with clear functions in gene expression: H3K27ac/H3K4me for cis-regulatory elements indicative of active clusters of transcription factors (TFs), H3K4me3 for active promoters, H3K9me3 for silenced heterochromatin, and the TFs reported bound at those genomic locations. All cell lines presented H3K4me3 enrichment at the Abcc4 promoter and were depleted of H3K9me3. The high Abcc4-expressing cell lines, such as PANC1, consistently showed H3K27ac/H3K4me enrichment at specific locations of intron1, which were not detected in low Abcc4-expressing cell lines, such as HPAF2. We overlapped these regions with the TFs peaks reported in high Abcc4-expressing CFPAC1 cell line and defined three TFs clusters for further analysis. We generated HPAF2, BxPC3 and PANC1 xenografted tumors in NGS mice and evaluated Abcc4 mRNA expression (RT-PCR) and chromatin enrichment (ChIP-PCR) of H3K27ac, FoxA1 and GATA2 at the intron1 clusters. We found Abcc4 mRNA levels as expected: low in HPAF2 and increased in BxPC3 and PANC1. H3K27ac showed enrichment at the three clusters in all tumors, indicative of active/poised state, but only high Abcc4-expressing BxPC3 and PANC1 showed enrichment of FoxA1/GATA2 at these genomic locations. FoxA1 was found enriched at all clusters only in BxPC3. GATA2 showed enrichment at clusters 2 and 3 in BxPC3 and PANC1, and at cluster 1 only in PANC1. These findings suggest that FoxA1 and GATA2 may contribute to aberrant Abcc4 expression, PDAC aggressiveness and progression.

6. (150) **B2-BRADYKININ RECEPTOR NON-PEPTIDIC LIGANDS AS NEW DRUG-REPURPOSING STRATEGY AGAINST COVID-19 AND OTHER ARDS-INDUCING LUNG INFLAMMATORY INFECTIONS.**

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INTRO: Dysregulation of kallikrein-bradykinin pathway has been linked to hyperinflammatory phase of several lung infections causing adult respiratory distress syndrome (ARDS) including COVID-19, SARS, MERS and Hantavirus Respiratory Syndrome. The injectable synthetic decapeptide Icatibant (Firazyr) is the only currently approved antagonist for B2-bradykinin receptor (B2-bkR), but its

high cost makes it prohibitive for most healthcare systems of the region, particularly in the current pandemic context. AIM: To find small oral bioavailable, non-peptidic repurposing drug candidates for competitive inhibition of B2-bkR. M&M: By using 3 refined atomic models of B2-bkR obtained by homology and threading methods (SWISS-model/FG-MD and GPCR-I-TASSER) a high-throughput molecular docking (AutoDockVina) virtual screening was performed against all 2893 FDA-Approved, 3153 Investigational, 2414 in-trials and 440 harmless natural compounds (Drug Bank). Strong binders ($\Delta G_{binding} \leq$ -11kcal/mol) were later scored by integrating the ligand-receptor contact forces (AutoDock tools, LigPlus) with the available toxicity, pharmacokinetic (FK) and pharmacodynamic (FD) data. By means of a high performance computing system (FIUNER cluster: 10 nodes, with 24 cores each), 20 nanoseconds molecular dynamics simulations (MDS) were run for top-10 ranked ligand-receptor complexes (NAMD/VMD). MDS trajectories were analysed by uni- and multivariate statistics using RMSD, RMSF, H-bonding and 2D-PCA as reaction coordinates (R). RESULTS & DISCUSSION: Starting from a large library of compounds, virtual screening achieved 41 putative ligands which, after filtering by FD, FK and thermodynamic criteria lead us to 6 oral-bioavailable and cost-effective promissory repurposing drugs. In order to experimentally test these candidates, a live cell imaging Ca^{2+} mobilization inhibition bioassay is under implementation.

7. (166) **IDENTIFICATION OF NON-CODING RNAs WITH CLINICAL IMPLICATIONS IN MOLECULAR SUBTYPES OF COLORECTAL CANCER. AN IN-SILICO APPROACH.**

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Colorectal cancer (CRC) is a frequently lethal disease with heterogeneous outcomes and drug responses. Multiomics studies have revealed the molecular landscape of CRC, enabling the classification of patients according to 4 consensus molecular subtypes (CMS1-4). CMS1-immune comprises most tumors with MSI. CMS2-canonical and CMS3-metabolic both show epithelial characteristics. CMS4 comprises the more mesenchymal-like cancers, with poor patient prognosis. CMS1 and CMS4 also show poor response to standard therapies. Non-coding RNAs (ncRNAs) constitute more than 70% of the transcriptome. Two main classes have been largely associated with cancer; miRNAs and lncRNAs. Since some of these ncRNAs can be detected in human body fluid and have good specificity and accessibility, they have been suggested to be used as novel potential biomarkers for CRC diagnosis and prognosis as well as in the prediction of the response to therapy. In this study, we performed a classification of 688 CRC tumors obtained from TCGA into the 4 CMS employing the CMScaller bioinformatics tool. We characterized each subtype according to its main features. We then conducted an exhaustive gene expression profile analysis to identify the top upregulated lncRNAs and mature miRNAs in each subtype (CMSk) compared to the rest of the groups (CMSk-1; pvalue<0.01), defining a CRC-CMS signature of ncRNAs. Applying different filtering criteria, we look for those ncRNAs with potential clinical implications. We identified two lncRNAs, AFAP1-AS1 and MIR99AHG, overexpressed in CMS1 and CMS4 (p<0.01), respectively, and associated to poor prognosis (p<0.05); and three miRNAs: miR-99a-5p, let-7c-5p, and miR-125b-5p, all upregulated in CMS4 and associated to bad prognosis (p<0.05). Overall, we defined the most relevant CMS specific lncRNAs and miRNAs in CRC, and selected a group of candidates with clinical implications to further evaluate in-vitro in CRC cell lines and ex-vivo in blood and tumors patient samples.

8. (185) **WHOLE-EXOME SEQUENCING LANDSCAPE OF A RAPIDLY-PROGRESSING CUTANEOUS MELANOMA PATIENT**

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Cutaneous Melanoma (CM) is a skin cancer with a high Tumor Mutational Burden (TMB) and a high-risk metastatic rate; the genetic landscape of a CM patient with rapid clinical evolution is described here.
Methods: Whole-exome sequencing analyses on gDNA from germline, precursor-nevi, primary CM and lymph-node metastasis (LN-mts) microdissected-tumor biopsies were performed. Data were analyzed following GATK guidelines (GRCh37/HG19 reference). Detection algorithms: HaplotypeCaller v3.3.0 for germline SNP; Mutect2 v3.8-0 for somatic SNPs/INDELs; FACETS v0.6.0 for CNV. Differential alterations in the allelic frequency (AF) of SNP/INDELs as well as in the cellular fraction (CF) of copy-number-variation (CNV) were discerned in genes with impact on cancer hallmarks in each step of tumor transformation and progression.
Results: Germline trunk mutations with known effect on susceptibility and poor-prognosis in CM were detected, early affecting genome stability (n=60). Regarding somatic gene alterations, CNV prevailed over SNP/INDELs, both showing an increasing number of affected genes in the path from nevi to metastasis. Accordingly, TMB tripled with progression (2.875X). The main somatic trunk driver was the oncogene BRAFV600E, with an increasing AF and CF in primary and LN-mts, sustaining proliferative signaling. At CNV level, deletion prevailed over gene amplification (8.42X). Metastasis-persisting genes exhibited increasing CF variation throughout progression (1.65X), supporting a functional selection of these altered-genes. Amplified genes (n=494) mainly affected cell proliferation, invasion & metastasis, angiogenesis and metabolism hallmarks. While deleted genes (n=4161) mainly affected regulation of cell proliferation, cell death and immunity hallmarks.
Conclusion: in this gradual although rapidly-progressing CM case, WES analysis allowed us to disguised differential alterations with impact on cancer hallmarks in each step of tumor transformation and progression.

9. (276) ***SERRATIA MARCESCENS*** **INVOLVED IN THE EARLY RESERVOIR OF GENETIC PLATFORMS RELATED TO DISSEMINATION OF ANTIMICROBIAL RESISTANCE MECHANISMS**

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Serratia marcescens is a Gram-negative, facultative anaerobic bacillus of the *Enterobacteriaceae* family. It has a ubiquitous distribution and is a frequent cause of hospital-acquired infections.
S. marcescens SCH909 is a multidrug-resistant strain isolated in 1988 that was sequenced by Miseq and PacBio. The chromosome (5.315.598 bp) and the pSCH909 plasmid (83.750 bp) were assembled with SPAdes 3.9.0. The genome was annotated with Prodigal and RefSeq database for the genome annotation which was completed with specific analysis by using Blast. Insertion sequences were searched by ISfinder and phages with PHASTER. Antimicrobial resistance genes were identified using RESfinder, CARD and Blastn with a cut-off e-value of e^{-10}.
Different sources of genetic platforms related to the diffusion of antibiotic resistance mechanisms were found including the IncL-type pSCH909 plasmid, a new transposon Tn*6824*, 13 insertion sequences in the genome.Also four integrons were found, one of them the class 2 integron *dfrA1-sat2-ybeA-ybfA-ybfB-ybgA*.and three class 1 integrons, two of them were "*head to head*" in pSCH909 (*dfrA1-aadA1* and *aadB-Se.ma*.I2-*aadA11/aac(6')-IId*-orfO-Δbla_{OXA-10}) and a third (*aacC1*-orfP-orfP-orfQ-*aadA1*) which was found within a novel genomic island named SmaR. SmaR, is closely related to Multiple

Antimicrobial Resistance Region (MARR) usually found in AbaR0-type and AbGRI2-0 from global clones of *Acinetobacter baumannii*, and in IncM plasmids circulating in *Enterobacteriaceae*.
Maintenance studies showed that the three class 1 integrons were maintained over one month without antimicrobial pressure. These findings, and the fact that *S. marcescens* is considered a relevant nosocomial pathogen that can have a wide range of niches –human, plant, animal, soil, and inanimate surfaces, showed the ability of this species to capture, maintain and spread a broad variety of antimicrobial resistance platforms.

10. (279) **MULTIPLE MULTIDRUG-RESISTANT *SERRATIA MARCESCENS* CLONES IN ARGENTINIAN HOSPITALS**

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Serratia marcescens, is a Gram-negative bacterium responsible for a wide range of nosocomial infections. The emergence of multidrug-resistant strains is an increasing danger to public health.
Utilizing whole-genome sequencing, we characterized the population structure and variation, as well as the antimicrobial resistance determinants, of 150 *S. marcescens* strains associated with differents infections from 14 hospitals across Argentina isolated from 1997 to 2018.
The 150 strains were sequenced by Miseq. The sequences were assembled with SPAdes 3.9.0. The genomes were annotated with Prodigal and used the RefSeq database for the genome annotation which was completed with specific analysis by using Blast. The plasmids were identified with PlasmidFinder. Antimicrobial resistance genes were identified using RESfinder, CARD and Blastn.
One important feature of pathogens belonging to this genus is their intrinsic and acquired resistance to a wide variety of antibiotic families, including β-lactam, aminoglycosides, quinolones and aminoglycosides. Our results showed that clinical isolates carried a great diversity of plasmids including identical plasmids circulating in two different hospitals carrying bla_{KPC-2}. The gene $bla_{CTX-M-2}$ was found inserted into both the plasmid and the chromosome associated with differents complex integrons. The $bla_{CTX-M-8}$ and $bla_{CTX-M-15}$ genes were also found to be associated with these platforms.
On the other hand, in this study, the most frequently occurring aminoglycoside-modifying enzymes included AAC(6')-I, AAC(3)-I and ANT(3")-I. Also, unusual combinations were also found such as AAC((6')-Ie/APH(2") in one of the isolates.
From the molecular point of view, the genomic plasticity of *S. marcescens* allowed us to presume that it is a reservoir of antibiotic resistance genes.

11. (294) ***E. coli* ST 131 IS ABLE TO ACQUIRE *mcr-1* GENE MEDIATED VIA OUTER MEMBRANE VESICLES**

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The role of outer membrane vesicles (OMVs) in the evolution to extreme and pandrug resistance in clinical samples is still unknown. That is why we propose to investigate the transmission of the *mcr-1* encoding gene through OMVs to the pandemic clone *E. coli* ST 131. This gene confers resistant to colistin, an antibiotic usually used as last resort in infections associated to multidrug resistant Gram-negative bacteria. First, we isolated and characterized the OMVs from *E. coli*_mcr-1 that has a plasmid, harboring the *mcr-1*- genes. The vesicles treated with DNAase were studied and characterized by microBCA, NTA, and TEM. NTA results showed a major peak between 100–200 nm in diameter.These results were confirmed through TEM images. Second, the DNA was purified from the OMVs and used for PCR analysis. Third, these OMVs were used to transform multidrug resistant clinical strain *E. coli* SM5 belonging to pandemic clones ST131 which harbors pDCAG1 plasmid with the extended-spectrum beta-lactamase $bla_{CTXM-15}$ gene. The checking of the transfer was performed by colony PCR for the *mcr-1* gene of different colonies that grew in plates with 50ug/ml of colistin. Further, gene maintenance experiment was conducted at increasing concentrations of colistin. In the present study for the first time, we characterized OMVs harbouring the *mcr-1* gene from *E. coli*_mcr-1 clinical strain. In addition, the transfer and maintenance of colistin resistance through OMVs to pandemic clone *E. coli* ST 131 demonstrates the threat of this still not completely studied mechanism of the Lateral Genetic Transfer.

12. (308) **MOM IS THAT YOU? PROTEOMIC APPROACH TO ASSESS THE RELATIONSHIP BETWEEN MESENCHYMAL STEM CELLS AND EWING´S SARCOMA CELLS: TRACKING THE CELL OF ORIGIN.**

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Ewing's sarcoma (ES) is a primitive neuroectodermal tumor that mostly affects children and young adults between 5 and 30 years. Understanding the biology and mechanisms involved in ES growth and progression may lead to the identification of new therapeutic targets. Previous results from our group have shown that a functional link exists between ES and bone marrow (BM)-derived stem cells. In spite of its characterized molecular pathology, many ES development mechanistic aspects remain unknown, without a well-defined etiology, and there is a need to elucidate mechanisms associated to tumor cell survival and cancer progression. Further, markers associated to clinical progression need to be identified. Given that mesenchymal stem cells (MSC) play a central role in regulating osteogenesis and hematopoiesis, sharing functional and hierarchical relationship to ES, we undertook a proteomic analysis with a shotgun approach aimed at comparing BM-MSC and TC71 ES cells. We identified 565 proteins shared by BM-MSC and TC71 cells, while TC71 had 628 and BM-MSC 484 unique proteins. Gene ontology analysis revealed that major differences were found in metabolism pathways, with emphasis in the citric acid cycle, electron transport and ATP synthesis that may relate to the aggressiveness of the disease in terms of rapid growth and metastases development after diagnosis. We also found differences related to protein cellular distribution, with a higher percentage of mitochondrial proteins in ES cells (43,71% TC71 vs 27,86% BM-MSCs), pointing that metabolism governs major divergences in ES cells. In terms of the ES signature EWS-FLI1 translocation that leads to cluster ES tumors as rather homogeneous, further exploration on the transcriptional activity of this oncoprotein in relation to the elusive cell of origin, will lead to improve the management of this sarcoma in soft tissues other than bone, as well as to identify markers associated to progression and therapy response.

13. (358) **COMPARATIVE GENOMICS OF HETERO AND HOMOGENEOUS METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS**

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S. aureus is a major human pathogen. Misidentification of heterogeneous Methicillin Resistant *S. aureus* isolates (HeMRSA) may lead to inappropriate clinical treatments. We study a clinical *mecA* positive HeMRSA strain (SA454He), susceptible to betalactams and two *in vitro* derivatives obtained from SA454He under oxacillin and rifampin selective pressure (SA454Ho and SA454RIF) expressing a homogeneously MRSA (hoMRSA) phenotype.
The aim of this study is to unravel genetic changes associated with the phenotypes observed for the aforementioned strains using whole-genome sequencing (WGS).
WGS was conducted on genomic DNA using Illumina MiSeq. Quality control (QC) of reads was carried out with FastQC and Kraken, *the novo* assembly with SPAdes, assemblies´ QC with QUAST and annotated with Prokka. ARIBA with different databases was used for the analysis of antimicrobial resistance determinants, plasmids, and MLST. Snippy was used to determine single nucleotide polymorphisms (SNPs) and INDELs against *S. aureus* MW2 and SA454He as reference sequences.
All strains belong to ST1, harbour *mecA* on SCC*mec*V and carry an average of 1576 SNPs each when compared to MW2. The *blaZ* operon was detected in SA454He, in a *rep*5a plasmid of approximately 22 kb, with a truncated *bla*R1 gene. This operon was absent in SA454Ho and SA454RIF genomes. SA454RIF and SA454Ho only carried one SNP each in *rpoB*, when compared with SA454He (Ser486Leu and Ala576Val, respectively).
Both the loss of *bla*Z operon and *rpoB* mutations might be related to the hoMRSA phenotype in mutant strains, which could possibly be selected in clinical settings. We highlight the potential use of WGS to identify genetic mechanisms of heteroresistance in routine bacteriology laboratories, and to detect these types of strains which represent a diagnostic and epidemiological challenge.

14. (388) **ASSAYS FOR GEL-BASED, SENSITIVE SCREENING OF REPRODUCIBLE DIFFERENCES IN CYTOSOLIC PROTEOMES BY COMPLEMENTARY, CELL-FREE REACTIONS LABELLING PROTEINS WITH NUCLEOTIDES. INFECTED MACROPHAGES AS MODEL.**

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AIMS: To optimize several conditions for novel, complementary biochemical assays allowing the screening of proteome differences between cell treatments by using cell-free, in vitro radiolabeling assays outperforming Coomassie and silver staining in sensitivity. METHODS: As model, macrophages were cultured and stimulated/infected or not with different treatments, bacteria, lipopeptides and times. Sub-fractionated cytosols were incubated in parallel with different radiolabeled nucleotides and a large battery of combinatorial reaction components, additives and conditions. Cell-free reactions were separated in 1D and 2D gels comparing proteome profiles by staining versus radiolabeling, analyzing effects of reaction components and culture conditions. The sample size was 3-6 and 4-9 for each reaction and culture condition, respectively. After systematic iteration, we selected the reactions detecting infection time-dependent proteome differences with 90-100 % reproducibility and >20 % dysregulated level. RESULTS: The assays were versatile, robust and suited for cytosols, detecting different complementary proteome profiles depending on the in vitro labelling covalent linkage (phosphorylation, nucleotidylylation, ribosylation, AMPylation). Nucleotides labeled in γ or α phosphate worked differently. In vivo phosphorylations were evidenced by coupled phosphatase assays (in vivo sites were excluded from reactions unless dephosphorylated). Intentionally phosphorylating non-physiologic sites unoccupied in cells some proteins were labeled reproducibly, regardless of culture conditions, sensitively quantitating their expression level. Proteome radiolabeling outperformed staining in sensitivity for low-abundance proteins uncovering 5 novel, reproducibly altered proteins with time-dependent up-/down-regulation. CONCLUSION: the assays uncovered new markers and pathways of innate immune responses, one having a novel PTM and possible uses in vaccine/adjuvant testing and infectology.

15. (414) **NEW INSIGHTS IN ULCERATIVE COLITIS ASSOCIATED GUT MICROBIOTA IN SOUTH AMERICAN POPULATION: *AKKERMANSIA* AND *COLLINSELLA*, TWO DISTINCTIVE GENERA FOUND IN ARGENTINE SUBJECTS.**

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Ulcerative colitis (UC) is the most common form of intestinal inflammation, which is believed to be the result of a deregulated immune system response to commensal microbiota in a genetically susceptible host. In the present study we aim to describe the gut microbiota of patients with UC in comparison with non-UC controls. We evaluated 46 individuals, 26 non-UC controls and 20 UC patients, from the metropolitan area of Buenos Aires (BA), Argentina. The hypervariable regions V3-V4 of the bacterial 16SR gene were sequenced using a MiSeq platform and sequences were analyzed using the QIIME2 environment. In addition, we looked for differential functional pathways using PICRUSt and compared the performance of three machine learning models to discriminate the studied individuals, using taxa and functional annotations. We found no significant differences in gut microbiota richness or evenness between UC patients and non-UC controls (alpha diversity). Remarcably, beta diversity showed significant differences. At the phylum level, *Verrucomicrobia* was overrepresented in controls while *Actinobacteria* was distinctive of UC patients; At the genus level *Bacteroides* and *Akkermancia* were significantly more abundant among controls while *Eubacterium* and *Collinsella* in UC patients. In addition, our results showed that carbohydrates metabolism was preponderant in UC patients, not observing a distinctive biochemical pathway for the healthy non-UC controls. Finally, in order to define a robust classifying method in our population, we evaluated the capability of three machine learning random forest models to classify individuals. Our results reinforced the idea of functional compensation in microbiome communities, as models that used KEGG orthologs annotations had better capabilities than taxonomy to distinguish UC patients. Our study provides new knowledge on the differences and similarities of the gut microbiota of UC patients as compared to non-UC controls of our population.

16. (433) **EVOLUTIONARY RESTRICTIONS IN THE SEDENTARY INTEGRON OF *Pseudomonas punaustralis* Ca10SN1**

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Integrons are platforms of recombination and expression of gene cassettes. *Pseudomonas punaustralis* Ca10SN1 is a recently discovered bacterium that carries a sedentary integron. In this work, we studied the distribution, structure, and recombinogenic characteristics of the *attC* sites of *P. punaustralis* Ca10SN1 to discuss the

evolutionary role of its sedentary integron. For this, the *P. punaustralis* Ca10SN1 genome was sequenced, assembled and annotated. Integron related regions and their components were predicted using *Integron finder*. The dissemination of the *P. punaustralis* Ca10SN1 *attC* sites was assessed using MegaBLAST, counting the number of species whose hit was ≥97% of identity and coverage. The number of mutations in the R and L boxes of the *attC* sites was estimated by comparing them with the *attC* site associated with the integrase (IntIPpun). Secondary structures were obtained using the RNAfold program of the Vienna RNA 2 package. Moreover, the probability of *attC* sites of folding a recombinogenic structure (pfold) was calculated with previously published equations. One integron integrase was predicted (IntIPpun), and 26 *attC* sites were identified, being one *attC* site associated with IntIPpun, and 25 *attC* sites scattered throughout the genome in CALINs. Regarding dissemination, 50% of the predicted *attC* sites were unique of *P. punaustralis* Ca10SN1 genome, 38.46% were exclusive to the genus *Pseudomonas* and 7.7% were found outside the genus *Pseudomonas*. The mean mutations in the 1L, 2L, 2R, and 1R boxes were 2.16±0.21, 2.2±0.35, 1.28±0.29, and 1.64±0.21bp, respectively. 2L and 2R showed significant differences (P<0.1) through a two-way ANOVA test. The pfold range of the *attC* sites was between $9.84x10^{-1}$-$6.45x10^{-31}$. Our results suggest that could be a threshold of mutations that may restrict the activity of IntIPpun on *attC* sites, evolutionarily constraining their dispersal to other species and genera.

17. (436) **CELLDEATH: A TOOL FOR SIMPLE DETECTION OF CELL DEATH IN TRANSMITTED LIGHT MICROSCOPY IMAGES BY VISUAL DEEP LEARNING ANALYSIS**

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Programmed cell death (PCD) is a highly regulated and controlled process that occurs in physiopathological contexts of multicellular organisms. Detection of PCD or its features is far from an impossible task, though it often requires some degree of experience and surely consumable reagents. In the present work, we aimed to develop a simple and fast way to accurately detect PCD on transmitted light microscopy (TLM) images from cell cultures using convolutional neural networks (CNN). We trained our network to detect PCD on four cancer cell lines and three pluripotent stem cell lines treated either with a topoisomerase I inhibitor -camptothecin (CPT)- or DMSO (vehicle) for 1, 2 and 3h. TLM images were taken immediately before adding the treatments (untreated) and every hour. PCD in every cell line was confirmed by flow cytometry analysis (Annexin-V+/7-AAD-) and immunostaining of γH2AX and p53. Classification was initially performed labelling both cell line and condition, reaching a final accuracy of 98.8 % in the validation set, and 98.7% in the test set at 1h of treatment with CPT. Comparing all non-exposed cells versus all exposed cells images showed an even higher accuracy of classification (99.4%). Finally, we ran an analysis treating each group independently, again classification was in general excellent. Considering the minor morphological changes at 1h, we challenged 5 experienced researchers (who had never seen the images before) to correctly classify CPT exposure in a random set of 50 images (pre-training). We then allowed them to train by looking at 500 labeled images and classify another set of 50 images (post-training). Performance by investigators was completely random, both before and after training, indicating that features detected by CNN are not easily recognizable. In conclusion, CNNs are able to detect subtle morphological changes consistent with PCD. Furthermore, we set up a script to easily train a CNN for such assays.

18. (457) **FACING THE CHALLENGES OF ANTIVIRAL CRISPR-CAS13 COVID-19 THERAPY IN SOUTH AMERICA**

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Introduction. CRISPR technology has generated great expectation in the field of gene editing. Since March 2020, Stanford's laboratory has become a world referent by offering an antiviral therapy. Up to date COVID-19 continues spreading relentless worldwide, mainly over winter time of the southern hemisphere. During this time, the virus has undergone mutations with uncertain impact on the efficacy of the crRNAs originally designed by Abbott. Our aim is to analyze the performance of Stanford's crRNAs in 905 SARS-COV-2 South American Genomic Sequences (SAGS).
Methodology. The top 40 crRNAs targeting the conserved RdRP and N regions of SARS-COV-2 RefSeq selected by Abbott were used for alignment studies with the SAGS published until September 2020 in GISAID. The bioinformatics tools Bowtie, MAFFT, Jalview and Cas13design were used for aligning and analyzing crRNAs to SAGS with a criterion of maximum complementarity (-v 0) and fast progressing method (FFT-NS-2).
Results. The clade distribution analysis of the SAGS revealed the main contribution of GR (53%), G (24%), GH (15%) in accordance to GISAD. The alignment of crRNAs to SAGS resulted in 4 out of 40 crRNA (10%) presenting an alignment efficiency lower than the 99% cutoff. These 4 crRNAs originated 269 misalignments mainly on sequences from Brazil (74%), Peru (10%) and Chile (8%). Misaligned Brazilian sequences had a distinctive mutation (T29148C). The Cas13design analysis predicted a high efficacy score for 2 of the 4 crRNAs (Q4-Q3) and low for the remaining 2 (Q2-Q1).
Conclusion. The evolution of the SARS-COV-2 clades in South America diverged from the scenario predicted in January 2020 in the Northern Hemisphere. However, the projected impact represents only a 1% loss of efficacy, the T29148C mutation circulating in Brazil being the main cause of misalignment in that country. Therefore, the combination of several crRNAs consolidates as the global antiviral CRISPR-based therapeutic strategy.

19. (460) **EXPLORING THE INHIBITOR OF APOPTOSIS BIRC6 AS A TARGET FOR LUNG CANCER THERAPY.**

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Inhibitors of apoptosis (IAP) have been shown to play a central role in the development and aggressiveness of different tumors. In particular, overexpression of BIRC6, a member of the IAP family, is associated with a poor prognosis in different tumors. The aim of this work was to select a possible therapeutic target through a transcriptomic analysis of the IAP family in lung cancer and develop baculovirus-based gene therapy vectors.
We focused the transcriptomic analysis on Lung Adenocarcinoma (LAC) and Lung Squamous Cell Carcinoma (LSCC). Two TCGA databases were analyzed for each cancer type and seven IAP were queried (cBioPortal and Xena platform). Our results demonstrated that at least two (BIRC5 and BIRC6) of the seven IAP have a higher expression in tumor compared to normal tissue in both types of tumors (ANOVA). Also, our results showed that LAC patients with alterations in the *birc6* gene copy number have a shorter median months of each disease status compared to the unaltereted group. Moreover, we observed that a higher copy number of *birc6* was associated with resistance to radiotherapy and tumor recurrence (χ 2). In order to characterize the role of BIRC6 in LAC, we designed three shRNAs targeting different regions of the *birc6* gene. Using bioinformatic methods we evaluated different parameters of the shRNAS (structure, stability, etc). Next, three recombinant baculoviruses (BV) were generated carrying each of these shRNA sequences and the reporter gene *dTomato*. The effect of BV-mediated *birc6* silencing was evaluated in the human LAC cell line A549 by immunofluorescence and flow cytometry. Apoptosis levels were measured by

TUNEL. One of the recombinant BV effectively reduced BIRC6 expression and induced apoptosis in 30% of the A549 treated cells (*t test*).
These results are encouraging and open the way to future preclinical studies, postulating BIRC6 as a promising therapeutic target, and the recombinant BV as a possible gene therapy vector against LAC.

20. (476) **DISCOVERY OF IONIZING RADIATION-RESPONSE AND RADIOSENSITIVITY BIOMARKERS**

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In view of the widely use of ionizing radiation (IR) in different fields, we aimed to establish a pipeline to study at a genomic level: IR-exposure, absorbed IR-dose and intrinsic radiosensitivity. Moreover, this study could help to detect the responsible molecular pathways of the biological IR effects. We performed a meta-analysis of raw data from public microarrays of different IR-exposure conditions: *ex vivo* irradiated human peripheral white blood cells (WBC) of healthy individuals and cancer patients, as well as total body irradiation or localized radiotherapy patients. The analysis was assessed using R commander and Bioconductor packages. We identified 275 differentially expressed genes (DEGs) (FDR<0.05) after IR-exposure enriched in processes associated with the radioinduced-response. We found a cluster of 21 DEGs which have a high potential to be used as biodosimeters and we identified 28 DEGS as possible 2 Gy cut-off indicators. Next, we detected eight overrepresented transcription factors (TFs) associated with the IR response (TP53, E2F7, NFIA, TCF4, HSF1, JAZF1, KDM4B and SMPX). These results were validated by qRT-PCR in *ex vivo* irradiated WBC. The expression levels of DRAM1, NUDT15, PCNA, PLK2 and TIGAR were confirmed at 1-4 Gy. Particularly, PCNA increased dose dependently. Curiously, TCF4, detected as a new TF implicated in the IR effects, significantly decreased post-irradiation.
This pipeline was implemented in cancer patients. We identified six DEGs which radiomodulation differs between radiosensitive and non-radiosensitive patients. Among these, two TFs: AFF3 and ATF3 and NOLC1 which has not yet been linked to the IR-response. Finally, we put together these results to identify a conservative gene profile. We found 32 genes modulated in *ex vivo* and *in vivo* sceneries. In conclusion, herein, we found new potential biomarkers of exposure and susceptibility which could improve the management of nuclear accident victims and radiotherapy patients.

21. (478) **GENOMIC ANALYSIS OF A KPC-2-PRODUCING *KLEBSIELLA PNEUMONIAE* ST15 FROM ARGENTINA**

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The isolation of colistin and carbapenem resistant *Klebsiella pneumoniae* (CCR-Kp) is increasing in hospital settings worldwide, and is related to increased morbidity, mortality and health care costs. *K. pneumoniae* ST15 is a less common Sequence Type than ST258 and ST11 nevertheless is also reported around the globe as responsible for CaR-Kp outbreaks. The aim of this work was to carry out the whole-genome sequencing (WGS), genomic and phylogenetic analysis of a multidrug resistant (MDR) CCR-Kp from Argentina. The MDR CCR-Kp strain KpS26 was isolated from a bloodstream infection at a hospital setting from Ciudad Autónoma de Buenos Aires, in February 2020. WGS was carried out using Illumina MiSeq-I with Nextera XT libraries. De novo assembly was carried out using SPADES v.3.11. Contigs were re-ordered using the ST15 reference genome *Klebsiella pneumoniae* PMK1 (GenBank CP008929) and oriented with MAUVE Contig Mover. The coding regions were detected with the Rapid Annotations using Subsystems Technology (RAST) server. Multilocus sequence typing (MLST) was performed in silico using the MLST database and schema for *K. pneumoniae* at the Pasteur MLST website. A maximum-likelihood tree was created with MEGA7 based on core SNPs from whole-genome alignment obtained with SNP-sites. The genomic, resistome, plasmids, IS and integrons content was analyzed with PathogenFinder, Resfinder, ISFinder, plasmid-SPAdes, PlasmidFinder and IntegronFinder. The KpS26 analysis revealed a genome of 5,815,319 bp (5342 CDS, 121 RNAs)of ST15. The Col(pHAD28), IncFIA(HI1), IncFIB(K), IncFII(K) and IncM1 plasmid replicons and 14 transferable associated antimicrobial resistance genes (ARGs), including bla_{KPC-2}, comprising six drug classes were detected. A class 1 integron with *dfrA14* as well as other gene cassettes were identified. The MDR CCR-Kp analyzed here shows that ST15 disseminates bla_{KPC-2} in Argentina alongside other antimicrobial resistance genes through Lateral Genetic Transfer.

22. (481) **GENOMIC ANALYSIS OF A CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* ST13 HARBORING $BLA_{OXA-163}$ FROM ARGENTINA**

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Carbapenem resistant *Klebsiella pneumoniae* (CR-Kp) causes outbreaks in hospital settings worldwide, and is related to increased morbidity, mortality and health care costs. CR-Kp ST13 are not predominant but nevertheless are reported around the globe. The aim of this work was to perform the whole-genome sequencing (WGS) of a multidrug resistant (MDR) CR-Kp from Argentina to carry out a genomic and phylogenetic analysis. The strain KpS27 was isolated from a bloodstream infection at a hospital setting from Ciudad Autónoma de Buenos Aires, in January 2020. KpS27 strain was an MDR CR-Kp, susceptible only to amikacin, tigecycline and colistin. WGS was carried out using Illumina MiSeq-I with Nextera XT libraries. Reads were quality trimmed and analyzed with fastqc, trimmomatic and cutadapt. Assembly were done with SPADES v.3.11. Contigs were re-ordered and oriented with MAUVE Contig Mover. The coding regions were detected with the Rapid Annotations using Subsystems Technology (RAST) server. Multilocus sequence typing (MLST) was analyzed in silico using the MLST database and schema for *K. pneumoniae* at the Pasteur MLST website. A maximum-likelihood tree was created using MEGA7 based on core SNPs from whole-genome alignment obtained with SNP-sites. The genomic, resistome, plasmids, IS and integrons content was analyzed i.e. with PathogenFinder, Resfinder, ISFinder, plasmid-SPAdes, PlasmidFinder and IntegronFinder. The phylogenetic analysis showed that

KpS27 belonged to ST13. The Col(pHAD28), IncC and IncFIB(pQil) plasmid replicons and 15 transferable associated antimicrobial resistance genes (ARG) comprising eight drug classes were detected. Among the ARG we highlight the presence of $bla_{OXA-163}$ a $bla_{OXA-48-like}$ gene, which codes for a carbapenemase. A class 1 integron as well as the gene cassettes *aac(6')-Ib-cr* and *dfrA14* were identified. This MDR CR-Kp strain carries a variety of mobile elements and shows that ST13 with $bla_{OXA-163}$ are nowadays disseminating in Argentina.

23. (505) **BUILDING A PREDICTIVE MODEL BASED ON GLYCOGENE EXPRESSION PROFILES OF MELANOMA PATIENTS FROM TCGA-SKCM PROJECT**

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Objectives. We aim to study glycoimmune pathways involved in resistance to immune checkpoint blockade (ICB) therapies for melanoma to establish a signature for patient classification.
Materials & Methods. Analysis were run using R software v3.6. Deconvolution was performed using MIXTURE tool and the glycoimmune pathways analysis was based on GlycoV4 chip (834 genes). Signature score was calculated as the geometric mean of the expression of genes in each signature. Cluster1 and Cluster2 comparisons was performed using Wilcoxon test.
Results. Metastatic tumor biopsies (n=357) were clustered using 78 high-variable glycogenes resulting in a Cluster2 of low Overall Survival (OS), and a Cluster1 of high OS ($p<0.05$). Next, we characterized the tumor microenvironment (TME) using deconvolution tools. Cluster2 showed a lower absolute score ($p<0.001$) and proportion of cell types associated with immune activation (activated CD8+, CD4+ and M1 macrophages), while showing higher proportion of M2 macrophages and resting CD4+ cells. Cluster2 also showed lower Cytolytic Score, lower TMB and higher Intratumor Heterogeneity ($p<0.01$). When analyzing gene signatures, Cluster2 showed lower score of apoptosis and interferon-γ with higher score of proliferation ($p<0.01$). We built a predictive model based on a Bayes Naïve classifier (AUC=0.902) using 20 features identified by recursive feature elimination and used it to classify 73 baseline biopsies of a separate cohort (anti-PD1, n=41; Combo, n=32). Patients classified as Cluster2 consistently had lower OS ($p<0.01$). By performing a Fisher's test, we found a significant association between Cluster2 and non-responding patients ($p<0.05$).
Conclusion. Dysregulation of 78 glycogenes correlates with distinct profiles of TME and biomarkers in association with OS. These profiles were also found in a cohort of ICB-treated patients. Further analysis is required to validate these findings in order to unveil new mechanisms of resistance to immunotherapy.

24. (525) **MIR-29B EXPRESSION IN BREAST TUMORS MIGHT INDUCE ACUTE MYELOID LEUKEMIA THROUGH TET GENE-TARGETING**.

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Acute Myeloid Leukemia (AML) is a hematopoietic malignancy that can arise as a secondary cancer after breast cancer (BrCa) therapy with alkylating agents or radiotherapy. This type of AML has usually poor prognosis and is refractory.
MicroRNAs (miRNAs) are small non-coding RNAs that target mRNA to reduce protein expression. It was reported that miR-125b, -29b, -29c, -101, and -7 are overexpressed in the bone marrow (BM) from AML-patients.
Ten-Eleven-Translocation (TET) family genes, including TET1, TET2 and TET3, codify for DNA demethylation enzymes. Particularly, TET2 is a tumor suppressor frequently mutated in AML, and its expression can be controlled by miRNAs.
Our aim was to identify TET target miRNAs released by breast tumors and their impact on AML.
We evaluated the incidence of BrCa in patients who later developed hematopoietic disorders. Through cBioportal software we found that 5.3% of the patients with leukemia and myelodysplastic syndromes (n=13/292) had developed BrCa previously. Then we analyzed miRNAs expression in breast primary tumors (BPT) or normal adjacent tissue (NAT) from patients obtained from the TCGA Breast Cancer cohort using UCSC Xena resource (n=1,229). Among the five miRNAs overexpressed in AML, only miR-29b was differentially expressed in BPT vs. NAT ($p<0.001$). Using DIANA-TarBase tool, we found that hematologic neoplasms, which include AML, were highly related to miR-29b, and TET1 was one of the most targeted genes (prediction score=0.98), validated in BM. KEGG analysis using DIANA-miRPath showed that miR-29b is involved in several types of cancer including chronic leukemia and AML. Gene ontology analysis revealed that extracellular matrix processes and DNA demethylation were significantly regulated by miR-29b ($p<0.01$). MiR-29b was found in exosomes from BrCa, using EVmiRNA software.
In summary, our results suggest that AML might emerge as a secondary neoplasm induced by BrCa through circulating miR-29b, which targets TET gene.

25. (537) **STUDYING THE ROLE OF THE LIVER X RECEPTORS IN THE MAMMARY GLAND DEVELOPMENT: AN IN SILICO APPROACH**

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Liver X Receptors (LXRs) are ligand-activated transcription factors of the nuclear receptor superfamily, being the oxysterols their endogenous ligands. They play a key role in maintaining the lipid homeostasis by inducing the expression of genes involved in cholesterol transport and the *de novo* synthesis of triacylglycerides, as well as regulating immune and inflammatory responses. In the mammary gland, these processes are tightly regulated postnatally. During lactation, the gland is endowed with an enormous capacity to synthesize and secrete lipids, however, after weaning, it undergoes a rapid involution in which inflammatory cytokines play a major role. We have previously shown that LXRα is expressed in the lactating murine mammary epithelium and is an important regulator of cholesterol incorporation into the milk (Grinman et. al., 2019), however, little is known about its role in other stages of this organ development. Based on an integrative analysis of public data from previous reports which use technologies such as bulk and scRNA-seq, ChIP-seq and ATAC-seq, we aimed to study at the single cell level, the role of the LXRs in the mammary gland development. We hypothesized that the LXRs pathway is upregulated from pregnancy to lactation and further downregulated towards involution. Using genes found to be regulated upon LXRs activation by Boergesen et. al., 2012, we tested if such LXR signature was enriched in the transitions between cell clusters of the scRNA-seq data from Bach et. al., 2017. Preliminary results from our analysis show a significant increase in the expression of the lipogenic *Srebf1, Thrsp, Fasn* and *Me1* genes

in mammary epithelial luminal progenitors in the transition from of virgin mice to lactation, and a concomitant decrease of the same genes from lactation to post-involution. These results support the role of the LXR as an important modulator of the lipid homeostasis in the lactating gland, modulating milk requirements to feed the newborn.

CARDIOVASCULAR Y RESPIRATORIO

26. (18) **TRIIODOTHYRONINE (T3) IMPROVES POST-ISCHEMIC MECHANICAL RECOVERY AND MITOCHONDRIAL FUNCTION PRESERVATION BY ENHANCING AMP-ACTIVATED PROTEIN KINASE (AMPK) ACTIVATION.**

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Experimental evidence has shown that T3 can regulate cardioprotective signaling pathways, increasing myocardium resistance to ischemia-reperfusion injury. Although novel studies have suggested that T3 enhances the activation of AMPK, a key enzyme regulator of energy balance, its role has not been elucidated yet.

In the present study the role of AMPK in the effects exerted by acute treatment with T3 on ischemic-reperfused myocardium was investigated. For this aim, rat left atria were subjected to 75 min simulated ischemia (I)-75 min reperfusion (R), in the presence of T3 (60 nM) and AMPK inhibitor, Compound C (CC; 10 μM). ANOVA, followed by Tukey, n=8/group.

Results showed that CC prevented the increase of AMPK activation induced by T3 (End stabilization period (ESP):1.33±0.02, I-R:2.20±0.11*, I-R+T3:2.79±0.10*#, I-R+T3+CC:1.17±0.10 AU;*p<0.05 vs ESP, I-R+T3+CC; #p<0,05 vs I-R). At the end of R, CC abolished the increase of contractile function recovery (Peak force (%) I-R:36±3, I-R+T3:51±2*, I-R+T3+CC:39±4;*p<0.05) and cellular viability conservation produced by T3 treatment (I-R:66±4, I-R+T3:79±3*, Is-Rs+T3+CC:54±3 %;*p<0.05). CC also prevented mitochondrial ultrastructure preservation exerted by T3, as well as mitochondrial ATP production rate and tissue ATP enhancement (I-R:24±1, Is-Rs+T3:59±6*, I-R+T3+CC:40±2 nmol/min/mg mitochondrial protein; I-R:420±52, I-R+T3:608±94*, I-R+T3+CC:266±46 pmol/mg protein;*p<0.05). In addition, CC reverted the increase induced by T3 in the calcium amount required to trigger massive mitochondrial calcium release (I-R:77±9, I-R+T3:114±12*, I-R+T3+CC:82±8 nmol/mg protein;*p<0.05) and the phosphorylation and inactivation of GSK-3β, master switch enzyme that limits mPTP opening (I-R:1.5±0.2, I-R+T3:2.2±0.1*, I-R+T3+CC:1.6±0.2 AU;*p<0.05).

Results suggest that AMPK is involved, at least in part, in the protective effects exerted by T3 in the ischemic-reperfused myocardium, contributing to mitochondrial structure and function preservation.

27. (251) **DIFFERENTIAL BINDING TO EXTRACELLULAR MATRIX COMPONENTS OF A NATURAL APOLIPOPROTEINA-I VARIANT ASSOCIATED WITH CARDIAC AMYLOIDOSIS**

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Specific interactions of apolipoproteins with components of the extracellular matrix (ECM), as proteoglycans (PGs), are associated with amyloidosis or atherosclerosis. These interactions seem to depend on age, cellular differentiation, and pathological conditions, which might modify glycosaminoglycans (GAGs) composition.

We previously showed that apoA-I Arg173Pro (a natural mutant involved in cardiac amyloidosis) but not the wild type protein (Wt) bound heparin at pH 7.4 This indicates that selective interactions of this variant may occur with GAGs. To study the specific role of the matrix?s charge on the interaction of apoA-I with GAGs, we synthesized polymers having different ratios of sulfated (sodium 4-styrene sulfonate, (SSNa) or hydroxilated monomers (2-hydroxyethyl methacrylate, (HEMA)) and studied the binding of fluorescently labelled apoA-I Wt or Arg173Pro. We show that both proteins are highly retained as long as the negative charge increases (50% with $p \leq 0.05$ as negative charge of the SSNa increased from 0.25 to 0.75 M). In addition, Arg173Pro remained in the matrix 10 % more than Wt ($p < 0.01$), indicating that the retention of specific proteins in the ECM could be part of the pathogenicity. To analyze the differential interaction with GAGs, Wt or Arg173Pro were incubated in the presence of Dermatan Sulfate (DS) or sodium heparin (HEP). The samples (n=4), were centrifuged, and pellets and supernatants analyzed by polyacrylamide gel electrophoresis. GAGs were visualized by staining with toluidine blue and the proteins with Silver stain. We observed that Arg173Pro had more interaction with DS and HEP than the Wt variant, which is interesting, since both GAGs are associated with amyloid deposits and heart disease, respectively. We conclude that the interactions of apoA-I variants with GAGs offer a challenging field to understand, not only the pathology but also possible therapeutic strategies to treat this disease.

28. (252) **HUMAN APOLIPOPROTEIN A-I IN ATHEROSCLEROSIS. THE ROLE OF OXIDATION OR NATURAL VARIANTS SYNERGIZING ITS DYSFUNCTION.**

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Oxidation of human high density lipoprotein and its major protein apolipoprotein A-I (apoA-I) was proposed to cause their failure to protect against cardiovascular disease. However, multiple and complex events might contribute to the breakdown of this protein to fulfill its protective role. To set light on this topic, we took advantage of the study of a natural variant with a deletion of the lysine 107 (K107del) associated with atherosclerosis and amyloidosis. We oxidized the variants by controlled incubation with H2O2 and determined structural and biological parameters by biophysical and biological approaches.

Both variants oxidized under these conditions preserved or even induced an increase in the lipid clearance with respect to untreated proteins (20 % with $p \leq 0.05$), and decreased the yield of the physiological dimeric conformations. Following 30-day incubation at 37oC K107del but not Wt acquired a well-defined fibrillar conformation which is the main signature of the amyloid pathology. These conformations (but not freshly folded proteins) activated neutrophils into the formation of neutrophil extracellular traps (NETs) ($p<0.05$), which was drastically elicited in the case of the K107del ($p<0.001$).

To initiate the search of possible pathways involved in cellular activation, we tested a cellular model of macrophages. Fresh and oxidized Wt promoted the increase of p62 ($p \leq 0.005$), a protein described as anti-inflammatory. In the presence of ATRA (an inhibitor of Nrf2-Keap pathway), only Wt effect was blocked (by 40%). Thus, it may be suggested that the oxidation of apoA-I resulted in the loss of one of its key functions. Altogether, our data support that post translational apoA-I modifications (probably chronic and progressive) raised a protein conformation with significant loss of function and increased aggregation tendency. Oxidation may help favoring

this conformation. The results learnt here strength a close association between amyloidosis and atherosclerosis.

29. (261) **COBALT CHLORIDE PROTECS THE HEART AFTER A GLOBAL ISCHEMIC INSULT.**

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Introduction: Ischemia-reperfusion (I/R) is one of the main cardiovascular risk factors and leads to heart contractile and energy dysfunction. I/R-induced damage is reduced by ischemic postconditioning. $CoCl_2$ has properties to function as a postconditioning agent, since it can trigger transcriptional changes that resemble the response to a hypoxic event under normoxic conditions.
Objectives: To evaluate $CoCl_2$ as a postconditioning therapeutic tool after a myocardial and arterial I/R.
Materials and Methods: Isolated adult Wistar rats hearts were arterially perfused at 37ºC by Langendorff method, paced at 3 Hz, exposed to 30 min ischemia followed by 45 min reperfusion (R) in the presence or absence of 0.23 mM $CoCl_2$ which was maintained or removed after 20 min of R.
Aortic contractility was evaluated in an isolated organ bath trough incubation with cumulative noradrenaline (NA) doses. After NA washing, 20 min of simulated arterial ischemia (SI) and R with or without $CoCl_2$ was performed, and NA response was re-evaluated.
Results: During R, the presence of $CoCl_2$ did not alter the cardiac resting pressure, nor the perfusion pressure, but increased the developed pressure ($p<0.05$) until 20 min which then descend reaching controls values. This decrease was prevented when $CoCl_2$ was eliminated at 20 min of R. $CoCl_2$ in R increased the contractile economy (P/Ht) and decreased the cardiac damaged area ($p<0.05$) and the incidence of arrhythmias ($p<0.001$).
Post-SI arterial contractility increased at the lowest NA dose but not at higher ones. $CoCl_2$ in post –SI R did not affect arterial force, but decreased NA sensitivity (EC 50: control: $10^{-7.5}$, $CoCl_2$: $10^{-6.5}$ M) and the maximum contractile response.
Conclusion: The use of $CoCl_2$ after an ischemic event attenuates the cardiac damage produced at least during the first 25 min of R and reduces the arterial adrenergic contractile response. These results support the use of $CoCl_2$ as a potential cardioprotective tool of clinical relevance.

30. (267) **H_2O_2, NO AND $ONOO^-$ IN THE CARDIAC MITOCHONDRIAL DYSFUNCTION IN A TYPE 1 DIABETES MODEL**

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AIM: To study the changes of mitochondrial production rates and/or steady-state concentrations ($[X]_{ss}$) of $O_2^{\cdot-}$, H_2O_2, NO and $ONOO^-$ in the temporal evolution of cardiac mitochondrial dysfunction in a type 1 diabetes model. **METHODS:** Diabetes was induced by a single dose of Streptozotocin (STZ, 60 mg/kg, ip) in male rats. Glycemia (mg/dl) was determined after 72 h (C:130 ± 5; DM:415 ± 23). The animals were sacrificed after 10 or 28 days of STZ-injection (7 or 25 days of hyperglycemia). Mn-SOD activity, and H_2O_2 and NO production rates were determined in the cardiac mitochondrial fraction. $[O_2^{\cdot-}]_{ss}$,$[NO]_{ss}$ and $ONOO^-$ generation were estimated from experimental data. **RESULTS:** When animals were sacrificed 10 days after STZ-injection, heart mitochondrial NO (30%) and H_2O_2 (117%) productions were higher and Mn-SOD activity was lower (15%) than control values. Moreover, mitochondrial $[O_2^{\cdot-}]_{ss}$ was 2.5-fold higher in heart from diabetic rats, along with a 30% increase in $[NO]_{ss}$. Thus, $ONOO^-$ production rate resulted 3 times higher. When animals were subjected to 25 days of hyperglycemia, Mn-SOD activity was really reduced (50%). While H_2O_2 generation was extremely augmented (128%), the increase in NO generation (23%) was similar to the one observed at 7 days. Increases in $[O_2^{\cdot-}]_{ss}$ (350%), $[NO]_{ss}$ (25%), and $ONOO^-$ production (450%) were obtained. Moreover, nitration of tyrosine residues of mitochondrial proteins was observed in diabetic animals sacrificed at day 28. **CONCLUSIONS:** Heart mitochondrial production rates of H_2O_2, NO and $ONOO^-$ were higher in diabetic than in control animals, both after 7 and 25 days of hyperglycemia. No difference in the increase -over the control values- of $[NO]_{ss}$ was observed over time, while a much greater rise in $[O_2^{\cdot-}]_{ss}$ was detected after 25 days of sustained hyperglycemia respect to the enhancement obtained at 7 days, intensifying the difference in $ONOO^-$ generation. Therefore, $ONOO^-$ generation rate is mainly controlled by $[O_2^{\cdot-}]_{ss}$ rather than by $[NO]_{ss}$

31. (334) **HYPERBARIC OXYGENATION THERAPY IN THE TREATMENT OF COVID-19 (PRELIMINARY REPORT)**

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Introduction: Hyperbaric oxygenation therapy (HBOT) has been shown to reduce the production and release of pro-inflammatory cytokines. Its use in patients with CoViD-19 and hypoxemia in China showed promising results although its use was poorly evaluated. Methods: A randomized, controlled study was started, comparing standard care (Control Group which includes non-hyperbaric oxygen supply) versus standard care plus HBOT (Test). The HBOT was carried out with Biobarica chambers of national development and medium pressure (1.45 atm) in sessions of 90 minutes per day for at least 5 days. Baseline daily oxygen saturation (SatO2) breathing ambient air (FiO2 = 0.21%) prior to HBOT was recorded. Results: 18 patients were included (ratio 1:1). The comparison between the groups did not show significant differences in terms of clinical status, general compromise, laboratory, age, comorbidities or history. The evolution of the HBOT group showed a rapid increase in SatO2 with a significant difference between groups from day 4 onwards. On day 5, the HBOT group presented SatO2 94.4 ± 2.7 (91.0-98.0)% Vs 89,8±2,3 (87,0-94,0) in control group. The time to normalize oxygen Saturation was significantly shorter in the HBOT group [mean±SD (min-max)]: 3,3±1,4 (1,0-5,0) VS 5,8±1,4 (3,0-7,0) days (P=0,002). The ascending slope for SatO2 in HBOT group was significantly higher than the control group: 2,1±0,6 (1,3-3,2) VS 1,5±0,6 (0,8-2,8) %/day (P 0,04). No adverse reactions were recorded. Discussion: HBOT shown to be more effective than oxygen suppy at ambient pressure. Although the study continues to recruit individuals, initial clinical results show a clear beneficial effect.
Reference: Rui-Yong C et al. Efficacy analysis of hyperbaric oxygen therapy in the treatment of severe coronavirus disease 2019 patients. Acad. J. Second Mil. Med. Univ. ; 6(41): 604-611, 2020.

32. (429) **EFFECTS OF REMOTE ISCHEMIC PRECONDITIONING ON EARLY MYOCARDIAL POST-INFARCTION REMODELING**

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Introduction: It is known that remote ischemic preconditioning (rIPC) reduces infarct size in experimental models of myocardial infarction (MI); while its effect is controversial in the clinical setting. Particularly, 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, underwent MI by permanent ligation (for 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. Finally, a third group was undergoing to left thoracotomy, but without myocardial ischemia (Sham). Infarct size was measured with triphenyltetrazolium, ventricular function by hemodynamic and echocardiography, and MMPs 2 and 9 activity was evaluated by zymography.
Results: There were no significant differences in the risk area and infarct size between groups. IM 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). 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). We did not observe significant differences between groups in the end-systolic stress values (afterload index). The relationship between end-systolic stress and ejection fraction was plotted, observing a significant improvement in the rIPC group, compared to the MI group. Finally, rIPC reduced significantly MMP-9 activity in the left ventricle (infarct area), but not MMP-2.
Conclusion: We have showed that rIPC has a beneficial effect on early remodeling, reducing myocardial post-infarction expansion and improving ventricular function. This beneficial effect could be related to a lower MMP-9 activity.

33. (472) **GENETIC ANALYSIS ALGORITHM FOR THE STUDY OF PATIENTS WITH CONGENITAL HEART DISEASE**

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Introduction: Congenital heart diseases (CHD) are structural anomalies of the heart and great vessels. They are present at birth and encompass a broad spectrum of anomalies affecting 0.6%-0.9% of all live births worldwide. In Argentina, CHD are the most frequent congenital anomalies representing 1/3 of all birth defects. Their etiology is heterogeneous; however, family recurrence has been observed suggesting the influence of genetic factors.
Objective: To analyze the presence of chromosomal abnormalities, genomic imbalances and/or sequence variants in a group of Argentine CHD patients.
Methods: A total of 378 CHD patients up to 16 years old were included. DNA from peripheral blood was obtained from all patients. Karyotyping was performed for the 126 CHD patients presenting multiple congenital anomalies (MCA). Samples from patients with conotruncal CHD or DiGeorge phenotype (N=215) were analyzed by multiplex ligation-dependent probe amplification (MLPA). Sixty-three MCA samples were selected for array-CGH analysis and 17 for targeted or exome next generation sequencing (NGS).
Results: A total of 294 patients were studied by at least one technique. Cytogenetic abnormalities were present in 10 MCA patients, while 10 had clinically relevant imbalances detected by array-CGH. Forty-five patients presented deletions or duplications by MLPA (42 in the 22q11 region). Clinically relevant nucleotide variants were found in 10 patients after NGS analysis, 4 of them novel, in *KAT6B, MYH11, MYH7* and *EP300* genes.
Conclusions: Using this algorithm that combines a technical and a clinical strategy, 26% of the patients analyzed were diagnosed.
Key words: congenital heart disease, genetic analysis chromosomal abnormalities, array-CGH, next generation sequencing

34. (519) ***TRYPANOSOMA CRUZI* PROMOTES VASCULAR ALTERATIONS ASSOCIATED WITH RISK OF CARDIOVASCULAR DISEASE**

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Chagas disease, caused by the parasite *Trypanosoma cruzi*, is an important cause of cardiac disease in endemic areas of Latin America. About 35% of infected patients develop chronic myocardiopathy that can lead to cardiac failure and stroke. The other infected patients remain asymptomatic (indetermined form), although increased aorta stiffness (AS) has been described in all infected patients suggesting that the infection could contribute to vascular alterations. Despite the importance of the vasculature in regulating the homeostasis of the cardiovascular system, little is known about its changes in response to this infection. The aim of this work was to study vascular cell population changes during the acute phase of *T. cruzi* infection. Thoracic (Athor), abdominal aorta (Aabd), aortic arch, and brachiocephalic artery (BCA) were obtained from BALB/c mice infected with 500 tps of *T. cruzi* at day 16 post-infection with non-infected mice (NI) used as controls. Arterial segments were analyzed by multiparametric FACS followed by t-SNE analysis to identify different cell populations. Similar cell clusters were observed in BCA, Aabd, and arch but not in Athor of NI mice. In addition, Athor was the segment presenting the major differences in cell clusters composition after infection. Thus, *T. cruzi* infection increased the percent of α-SMA+ (smooth muscle cells, SMC) and F4/80+ CD11b+ (macrophages, Mo) cells, and the expression of markers of active immune cells. Interestingly, cell clusters co-expressing SMC- and Mo-markers, suggesting SMC-transdifferentiation into Mo or *viceversa,* were also observed in Athor. Taken together, these results suggest that *T. cruzi* infection induces vascular changes, being Athor the most affected segment. Besides, transdifferentiation and immune cell activation could be mechanisms involved in these vascular alterations.

35. (523) **LONG NONCODING RNAS AND YTHDF2 REGULATE HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS DIFFERENTIATION**

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Alteration of smooth muscle cell (SMC) plasticity from a contractile-differentiated to a proliferative-dedifferentiated phenotype constitutes a key factor in the development of cardiovascular diseases. Epigenetic mechanisms, such as long noncoding RNAs (lncRNAs) and RNA modifications such as N^6-methyladenosine (m^6A) regulate a number of biological processes in both physiological and pathological settings, but their participation in SMC differentiation is just starting to emerge. The aim of this study was to investigate molecular mechanisms involved in SMC differentiation mediated by novel lncRNAs. We used an *in vitro* model of cell-to-cell contact-induced SMC differentiation of pulmonary artery-derived SMC (hPASMC, Lonza). LncRNAs expression was assessed by Illumina RNA deep-sequencing of total RNA and RT-qPCR. The

lncRNA4 (*TCONS_00006193*) expression showed a significant increase during differentiation when compared with proliferative SMC (p<0,0001) by RT-qPCR and Northern Blot, and decreased during SMC dedifferentiation (p=0,0073), analyzed by RT-qPCR. Silencing of the lncRNA4 using siPool-RNAs resulted in SMC differentiation defects, evidenced by decreased expression of the SMC marker genes *MYOCD* (p=0,0019), *CNN1*(p=0,0041) and *SM22*α (p=0,0006), measured by RT-qPCR and by increased proliferation analyzed by flow cytometry. LncRNA4 pull-down showed its association with the methylation machinery. Interestingly, the levels of the m6A reader YTHDF2 decreased throughout differentiation (p=0,0031). YTHDF2 silencing using siPool-RNAs induced a significant increase of the candidate lncRNA4 (p<0,0001), as well as of *MYOCD* (p=0,0008) and *CNN1* (p=0,0136) evaluated by RT-qPCR indicating an induction of SMC differentiation. These findings support that lncRNA4 and YTHDF2 are key to SMC differentiation and therefore could have a role in the development of cardiovascular diseases.

36. (539) **ORAL ADMINISTRATION OF STEVIOSIDE AS A CARDIOPROTECTIVE NUTRACEUTICAL STRATEGY: AN INSIGHT INTO MITOCHONDRIA STATUS AND ITS RELATIONSHIP WITH PROTEIN KINASE B (AKT)**

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Stevioside (S), a diterpenoid glycoside, is the main non-caloric sweetener extracted from Stevia rebaudiana Bertoni leaves. In previous studies, we demonstrated that the oral administration of S improved the recovery of hearts subjected to ischemia-reperfusion (I-R), and increased phosphorylation of Akt and GSK3β. These effects were partially reverted by the administration of wortmannin (W), an upstream inhibitor of Akt.

Since mitochondrial dysfunction plays a key role in IR injury, we aimed to investigate the effects of oral administration of S (168mg/kg/15days) on several mitochondrial parameters from Langendorff-perfused rat hearts subjected to I-R.

Hearts from female Wistar rats (200-250g) fed ad libitum were used. W (100nM) was added 15 minutes before I. The mitochondrial ultrastructure was analyzed by electron microscopy, the measurement of mitochondrial ATP synthesis was performed by the luciferin-luciferase method and calcium-triggered mitochondrial swelling was determined as % of light scattering decrease at 540nm (%LSD). We also studied calcium retention capacity (CRC) by exposing mitochondria to small pulses of calcium using the fluorescent dye: Calcium Green-5N. ANOVA, n=8/group.

Results showed, at the end of reperfusion, an increase in mitochondrial ATP synthesis rate of hearts treated with S (C:66.3±6.5, W:59.5±6.1, S:87.3±3.7*, S+W:64.6±6.9 nmol/min/mg of mitochondrial protein; *p<0.05 vs all groups). Likewise, electron micrographs showed better mitochondrial conservation in the S group. In addition, both %LSD produced by calcium overload (300μM) and CRC were significantly lower with S treatment compared to the other groups. (%LSD: C:2.6±0.3, W:2.5±0.4, S:1.3±0.3*, S+W:2.2±0.3%; CRC: C:31.0±3.1, W:30.7±3.7, S:46.6±5.4*, S+W:36.8±5.4 nmol calcium/mg protein; *p<0.05 vs all groups).

These findings suggest that oral administration of S presents cardioprotective effects due to better mitochondrial preservation and could be partly mediated by Akt activation.

37. (547) **NADPH OXIDASE-5 INDUCES OXIDATIVE STRESS IN HUMAN ENDOTHELIAL CELLS AND IS POSITIVELY REGULATED IN PRO-ATHEROGENIC CONDITIONS**

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Excessive production of reactive oxygen species (ROS) plays a detrimental role in the progression of atherosclerosis. Increased expression of the pro-oxidant enzyme NADPH oxidase (NOX) in the arterial wall has been hypothesized to correlate with vascular injury and progression of atheroma plaque. Since the gene encoding NOX5 is not expressed in the mouse genome, we examined the expression of NOX5 isoform in human endothelial cells (HUVECs) under pro-atherogenic and pro-inflammatory conditions. HUVECs were exposed to Angiotensin II (AngII) or to tumor necrosis factor (TNF-alpha) and incubated with selective NOX-inhibitors one hour prior treatment. NOX expression was determined by qRT-PCR and oxidative stress was evaluated using a fluorescent probe sensitive to ROS production. HUVECs expressed both NOX2 and NOX5 mRNA (2.4 ΔCT ±0.1 and 19.5 ΔCT ±1.8, respectively). AngII and TNF-alfa increased ROS generation in HUVECs which was inhibited by selective inactivation of NOX2 or NOX5. As enhanced activity of NOXs occur in many pathological conditions including hypertension and heart failure we next determined the expression of NOX in Human Mammary Arteries (HMA) of patients undergoing coronary bypass surgery. NOX2 and NOX5 expression in HMA were similar (7.42 ΔCT ± 0.55; and 8.32 ΔCT ± 0.43, respectively), and they exhibited a positive and significant correlation (r= 0.665; p=0.0007) between them. High NOX5 expression was found in patients with Hypertension, Dyslipidemia and Obesity. Our results suggest that NOX2 and NOX5 are up-regulated in pro-atherogenic conditions and contribute to endothelial dysfunction. Taking these results into account, it is attractive to speculate that small, selective and specific inhibitory molecules of different NOXs could exert a beneficial effect on vascular pathology

38. (556) **CARDIAC HYPERTROPHY AND MORTALITY ARE INCREASED IN AGED MICE WITH GENETIC DELETION OF GALECTIN 3**

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Aging is associated with highly prevalent cardiovascular pathologies such as hypertension and heart failure. We aimed to study the cardiovascular effects of genetic deletion of Galectin 3 (Gal3), a β-galactosidase binding lectin with proinflammatory and profibrotic effects, in aged mice. Wild type C57BL/6 (C57; n=54) and Gal3 knockout (Gal3KO; n=55) mice with access to water and food *ad libitum* were observed for 24 months. At 2 years of follow up, survival rate was quantified, systolic blood pressure (SBP) was measured by plethysmography, and echocardiography (ECO) was performed in sedated mice to measure left ventricular geometry and function. In addition, shortening fraction (SF, %) and cardiac mass (mg) were calculated. Then, animals were euthanized, the organs harvested weighted and the tibia length (TL) measured. Results (Mean ± SE): At 2 years, the survival was reduced in Gal3KO (p= 0.02 Gal3KO vs C57). SBP (mmHg) was 112 ± 3 in C57 vs 114 ± 3 in Gal3KO (p=NS). SF (%) was 61 ± 1 C57 vs 60 ± 2 Gal3KO (p=NS). Increased hypertrophy was observed in Gal3KO, posterior wall thickness (mm) was: 0.8 ± 0.02 in C57 vs 1.5 ± 0.2 in Gal3KO (p=0.0003) and 1 ± 0.02 in C57 vs 2 ± 0.1 in Gal3KO (p=0.0003) as measured in diastole and systole respectively, while anterior wall thickness (mm)

in systole was 1 ± 0.02 in C57 vs 2 ± 0.1 in Gal3KO (p=0.0005). Cardiac mass (mg) was 80 ± 10 vs 140 ± 20 in C57 and Gal3KO mice respectively (p=0.01). Left ventricular end systolic and end diastolic diameter (mm) were 1 ± 0.1 in C57 *vs* 1 ± 0.1 in Gal3KO and 3 ± 0.1 in C57 *vs* 3 ± 0.1 in Gal3KO (p=NS). At necropsy: Heart weight/TL and kidney weight/TL were 8 ± 0.3 and 9 ± 0.3 (p<0.05) and 25 ± 1 vs 34 ± 1 (p<0.05) in C57 and Gal3KO mice respectively. In summary, our data suggests that genetic deletion of Gal3 reduced survival rate and increased myocardial hypertrophy without changes in SBP in aged mice.

ENDOCRINOLOGÍA

39. (34) **RETINOIC ACID PATHWAY IN CORTICOTROPH TUMORS**

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Cushing disease (CD) is caused by excessive glucocorticoid secretion due to adrenocorticotropic hormone (ACTH) overproduction by a tumor of the pituitary gland. ACTH biosynthesis is coordinately controlled by different transcription factors at the level of the pro-opiomelanocortin (Pomc) gene.

Retinoic acid (RA) is being tested as medical treatment for CD. In previous studies we demonstrated that RA and Bone morphogenetic protein 4 (BMP-4) pathways control Pomc transcription by a potentiated inhibition.

It has been shown that about 25% of patients do not respond to RA treatment. RA inhibitory factors that are expressed in corticotroph tumoral cells could allow to predict the response. Among them we are exploring COUP-TFI and observed in a first series of patients that one out of four does expresses COUP-TFI, a strong inhibitory factor of RA action.

In the search for new factors involved in the effect of RA on Pomc and ACTH, that would allow predicting therapeutic efficacy and possible secondary events, we studied BMP-4, Nurr77 and octamer-binding transcription factor 4 (OCT-4).

We observed a potentiated inhibitory effect of 100nM RA in co-treatment with 100ng/ml BMP-4, on ACTH secretion in murine AtT-20 corticotroph cells, consistent with the potentiated effect described on Pomc transcription. BMP-4 (3.9-folds) or RA (1.9-folds) treatment increased the phosphorylation of SMAD1/5 (Western blot), which was not further altered by the co-treatment (4-folds). Using a luciferase reporter for the Pomc promoter, Nur77 enhanced Pomc transcription and also RA inhibition, but did not alter the co-treatment. OCT-4 has a dose dependent inhibitory effect on Pomc transcription, but also induces the loss of action of RA and modulates the BMP-4 action.

The regulatory interaction of RA with transcription factors as Smads, Nur77 or OCT-4 in the control of corticotroph gene expression, could contribute to define more precise biomarkers or targets for corticotrophinomas treatment.

40. (53) **DEVELOPMENT AND VALIDATION OF A PREDICTION RULE TO DIAGNOSE GROWTH HORMONE DEFICIENCY WITHOUT NEED FOR STIMULATION TESTS IN CHILDREN WITH RISK FACTORS**

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Introduction: Practice guidelines suggest, but cannot recommend, establishing a diagnosis of growth hormone deficiency (GHD) without performing growth hormone stimulation tests (GHST) in children with risk factors, due to the lack of sufficient evidence. Our objective was to develop and validate a prediction rule to diagnose GHD in children with growth failure and potential risk factors.

Design and Methods: Development of a prediction rule, following the TRIPOD methodology, in a cohort of children with growth failure, and its validation in a second, independent cohort, in a tertiary pediatric hospital.

Results: In the first cohort (n=770), after tuning the classification model trained using the 15 potential dichotomic predictors, 9 variables showed an odds ratio (OR) >5 and a p-value <0.0001. Using a gain ratio, entropy reducing algorithm to automatically build a decision tree from the selected variables, without reduce error pruning or limits on minimum number of records, the resulting prediction rule stated that a patient would have GHD if (s)he had: pituitary dysgenesis, or two or more pituitary deficiencies, or one pituitary deficiency plus: neonatal hypoglycemia or hypogenitalism, or diabetes insipidus, or midline abnormalities, or (supra)sellar tumor/surgery, or cranial radiotherapy >18 Gy. In the validation cohort (n=161), the specificity of the prediction rule was 99.2% (95% CI: 95.6-100%), its positive predictive value 95.2% (77.3-99.8%), its positive likelihood ratio 69.4, and the number needed to test was 1.19.

Conclusions: The clinical rule developed here predicts the existence of GHD with high specificity in children with growth disorders and clinically identifiable risk factors, thus providing compelling evidence to strengthen the recommendation that GHD can be safely diagnosed without resorting to GHST in neonates and children with growth failure and specific comorbidities.

41. (86) **THE ANDROGEN RECEPTOR (AR) EXPRESSION DETERMINES THE ANDROGENIC CONTROL OF HYPOXIA-INDUCED STROMAL CELL PROLIFERATION IN BENIGN PROSTATIC HYPERPLASIA**

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Benign prostatic hyperplasia (BPH) is characterized by an epithelial and stromal proliferative process, with testosterone being classically considered the most important factor involved in its pathophysiology. However, BPH occurs in older men, when androgen levels are usually dropped. Accumulated evidence indicates that BPH is associated with a hypoxic microenvironment which contributes to cell proliferation. We therefore investigated the effect of hypoxia on BPH stromal cell proliferation and the role of testosterone on this hypoxic context. Prostatic stromal cells, surgically harvested from patients with BPH (n=12, obtained under informed consent and approved by the Comité Institucional de Ética de Investigación en Salud of Sanatorio Allende), were isolated, cultured, and stimulated with $CoCl_2$ (200 μM), a stabilizer of hypoxia-inducible factor-1 (HIF-1) that mimics hypoxia, alone or in combination with testosterone at physiological doses (0.1 μM) for 24h.

As expected, $CoCl_2$ induced the expression of HIF-1α, as determined by western blot, which was correlated to a 2-to-4-fold increase in cell proliferation (by ki67 and BrdU incorporation) in all cultures ($p<0.01$ vs. vehicle). Testosterone decreased both HIF-1α expression ($p<0.01$ vs. $CoCl_2$) and cell proliferation ($p<0.01$ vs. $CoCl_2$) induced by $CoCl_2$ in 8 out 12 patient-derived cell cultures. In the remaining 4 cases, the testosterone treatment resulted in the upregulation of HIF-1α and cell proliferation, which was associated with a low expression of the androgen receptor (AR), by western blot, when compared to the previous 8 cell cultures. Then, our aim was to increase the basal levels of AR in those cells by pre-treating them with testosterone for 8h. Afterwards, the cells were subjected to hypoxia, with testosterone being able to repress both $CoCl_2$-induced HIF-1α and cell proliferation and thus, changing its behavior. Our results indicate that a hypoxic context increases cell prolifera-

tion in BPH stromal cells and androgens play a dual role in hypoxic microenvironments which depend on the expression levels of the AR.

42. (105) **PULMONARY PERFUSION CENTELLLOGRAPHY AS A METHOD OF EARLY DETECTION OF PULMONARY THROMBOEMBOLISM IN DOGS WITH CUSHING'S DISEASE**

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Cushing's disease (CD) is a common endocrinopathy in dogs. Pulmonary thromboembolism (PT) is one of the alterations with a high mortality rate. Pulmonary perfusion centellography is an imaging method that allows evaluating blood perfusion in the lung and detecting non-perfused areas, compatible with thrombi. We determined the usefulness of centellography as a method to detect insipient pulmonary thromboembolism and its association with coagulation markers. 12 dogs with CD were studied at School Hospital FCV-UBA. All presented typicl clinical signs of CD. 4 dogs showed continuous panting at rest that was accentuated when walking and increased respiratory rate. This panting occurred after the appearance of clinical signs of CD(1 to 3 months after). Pulmonary centellography was performed on all of them and D dimer(DD), fibrinogen, activity of antithrombin III(AT-III) and factor VIII(F-VIII) were evaluated as a marker for the presence of clots. These values were compared between the 4 dogs with respiratory problems and the remaining 8 animals. The comparison was made using the Mann-Whitney test, considering $P<0.05$ significant. The results were expressed as median and minimum and maximum ranges. The 4 dogs with intense panting showed a pulmonary lesion (lack of perfusion) compatible with PT on centellography. The 4 dogs with PT presented positive DD(>400), lower values of Fibrinogen, AT-III and F-VIII compared to the 8 without respiratory distress ($P=0.02$; $P=0.008$ and $P=0.017$ respectively). Pulmonary centellography is a very useful imaging method to evaluate the perfusion of the lung and detect non-perfused areas compatible with the presence of thrombi. A positive DD together with a decreased AT-III and F-VIII activity demonstrate a state of hypercoagulability, being useful as markers for the presence of thrombi. In patients with CD who present intense panting at rest and tachypnea, it is recommended to send pulmonary perfusion centellography for early detection of PT.

43. (136) **TISSUE KALLIKREIN (KLK1) AND KININS ACTIVATE TGFβ IN EXPLANTS OF NORMAL PITUITARIES AND PROLACTINOMAS FROM MICE LACKING THE DOPAMINE RECEPTOR TYPE 2**

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The Kallikrein -Kinin System (KKS) was first described in the cardiovascular system. The main function of Tissue Kallikrein (KLK1) is the proteolytic cleavage of kininogen to release kinins: Bradykinin and Kallidin, which exert their effect through their receptors B1R and B2R. KLK1 was also described as an important activator of latent TGFβ1, a potent inhibitor of lactotroph cell proliferation and prolactin (PRL) secretion. We have previously found that the pituitary expression of KLK1, B1R, B2R, as well as local TGFβ1 activity, is reduced in prolactinomas. We hypothesize that the recovery of local KKS could improve pituitary TGFβ1 activity counteracting prolactinoma development. In the present work, we study the effect of KLK1 and the kinins on TGFβ1 activation in the pituitary gland. To this end, 8-month-old female and male mice lacking the dopamine receptor type 2 (Drd2KO) and WT counterparts were used. Only KO female develops lactotroph hyperplasia and hyperprolactinemia (prolactinoma). Pituitary explants from Drd2KO and WT mice were incubated with: KLK1 (1U/ml), the B1R specific agonist (des-Arg-bradykinin, 10-8M) or vehicle for 30 minutes. ELISAs were performed to assay active TGFβ1 in pituitary homogenates and medium. We found that KLK1 and the specific B1R agonist increase the levels of active TGFβ1 in both Drd2KO and WT female pituitaries. KLK1 had no effect on pituitary TGFβ1 activation in males. Active TGFβ1 was not detectable in medium Our results demonstrate that KLK1 and kinins, acting throw B1R, activate TGFβ1 in the pituitary, suggesting that the pharmacological manipulation of the pituitary KKS activity could represent a novel treatment for prolactinomas, in particular, for those which are resistant to dopaminergic drugs.

44. (148) **THE REPRODUCTIVE STRATEGY OF THE PLAINS VIZCACHA (*LAGOSTOMUS MAXIMUS*) IS ASSOCIATED WITH SEASONAL ENVIRONMENTAL VARIATIONS**

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Seasonal breeding is a common strategy among mammals. Day length (photoperiod) is a determining factor for seasonal reproduction. Climate and food availability indicate the optimal time of year for breeding. This is essential in large animals that live several years and that are exposed to different climatic conditions. The South American plains vizcacha is a rodent with seasonal breeding (twice a year). It shows a ~154 day-length gestation with reactivation of the reproductive axis at mid-gestation enabling ovarian estradiol (E2) and progesterone (P4) synthesis up to term-pregnancy. We developed a retrospective study with 1086 adult female vizcachas captured between years 2006 and 2019 in order to study their reproduction, focusing on ovulation and pregnancy rates, and hormonal dynamics. The effect of environmental conditions was evaluated. Ovulation and pregnancy rates, and serum E2 and P4 levels (measured by ELISA), showed seasonal differences: the gestational Cycle 1 (from April to August) depicted significant higher rates and P4 levels ($p<0.05$) than the gestational Cycle 2 (from October to February) whereas the latter exhibited significant higher E2 values ($p<0.05$). However, both Cycles exhibited similar hormone profiles along the gestation period with peaks around days 64 and 110. Differences between Cycles were associated with changes in light availability. On the other hand, significant variations in hormonal values were observed among years. High E2 values were significantly associated with high temperatures and low precipitations, whereas low E2 levels were linked to low temperatures and high precipitations. Strikingly, the association of climatic factors with P4 levels was not significant. In conclusion, the reactivation of E2 and P4 synthesis at mid-gestation enables delivery at the most favorable seasonal condition for breeding. Environmental variables as photoperiod, temperature and precipitations would be associated to its reproductive strategy.

45. (176) **EFFECT OF STRESS EXPOSURE IN THE PROGRESSION OF DIABETES. PARTICIPATION OF THE MICROBIOTA**

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Type 1 diabetes (T1D) is an autoimmune disease characterized by impaired insulin secretion. Recently, there was an increase in T1D suggesting the participation of environmental factors in its development. It is well known that the microbiota can modulate the immune system and multiple studies have shown a decrease in the microbial diversity prior and after the development of the T1D, but the mechanism involved remains unknown. In addition, it has been recognized the contribution of psychosocial factors in T1D. We have previously shown that chronic stress worsens the progression of T1D and multiple studies have shown that stress can modify the microbial composition. The aim of this project is to determine participation of the microbiota and stress on the development and progression of T1D and the mechanisms involved. To induced diabetes, we treated male BALB/c mice with multiple low doses of streptozotocin (stz)

and then, the animals were subject to chronic mild stress (CMS) by a daily application of different mild stressors. Fecal samples were collected and genomic DNA was extracted. 16s Bacteriodetes and 16s Firmicultes (most abundant component of the microbiota) were measured by qPCR using specific primers. We found an increase in 16s Bacteroidetes after CMS exposure in stz treated mice (DIAB + CMS group) compared to all the other treatments (Ctrol, CMS and DIAB groups. One-way ANOVA, $p<0.05$). We didn't find any difference on 16s Firmicutes. DIAB + CMS and DIAB mice showed higher levels of glycemia compared control and CMS groups during all the treatment (Two-way ANOVA, $p<0.05$). After 3 months CMS exposure, animals were sacrifice and lamina propria cells were isolated, stained with anti CD45-PerCP/Cy5.5 and anti CD3-APC antibodies and used for flow cytometry. We found a non-significant decrease in CD45 and CD3 cells in DIAB + CMS mice (One-way ANOVA, $p=0.08$ for CD45 and $p=0.07$ for CD3). These results suggest that CMS in a T1D context, can modulate the microbiota.

46. (238) **FUNCTIONAL EVALUATION OF INCIDENTAL ADRENAL TUMORS THROUGH THE 1 MG DEXAMETHASONE SUPPRESSION TEST DETERMINING CORTISOL IN SERUM AND SALIVA**

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The subclinical Cushing´s syndrome (SCS) is found in 20% of incidental adrenal tumors (AI). The 1 mg oral dexamethasone suppression test (DST) measuring circulating cortisol (F) is a sensitive method to rule out SCS. The assessment of salivary cortisol (SAF) as a surrogate of F became a non-invasive methodological advance. The aim of this study was to investigate the utility of salivary an serum cortisol (SAF_{dex} and F_{dex}) for the detection of SCS in patients with AI. Twenty subjects with AI (7 male and 13 women; 65.0 ± 11.0 y/o) were studied. Eight patients (1 male and 7 women; 20.0-60.0 y/o) with overt non ACTH dependent Cushing Syndrome (CS) were included as the reference group of active hypercortisolism. Subjects collected 24-hour urine for urinary free cortisol (UFC) and obtained whole saliva samples at 23 h for cortisol (SAF_{23}). Subsequently, they received 1 mg oral dexamethasone. The next day at 8 h, blood (F_{dex}) and saliva (SAF_{dex}) samples were obtained. F, SAF and UFC were determined by RIA and ACTH by IRMA. Reference values from our laboratory (n= 100): UFC ≤90.0 μg / 24hs; ACTH: 10.0-50.0 pg / ml, SAF_{23}: 0.5-3.8 nM / l; SAF_{dex}: 0.5-2.0 nM; F_{dex}: 13.8-50.0 nM. Statistics were performed by Mann-Whitney and Spearman tests, $p<0.05$ was considered significant. In AI: ACTH 22.0 ± 11.0 pg /ml; UFC: 47.0 ± 20.0 μg / 24hs; SAF_{23}:1.5 ± 0.9 nM); SAF_{dex}: 1.0 ± 0.5nM and F_{dex}: 35.6 ± 10.0 nM were normal and significantly different from CS: 5.6±1.8 pg /ml; 391.0 ± 406.0 μg / 24hs; 20.0 ± 32.0 nM; 27.0 ± 24.0 nM and 674.0 ± 339.0 nM, respectively; $p<0.05$ in all cases. A positive and significant correlation was demonstrated between SAF_{dex} and F_{dex} in AI ($r = 0.830$) and CS ($r = 0.905$); $p<0.05$.

Conclusion: SCS was excluded in all AI. The dexamethasone suppression test using serum and saliva as a diagnostic fluids was a sensitive and practical method to rule out hypercortisolism in these patients.

47. (246) **A PREPUBERTAL SERTOLI CELL LINE WITH STABLE EXPRESSION OF THE FSH RECEPTOR**

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CEDIE.

Background: FSH regulates Sertoli cell function during development through the FSHR, a 692-amino acid G protein-coupled receptor. In vitro studies using Sertoli cell lines are instrumental for the study of FSH action. However, no prepubertal Sertoli cell line exists that expresses the FSHR. Our group generated the murine SMAT1 cell line, as a model for in vitro study of prepubertal Sertoli cell physiology, but these cells have also lost FSHR expression. Transient FSHR transfection of SMAT1 cells is a suboptimal solution, resulting in cellular damage and heterogeneous results from one experiment to another. The objective of this work was to generate a prepubertal Sertoli cell line with stable FSHR expression.

Methods: 1x106 SMAT1 cells were transfected with 0.2 μg of pCDNA3-FSHR expression plasmid (8.1 kb) using 1 μl lipofectamine in a 24-well plate, and selected by resistance to geneticin (G418). Surviving cells were subjected to limiting dilution in a 96-well plate to obtain a single cell per well. Clones that expanded under G418 were tested for FSHR expression by RT-PCR.

Results: The selective condition was determined as 500 μg/ml of G418, concentration required to provoke massive death of non-transfected SMAT1 cells in 2 weeks. Upon limiting dilution, 21 wells containing 1-3 cells were identified and followed for survival and cell division for 6 weeks. Eight clones managed to expand in the presence of G418, 6 of which showed cDNA amplification when tested for FSHR expression by RT-PCR. One clone presented the expected product size for the FSHR amplified fragment (95 bp). Protein expression analysis are underway to fully characterize the obtained clone.

Conclusions: By liposome transfection, we have obtained a clone of prepubertal mouse Sertoli cell line SMAT1 with stable expression of the FSHR. Following full characterization, this cell line will represent a unique tool for the study of the physiological regulation of the prepubertal Sertoli by FSH.

48. (314) **IS SUBCUTANEOUS ADIPOSE TISSUE THE MOST THERMOGENIC DEPOT? COMPARATIVE ANALYSIS BETWEEN TWO RODENT MODELS**

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It is known that cold exposure and β-adrenergic stimulation activate white Adipose Tissue (AT) browning. Also, it has been largely described that Subcutaneous AT (SCAT) from rodents has greater browning capacity. However, most studies were carried out in mice. Here, we compared the thermogenic capacity of different white AT depots in rats and mice. Male adult rats and mice were housed at Room Temperature (Rat-RT and Mice-RT) or at Cold Temperature (4°C for 7 days, Rat-CT and Mice-CT). SCAT and Abdominal AT pads (Epididymal (EAT) for mice and Retroperitoneal (RPAT) for rat) were dissected and processed for UCP-1 and PGC1α quantification (qRT-PCR). We found that both thermogenic markers were less expressed in SCAT from rats vs. mice, being even lesser upon cold exposure (2way ANOVA, interaction $p<0.01$). In addition, we compared the thermogenic response of EAT or RPAT vs. SCAT after cold stimulation. We found that UCP-1 and PGC1α expressions were lower in EAT compared to SCAT in mice ($p<0.05$), as previously described, but the opposite effect was found in RPAT vs. SCAT from rats, i.e. high expression of UCP-1 ($p<0.01$) and a tendency toward higher levels of PGC1α ($p=0.06$). Next, we studied the thermogenic capacity of *in vitro* differentiated adipocytes from the different AT depots. To this aim, Stromal Vascular Fraction cells were cultured and differentiated with pro-beige cocktail. At the end of culture, adipocytes were incubated or not with forskolin and cells were processed for UCP-1 quantification (qRT-PCR). In stimulated conditions, UCP-1 levels were lower in mice EAT ($p<0.0001$), but higher in rat RPAT ($p<0.0001$) compared to their respective SCAT depots. Overall, we found a differential thermogenic response in AT depots and adipocytes coming from rats or mice. Our results showed, for the first time that the relevance of white AT browning is specie-dependent, being larger in the SCAT from mice and in the RPAT from rats. PICT 2015-2352, PICT 2017-2038, PICT 2017-2314 (1998)

49. (327) **EVALUATION OF CALCITRIOL IN COMBINATION WITH HISTAMINE AS A THERAPEUTIC STRATEGY FOR LEYDIG CELL TUMORS IN A SYNGENEIC MOUSE MODEL**

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Leydig cell tumors (LCT) are testicular neoplasms associated with endocrine dysfunctions in boys and adults. Current therapies for benign LCT entail side effects, while malignant LCT respond poorly to chemo/radiotherapy. Hence, new treatment options are needed. Calcitriol (CAL, the active form of vitamin D) has been tested as a therapeutic approach to manage various cancers and is often assayed in combination with other drugs to potentiate its effect. Histamine (HA) receptor H1 gene has a vitamin D-response element, and we have recently found that CAL induces its expression and decreases cell proliferation in Leydig tumor cells. In turn, HA is currently used as part of certain anti-cancer regimens, and our previous reports indicate that it can reduce the steroidogenic potential of the Leydig tumor cell line MA-10 *via* H1 receptor. Objective: To evaluate the effectiveness of a combined CAL+HA treatment in a syngeneic murine model of LCT. Methods: *In vitro*: MA-10 cells were treated with CAL (10^{-8} - 10^{-11}M) or HA (10^{-5} - 10^{-9} M). StAR protein expression, cell proliferation and steroid levels were evaluated by Western blot, SRB assay and RIA, respectively. *In vivo*: Six-week-old male mice CB6F1 with MA-10 cell-derived tumors were treated i.p. with CAL (0.05 μg), HA (0.5 mg/kg), CAL+HA, or vehicle, thrice a week. Results: CAL reduced LCT volume *in vivo* (52% inhibition, $p<0.01$), with no variation in plasma steroids *vs* vehicle-treated mice. While HA was ineffective over the time and dosing scheme studied, CAL+HA treatment promoted LCT growth (75% increase *vs* vehicle-treated mice, $p<0.001$). Conclusions: CAL emerges as a potential option for LCT treatment. HA has no anti-tumor effect. Importantly, combined CAL+HA treatment can be counterproductive for LCT patients. Although further research is needed, the explanation for this phenomenon could be related to the interaction between CAL and the histaminergic system in multiple cell types beyond Leydig tumor cells *in vivo*.

50. (357) **EFFECTS OF TWO *HOPS* EXTRACTS ON THE UTERUS OF OVARIECTOMIZED RATS**

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A botanical dietary supplement from Humulus lupulus (*hops*) contains mainly xantohumol (XN) but also 8-prenylnaringenin (8-PN), among others. These *hops extracts* exhibit chemopreventive, cytotoxic and anti-inflammatory properties while 8-PN is a potent phytoestrogen. Although the 8-PN concentration in *hops* is very low, XN (the most abundant compound) can be metabolized to 8-PN. Here we evaluated the potential estrogenic effects of *hops* and *knockout hops* (*KO-hops*) (reduced in XN and consequently in 8-PN) using the uterotrophic assay.
Female Wistar rats (7 weeks old) were bilaterally ovariectomized (Ovx). Fourteen days after the surgery, rats were treated for three consecutive days with 17β-estradiol (E2: 4 μg/kg bw/day) or fed with the vehicle (Ovx), *hops* or *KO-hops* at 8, 40 and 200 mg/kg bw/day. Animals were sacrificed 24 h after the last treatment. The uterus was removed, weighed and processed for histology and mRNA extraction. We determine the relative uterine weight (rUW), luminal epithelial cell height (LECH) and the mRNA expression of the estrogen receptor α (Esr1) and complement C3 (C3).
The rUW and LECH were increased by E2 respect to Ovx ($p<0.05$). The rUW was similar between *hops, KO-hops* and Ovx groups. An increase in LECH was shown in *hops 40* compared to Ovx ($p<0.05$). The mRNA expression of Esr1 and C3 was downregulated and upregulated, respectively by E2 treatment ($p<0.05$). Esr1 and C3 mRNA were changed neither by *hops* nor by *KO-hops* respect to the Ovx.
In general, no estrogenic uterine effects were observed in response to *hops* extracts, independently of their composition (*hops* and *KO-hops,* not showed estrogenic response). In conclusion, the absence of uterine estrogenic effects provides evidence for the safety of both extracts. Although this result encourage us to study the hops extracts for its chemopreventive properties, more experiments are needed to deeply characterize hops and their compounds.

51. (370) **EFFECT OF CHEMOTHERAPY ON ENDOCRINE TESTICULAR FUNCTION DURING CHILDHOOD AND PUBERTY IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA OR NON-HODGKIN LYMPHOMA: A PROSPECTIVE, LONGITUDINAL STUDY.**

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Introduction: Chemotherapy can have variable effects according to the proliferative state of cell populations. Testicular Sertoli cells show differences in proliferative status between infancy and puberty.
Aim: To determine if there is a differential effect of chemotherapy on Sertoli cell function between children and adolescents with hematologic malignancies during and after treatment. Secondarily, testosterone and gonadotropins were evaluated.
Methods: prospective cohort study in children with acute lymphoblastic leukaemia or non-Hodgkin lymphoma. Serum levels of AMH and FSH (direct and indirect marker of Sertoli cells, respectively) and of testosterone (T) and LH (direct and indirect markers of Leydig cells) were evaluated during chemotherapy and for 24 months after the end of the treatment. Results were analysed according to pubertal stage and expressed as median (range).
Results: 16 prepubertal boys, age 4.2 yr (0.7-7.1), and 5 pubertal, age 13.4 yr (11.7-14.8) were analysed. Follow-up was 4.6 ± 0.6 years. In prepubertal boys, AMH was pre-treatment (tx): 709 pmol/L (331-1333), at 3 months (m) of tx: 1191 (396-1660), 6 m: 934 (273-1325), 12 m: 577 (338-1474), 24 m (end tx): 1156 (625-1563); in the subsequent follow-up, 3 m: 1096 (532-1708), 12 m: 1212 (603-2601) and 24 m: 1011 (520-2035). 24 m after end of tx, serum AMH was higher than baseline in all patients. T, FSH and LH were always normal. In pubertal boys, AMH was pre-tx 74 pmol/L (39-121), 3 m: 76 (47-98), 6 m: 92 (64-173), 12 m: 74 (55-229), 24 m (end tx): 95 (46-118); in follow-up: 3 m: 124 (37-174), 12 m: 140 (76-157) and 24 m: 129 (32-173). T was always normal. FSH and LH increased during chemotherapy and normalised 24 m after the end of chemotherapy.
Conclusion: In prepubertal boys, no compromise of Sertoli cell function was observed during the entire follow-up. In pubertal boys, a transient elevation of gonadotropins was observed, which may reflect a reversible deleterious effect of chemotherapy.

52. (379) **SPEXIN IS THE NEW ADIPOKINE: ANALYSIS IN AN OBESE PEDIATRIC COHORT IN ARGENTINA**

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Overweight and obesity are associated with sedentary lifestyle and

unbalanced diet intake. According to the last survey about nutrition and health (2019) in Argentine, 4 out of 10 children are overweight/obese. Our aim was to evaluate, in a male pediatric cohort, plasmatic levels of Spexin (SPX), Leptin (Lep) and Adiponectin (Adipo) in order to find their relationship with obesity risk factors. The study was approved by the CIRPI of La Plata Children's Hospital. Physical, biochemical parameters and plasmatic adipokines from 70 children were evaluated. Patients were classified by their BMI z-score (BAZ), ranging from -1 to 1 for control (CTR), from 1 to 2 for overweight (OW) and higher than 2 for obese (OB). Results revealed no significant differences for glucose and lipid profile, while for hepatic TGP, GGT and PCR, OB children showed higher levels than CTR. Adipo levels were similar among groups, but SPX (CTR=0,79; OW=0,53; OB=0,38 ng/mL; P<0,0001) and Lep (CTR=1,95; OW=9,95; OB=17,25 ng/mL; P<0,0001) showed significant differences. Moreover, we found significant correlation for SPX vs. BAZ, Lep, insulin, triglycerides (TG), HDL and GGT and for Lep vs. BAZ, insulin, TG, SPX, VLDL, PCR, GGT. Finally, principal component (PC) analysis was assessed, showing that in PC1 SPX clustered with insulin, Lep and TG and correlated with BAZ (P<0,001); whereas in PC2, lipid profile and adiponectin clustered together, but did not correlate with BAZ. PC1 explained 39% of variance of the population, PC2 21% and all together reached 60%. In conclusion, we showed for the first time in an Argentinian pediatric cohort, that plasmatic SPX levels decreased as BAZ increase and SPX and Lep were able to discriminate among groups, according to BAZ range. Moreover, we demonstrated by PC analysis that SPX, Lep, Insulin and TG clustered together and explained in a higher proportion the variance of the population, reflecting the global metabolic status of patients. PICT2017-2314; PUE-2017.

53. (392) **FLNA EFFECT OVER NON-CANONICAL CYCLIN D1 EXPRESSION ON PITUITARY CELLS**

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Filamin A (FLNA) is an acting binding protein that is deregulated in pituitary tumours and plays a role in lamellipodia formation. Cyclin D1 (CD1) is increased in tumours, but is not always correlated with cell cycle progression. FLNA and CD1 localized on cellular protrusions and physically interact to regulate cell motility. We hypothesized that FLNA promotes CD1 expression with non-canonical functions in pituitary cells. The objective was to analyse the effect of FLNA over CD1 expression and function on lactotroph cells.
Transfected GH3 cells for FLNA overexpression (GH3F+) and pituitary glands from female rats treated with estradiol benzoate for 20, 40 and 60 days, as pituitary hyperplastic adenomatous model, were used. FLNA and CD1 expression was analysed by Western blot and subcellular localization by immunocytochemistry (ICC). Cell cycle progression was analysed by flow cytometry, Ki67 index by ICC, colony formation capacity by clonogenic assay, and cellular morphology was performed by optical microscopy. The statistical analysis used was ANOVA-Tukey.
GH3 FLNA overexpression induced CD1 increase with low proliferation rate detected by Ki67 index and clonogenic assay, suggesting that is not exerting canonical functions. Also, the GH3F+ morphological analysis showed a significant increase in cells with lamellipodia, doubling the pleomorphic cell number, which showed diffuse cytoplasmic CD1 distribution, suggesting an involvement of cytoplasmic CD1 in cell motility.
FLNA increased gradually until the 40 days of pituitary hyperplastic glands and declined at 60 days, similar to cyclin D1 expression pattern. Cell cycle analysis revealed a proliferation phase S/G2-M declined at 40 days, suggesting that CD1 expression does not correlate with cell cycle progression.
Our results suggest that FLNA enhanced CD1 expression, without affecting cell cycle progression, leading to non-canonical functions, possibly related to cell protrusion formation.

54. (409) **GENDER DIFFERENCES IN ERβ EXPRESSION AND PITUITARY CELL RESPONSE TO DEHP PERINATAL TREATMENT**

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Di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used phthalate, non-covalently bounded to PVC polymers, acting as endocrine disruptor by interfering with estrogen signaling. One of the most relevant targets of E2 actions is the pituitary gland, whose endocrine populations expresses receptors (ER) and is sensitive to E2 variations. The aim of this study was to analyze the effect and gender differences of perinatal DEHP exposure on pituitary ERβ expression, exploring the impact on lactotroph cell growth.
Female Wistar rats were exposed to DEHP (200mg/kg) or corn oil (controls) by gavage from day 0 of gestation, throughout pregnancy and lactation, until weaning. Male and female pups were used at 21 (prepubertal) and 75 (adult) postnatal (PND) days. Also, pituitary cultures from male and female rats were stimulated for 72h with 1, 10 or 100 nM of DEHP. The ERβ+ cells were determined by flow cytometry and the lactotroph (ki67+) were quantified by double immunostaining. The statistical analysis used was ANOVA Fisher (P<0.05).
Perinatal DEHP increased ERβ expression in prepuberal and adult male rats. However, in female rats while DEHP also increased ERβ expression in prepubertal rats, the ERβ decreased in adulthood. *In vitro* system, all tested doses of DEHP up regulated the ERβ+ cells in cultures from male, with opposite effects in female cultures, showing ERβ decrease in a dose-dependent manner. Also, DEHP reduced the lactotroph Ki67+ cells in male, with contrary effect in female rats showing that DEHP increased lactotroph Ki67+ cells.
These results showed that DEHP exposition during embryogenesis and perinatal development induces changes in the pituitary gland, with differential effect on ERβ expression in male and female rats and lactotroph cell growth. These changes in ER expression may be a mechanism underlying DEHP exposure in the pituitary gland, leading to cell growth deregulation.

55. (427) **ELUCIDATING PROP1 TRANSCRIPTIONAL COMPLEX THAT DIRECTS PITUITARY GLAND DEVELOPMENT**

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Prop1 is the first pituitary specific transcription factor that leads gland development and lineage differentiation into the hormone-expressing cell types, but little is known about the regulation of this process. Our aim is to elucidate Prop1 transcriptional complex (TC) and the genes that are regulated to guide pituitary development. We used the murine pituitary cell line GHFT-1, engineered to express biotinylated Prop1, and conducted RNA-seq and RIME experiments. Differential gene expression analysis indicated that Prop1 upregulated 240 genes and downregulated 201 genes in Prop1 cell line compared to control (fold change=|1.5|). DAVID analysis showed that the most regulated GO terms were related to extracellular matrix, cell adhesion and junctions and positive regulation of proliferation and cell migration, among others. These results are in accordance with previous reports showing that Prop1 guides pituitary development through an EMT-like process.
To study Prop1 TC we used RIME and identified 786 proteins that immunoprecipitated with Prop1. DAVID analysis showed enrichment in GO terms related to cell adherens junction, cadherin-binding involved in cell-cell adhesion, focal adhesion, RNA binding and splicing, spliceosome and chromatin binding. To further unveil Prop1 partners we used LISA which predicts transcriptional regulators using chromatin accessibility and histone mark ChIP-seq data. We used as input the genes that intersected from the ChIP and RNA-seq experiments and obtained 536 genes, DAVID analysis showed that the main GO terms were transcription regulation, in utero embryonic development, steroid hormone pathway, cell differentiation,

chromatin remodeling and stem cells commitment, among others. Of these genes 37 were present in the RIME, i.e.: Stat3, Nfib, Sin3a and Ctnnb1. Further experimental work is needed to validate Prop1 interaction partners. Understanding the TC formed will shed light on the pivotal role of Prop1 in pituitary development.

56. (467) **MOLECULAR DETECTION OF THE LEVELS OF THYROGLOBULIN MRNA IN THE FOLLOW-UP OF DIFFERENTIATED THYROID CANCER PATIENTS**

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Introduction: The postoperative follow up of patients with differentiated thyroid cancer (DTC) is based on the levels of serum thyroglobulin (Tg) measured by immunoassay and imaging studies, mainly neck ultrasound. However, antiTg antibodies interfere with this measurement and produce falsely low Tg levels that can be misleading in up to 20% of patients. The search for alternative methods to assess the presence of circulating Tg is warranted. **Objective:**Determine the sensitivity, specificity, and predictive values of mRNA of Tg (Tg- mRNA) levels measured by quantitative RT-PCR in patients followed for DTC. **Methods:**This is a prospective study of Tg-mRNA levels measured with qRT-PCR in patients followed for DTC without evidence of disease (69), and persistence of structural disease (23), as well as 28 patients without evidence of disease who had not received RAI ablation. RNA was obtained using Ribo Pure Blood and then the RT-PCR was performed. Results were analyzed using the Unity Real-Time program and expressed as fg/µg ARN. We used a ROC curve to identify the best cutoff level to distinguish between patients with structural disease persistence and without.**Results**:We recruited 120 patients who had undergone total thyroidectomy, and 92 had received RAI ablation. Patients with structural persistence showed Tg mRNA levels of [0.133 fg/µg ARN (0.07-0.33)] and those with no evidence of disease [0.10 fg/µg ARN (0.08-0.17)](P<0.06). Patients who had not received RAI ablation showed Tg mRNA levels similar to those who had [0.11 fg/µg ARN (0.05-0.27)]. Sensitivity was 69.6%, specificity was 59.4%,negative predictive value was 85.4%positive predictive value 36.4%.**Conclusions:** Tg mRNA levels were not significantly different between patients with structural persistence and those with no evidence of disease.Our experience shows that Tg-mRNA could be useful as a rule-out test in selected cases, but its low sensitivity and specificity precludes its usefulness as a first-line test.

57. (491) **SYSTEMATIC ANALYSIS OF THYROPEROXIDASE VARIATIONS IN PATIENTS WITH CONGENITAL HYPOTHYROIDISM.**

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The congenital hypothyroidism (CH) is the most common endocrine disease characterized by low levels of circulating thyroid hormones. The prevalence of CH is 1 in 2000-3000 live births. Variants in Thyroperoxidase (TPO) appear to be one of the causes of dyshormonogenesis with permanent CH. TPO is a membrane-bound glycoprotein. The TPO gene is located in chromosome 2 [2p25], comprises 17 exons, covers approximately 150 kb of genomic DNA and encodes 933 amino acids. The TPO enzyme activity depends on both proper folding and membrane insertion, and an intact catalytic site. In the present work, we present the analysis of 25 patients from 20 unrelated families with TPO variants identified in our laboratorory. We include the first case in which a variant in the TPO gene was identified worldwide, an argentinian boy presenting a homozygous duplication of a tetranucleotide GGCC in exon 8. All patients underwent clinical and biochemical evaluation. Sanger technique as well NGS technique using a custom panel targeting 8 genes associated with dishormonogenesis (TPO, IYD, SLC26A4, TG, DUOX2, DUOXA2, TSHR, SLC5A5) and bioinformatics analysis were performed. Our observation shows that variants in both TPO alleles were found in 16 families (2 as homozygote and 14 as heterozygote compound), whereas in 3 families only 1 variant was detected. In the remaing family, 1 TPO allele and 1 IYD allele showed variants. 20 different variants were identified of which 14 were novel (9 missense, 2 deletions, 1 nonsense, 1 duplication and 1 splicing). Additionally, we present an updated database from international bibliography and the Genome Aggregation Database (gnomAD). Our findings suggest monoallelic variants and oligogenic inheritance are also involved in the pathogenesis of CH. The identification and characterization of TPO variants is undoubtedly a valuable approach to study the TPO structure/function relations and for the elaboration of a clinical diagnosis and genetic counseling.

58. (512) **ASSESSMENT OF JAWBONE MICROARCHITECTURE IN BISPHOSPHONATE- TREATED SHEEP BY CONE-BEAM COMPUTED TOMOGRAPHY**

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The cone-beam computed tomography (CBCT) is a non-invasive 3D reconstruction technique that enables to assess jawbone micro-architecture. It is used to illustrate results through 3D images. In the present report, the combination of CBCT with other techniques was used to evaluate changes in composition and micro-architectural structure quantity of the ewe jawbone after oestrogen withdrawal (OVX) and/or chronic treatment with high doses of zoledronic acid (zol). Three groups of adult Corriedale ewes, 35 to 40 kg body weight were used: OVX: OVX ewes receiving saline solution; ZOL: OVX ewes treated with Zol (4 mg/month) for 28 months for high cumulative dose of ZOL in bones and SHAM: SHAM ewes receiving saline solution (control). At the end of the study, hemi-mandibles were extracted: Bone mineral density (BMD) and content (BMC) of the mandibles were evaluated ex vivo by DXA (Lunar DPX); CBCT was performed using Planmeca Promax 3D Classic. Results of CBCT: OVX as compared to SHAM ewes significantly decreased BMC and BMD (p<0.001); BV/ TV (%), Tb. Th, connectivity and anisotropy (p<0.0075; p<0.0075, p<0.001 and p<0.02, respectively) while Tb. Sp (p<0.0002) was significantly increased. Zol treatment did not show statistical differences when compared to SHAM group. Zol showed values of anisotropy significantly higher than OVX group (p<0.018) and Tb.Sp significantly lower than OVX groups (p<0.043). BV/ TV (%), Tb. Th and connectivity as compared to OVX group showed a clear tendency to be higher and almost reached significance (p=0.055; p=0.061 and p=0.054, respectively. Maxillary BMC and BMD were lowest in OVX ewes (p<0.05) and were significantly higher in ZOL than in SHAM ewes (p<0.05). Conclusion: The CBCT technique was useful to evaluate the deterioration of the bone quali-

ty by estrogen withdrawal and the recovery by ZOL treatment.

59. (548) **COVID 19 INFECTION AND SERUM TRIIODOTHYRONINE 548**

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Experimental and clinical evidence reveals an action of thyroid hormone to protect and adapt injured tissue. In acute disease, triiodothyronine (T3) is reduced with or without changes in the remaining thyroid profile and it is the Non-Thyroid Illness Syndrome (NTIS). Our main objective is describe T3 levels in patients with clinical picture compatible with COVID-19 infection. Materials and methods: 56 adults of both sexes (July-August 2020), admitted to public hospital, with a clinical/epidemiological presentation for COVID19. The demographic, clinical and confirmatory diagnosis data were recovered from the epidemiological file and the Notification Sistem. T3 was dosed by chemiluminescence in ARCHITECT-ABBOTT. The results were expressed as mean ± SD, T test, chi square and linear correlation, $p<0.05$. Results: In an adult population of 25 women and 31 men, 43 COVID19 cases (G1) were confirmed and in 13 (G2) it was ruled out. The mean time to onset of symptoms and consultation was 4 days. 59% of G1 and 69% of G2 had comorbidities. 21% of G1 (2 deceased) and 7.7% G2 required critical care, $p <0.05$. G1 referred cough, fever, respiratory distress and general discomfort and G2 added myalgia and gastrointestinal symptoms. T3 (ng/dL) in G1 and G2 were 65.8 ± 21.8 and 70.4 ± 23.1, presenting a significant difference with respect to the general adult population. 67% of G1 and 46% of G2 presented T3 below 70 ng/dL. No correlation was found between symptoms and T3. In febrile subjects, T3 (ng/dL) were 67.8 ± 23.7 in G1, 75.6 ± 14.3 in G2 and 71.6 ± 24.2 feverless. In critical patients G1, T3 was 53.0 ± 16.2, G1 mild/ moderate infection 68.8 ± 22.1, deaths less than 50.0 ng/dL, $p <0.05$. No difference between sexes was found. Conclusion: Significant decrease in T3 is observed in patients requiring hospitalization. NTIS is present in 50% of COVID19 patients. In critically ill COVID, T3 decreases sharply, possibly related to the severity and poor prognosis of the infection.

60. (553) **EXTRACELLULAR VESICLES SPREAD ADAPTIVE PRO-INFLAMMATORY RESPONSES TRIGGERED BY TRIIODOTHYRONINE (T3) ON MICE DENDRITIC CELLS (DCS)**

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T3 is the biologically more active thyroid hormone. DCs are specialized antigen presenting cells. T3 activates mice DCs (T3-DCs) inducing Th1 and Th17 proinflammatory, and cytotoxic responses, restraining Tregs. On this basis, antitumor antigen-specific T3-DCs based vaccination strategies were exploited successfully for melanoma and colon cancer in mice. Extracellular vesicles (EVs) are nano-sized membrane vesicles that play key roles in intercellular communication. We reported that EVs released by T3-DCs (T3-EVs) activated syngeneic DCs with a pro-inflammatory profile, contributing to paracrine DC communication *in vitro*. To go further, the aim of this study was to evaluate the adaptive modulatory role of T3-EVs. Bone marrow DCs obtained from C57BL/6 WT mice were stimulated (or not) with T3 (10nM) for 18h. DC-EV fractions were isolated by differential ultracentrifugation of culture supernatants (2,000g: 2K; 10,000g: 10K; and 100,000g: 100K). Allogenic splenocytes were obtained from BALB/c mice and stimulated with DC-EV fractions of T3-stimulated and Control (C) cells for 6 days. Intracellular and secreted cytokine production were analyzed by flow cytometry and ELISA. Statistical analysis: Sidak's multiple comparisons test. $P<0.05$ was considered statistically significant. Results showed that 100 K and 2K T3-EVs increased CD8 splenocyte subpopulation (vs their C). Moreover, the secretion of IFN-γ and IL-17 were augmented in splenocyte cultures after 2K T3-EVs stimulus (vs C). Similarly, 10K T3-EVs augmented IL17 secretion (vs C). We conclude that T3-EVs activated allogenic *splenocytes increasing* CD8 subpopulation *in vitro,* in an EV-fraction dependent manner. Besides, the secretion of splenocyte pro-inflammatory cytokines augmented after 2K and 10K T3-EVs stimulus. This study underscores the role of EVs in immune-endocrine crosstalk and provides initial tools for future designs of T3-DC based immunotherapies. Further research to enlighten this topic is under course.

61. (15) **NARINGIN AVOIDS THE HEPATIC MITOCHONDRIAL DYSFUNCTION CAUSED BY THE EXPERIMENTAL TYPE 1 DIABETES**

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In a previous work we have shown that naringin (NAR), a natural flavonoid, protects against the liver damage caused by STZ (streptozotocin)-induced diabetes. The aim of this study was to determine whether NAR modifies the redox state of liver mitochondria altering the Krebs cycle function, which could lead to perturbations in the mitochondrial dynamics and biogenesis. Male Wistar rats were divided into three groups: 1) controls, 2) STZ rats (diabetes induced by 60 mg/kg b.w. STZ), 3) STZ rats treated daily with NAR (40 mg/kg b.w.) for 30 days. Rat liver mitochondria were isolated from the different groups of animals. Oxidative stress parameters, activities of Krebs cycle enzymes and ATP synthase as well as mitochondrial dynamics and biogenesis markers were determined. Results were evaluated by ANOVA and Bonferroni test. STZ rats showed decreased isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH) activities affecting ATP synthesis. NAR completely blocked these alterations. STZ rats also presented hepatic mitochondrial oxidative stress, as indicated by a lower GSH level and higher superoxide anion and protein carbonyl contents and catalase activity. NAR administration avoided these changes. STZ rats decreased the gene expression of Drp-1 and Mfn-2, two molecules involved in the mitochondrial dynamics, an effect that was blocked by NAR. The gene expression of Pgc1-α and Tfam, two molecules involved in the mitochondrial biogenesis, remained unchanged in all groups. In conclusion, the experimental diabetes produces oxidative stress, decrease in the activity of enzymes from the Krebs cycle and ATP synthase and alteration in the mitochondrial dynamics. NAR protects the liver mitochondria against the dysfunction produced by STZ-induced diabetes.

FARMACOLOGÍA

62. (21) **ILEX PARAGUARIENSIS EXTRACT IMPROVES BIOCHEMICAL PARAMETERS IN OLD MICE: AN IN VIVO STUDY**

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Tea from Ilex paraguariensis (IP) is widely available and is a good source of caffeine and other bioactive compounds. The genus Ilex, are also traditional drinks, with lesser overall usage, but have attracted much recent attention and have been subjected to further study. Because the potential of IP has been suggested in the management of obesity, the aim of the present study was to evaluate the putative effect of IP extract on weight loss, biochemical parameters in old obese mice. Thirty animals were randomly assigned to three groups according to the treatment (water or IP extract 1.0 g/kg body

weight). The roasted IP extract was prepared according Pharmacopoeia, and administrated by intragastric gavage. The experiments were performed in accordance with the principles for animal experimentation. After treatment intervention (30 days), plasma concentrations of total cholesterol, high-density lipoprotein colesterol (HDL), triglyceride, and glucose were evaluated using WienerLab (Rosario, Argentina). Plasma LDL cholesterol concentration was calculated according to the formula: CHOL–(triglyceride/5 + high-density lipoprotein. Comparisons among groups of data were done using one-way ANOVA followed by the Dunnett Multiple Comparisons test. An associated probability (P value) of <5% was considered significant. The results found that old mice treated with IP extract exhibited attenuation of weight gain, and restoration of the serum levels of cholesterol, triglycerides, LDL cholesterol, and glucose. In conclusion, the data show that IP extract has potent bioactivity in vivo. There are data showing that animal models are a useful tool to evaluate the efficacy of potential compounds in the prevention and treatment of obesity.

63. (31) **EFFECTS OF CARROT FIBRE ON BIOCHEMICAL PARAMETERS IN MATURE MICE**

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Carrot is one of the important root vegetables rich in bioactive compounds like dietary fibers having significant health-promoting properties. Large quantities of carrots are annually discarded in different parts of Argentina because they do not meet market standards. Besides the economic loss to the producers, the discard poses an environmental problem. In order to decrease the environmental impact produced by carrot discards and increase the sustainability of this important primary crop. The Group of revaluation of discards (FIQ-UNL-CONICET), developed a process of extraction of by-products of discarded carrots, in particular fibers. It is known that; carrot fiber is a very suitable tissue for food supplementation. Thus, the aim of the present study was to evaluate the effects of carrot fibre on weight loss and related biochemical parameters in mature mice. Thirty-two animals were randomly assigned to four groups according to the treatment (standard food or fibre 1.0 g/kg body weight). The experiments were performed in accordance with the principles for animal experimentation. After treatment intervention (90 days), plasma concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL), triglyceride, and glucose were evaluated using Wiener-kits Diagnostics (Rosario, Argentina). Plasma LDL cholesterol concentration was calculated according to the formula: CHOL–(triglyceride/5 + high-density lipoprotein. Comparisons among groups of data were done using one-way ANOVA followed by the Dunnett Multiple Comparisons test. An associated probability (P value) of <5% was considered significant. During this first study, the consumption of the fibre resulted in an alteration of all parameters evaluated. No microscopic or macroscopic alterations were observed in the organs of interest, related to the treatments. These results suggest that orally administered fibre may provide beneficial effects on metabolism, without obvious undesirable effe

64. (109) **DRUGS USED FOR THE TREATMENT OF GASTRIC HYPERACIDITY IN AFFILIATES OF THE UNIVERSITY SOCIAL SECURITY INSTITUTE IN CORRIENTES CITY, 2019.**

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Drugs for the treatment of gastric hyperacidity are widely used, often without scientific basis, subjecting the patient to possible risks and generating an increase in healthcare costs. Objective: To characterize the dispensing of drugs used for the treatment of gastric hyperacidity in the pharmacy of the University Social Security Institute of Corrientes. Materials and methods: A quantitative, descriptive and cross-sectional study was carried out during 2019 in which outpatient dispensations of drugs used for the treatment of gastric hyperacidity were analyzed. The drugs were classified quantitatively according to the Anatomical, Therapeutic, Chemical Classification of Medicines of the World Health Organization. Dispensations that included drugs with action on the digestive system and metabolism (A), specifically the ones used for the treatment of gastric hyperacidity (A02), were selected, and the defined daily doses (DDD) were calculated. Descriptive statistics were performed. Results: Of 43,748 drugs in group A, 7,164 (16.38%) were A02. Of these, 6,404 (89%) were monodrugs and 760 (11%) were fixed-dose combinations (CDF). 55% were Women. Average age was 54.77 (SD +/- 12). The DDD of A02 as monodrugs: proton pump inhibitors (PPIs): Omeprazole (72,549), Esomeprazole (43,228), Pantoprazole (31,934), Dexlansoprazole (13,260) and Rabeprazole (1,442); H2 receptor antagonists: Ranitidine (20,190.40). Other agents against peptic ulcer and gastroesophageal reflux: Sucralfate (1,460). Of the CDFs, the A02s most dispensed were: Ranitidine (378), magnesium salts (140), Omeprazole (79), Famotidine (50) and Misoprostol (5). Conclusions: Within the A02s, PPIs represent the majority of the drugs dispensed. In recent years, there has been an increase in the prescription of drugs, making it necessary to request for them to be used only for accepted indications and for the appropriate time.

65. (110) **DRUG UTILIZATION STUDY: BENZODIAZEPINES PRESCRIBED IN A UNIVERSITY SOCIAL SECURITY INSTITUTE. CORRIENTES CITY, 2020**

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Benzodiazepines (BZD) are one of the most prescribed drugs in the world to treat anxiety and insomnia, but their use is not without risk. Objective: to characterize the prescription of BZD in a University Social Security institute. Materials and methods: a descriptive, cross-sectional study was carried out in which all BZD outpatient dispensations from January to May/2020 were analyzed. The Anatomical, Therapeutic, Chemical classification of Medicines (ATC-2020) of the World Health Organization was used. Subgroups N05CD (Derived from BZD) and N05CF (Related to BZD) were exclusively selected; and the defined daily doses (DDD) were calculated. Qualitative classification: Potential Intrinsic Therapeutic Value (Laporte-Tognoni) was used. Data was analyzed in Excel spreadsheet. The 8 mg DDD (as an anticonvulsant) was adapted to a non-antiepileptic dose of 1 mg/day. Results: 3,449 drugs with action on the central nervous system (CNS) were prescribed, of which 2,489 (72%) were BZD: 2,131 (85.6%) as mono-drugs and 358 (14, 4%) as fixed-dose combinations (CDF). Median age: 56 (SD +/- 14). Range: 3 to 94 years old, 1102 (44%) prescriptions were in patients ≥ 60 years. Prescriptions for females: 1,483 (59.6%). BZD most prescribed as mono-drugs and their respective DDDs: Clonazepam (62,071), Alprazolam (33,585), Lorazepam (4,574), Diazepam (2,550), Zolpidem (5,946), Bromazepam in CDF: 312. Conclusions: BZDs were the most frequent drugs prescribed within drugs of action on the CNS, the majority in women, with a median close to the third age, high percentage in people ≥ 60 years. Clonazepam and alprazolam were the BZD most prescribed as mono-drugs, bromazepam in irrational CDF. Its high consumption exposes this population group to serious health risks, ranging from memory disorders, traffic accidents and an increase in the probability of the appearance of Alzheimer's dementia.

66. (111) **ASSESSMENT OF THE INFLUENCE OF THE ANTIHISTAMINE AZELASTINE ON THE ONSET OF GLUCOCORTICOID-INDUCED ADVERSE EFFECTS. CONSEQUENCES ON BONE METABOLISM.**

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We have previously described *in vitro* that histamine H1 receptor ligands potentiate the anti-inflammatory effects of glucocorticoids (GCs) and established its therapeutic potential in a murine asthma model. Though, it is crucial to evaluate how this crosstalk alters the onset of GC-induced adverse effects to assess cotreatment safety. Considering that the therapeutic use of GCs is often limited by bone loss, we used the MC3T3-E1 osteoblastic cells differentiated with ascorbic acid and β-glycerophosphate as an *in-vitro* model to study the joint effect of dexamethasone (DEX) and the antihistamine azelastine (AZE) on the expression of bone biomarkers determinants of the balance between bone formation and resorption. Treatment of the cells with 0.1 nM DEX reduced osteoprotegerin (OPG) and increased receptor activator of NF-kB ligand (RANKL) expression in a 17% and 100% respectively, while pre-treatment with 10 μM of AZE reversed both effects by increasing OPG and decreasing RANKL expression in a 92% and 66% respectively ($p<0,05$). Additionally, treatment with 1 nM DEX reduced osteocalcin (OC) gene expression in 48%, while in cells pre-treated with 10 μM AZE this reduction was 16% ($p<0,05$). These findings suggest that co-treatment might represent an advantage in terms of bone impairment. We also performed the MTS metabolic assay to assess the effect of AZE on cell proliferation. Treatment with DEX inhibited cell proliferation in a concentration-dependent manner, reaching the maximal effect at 1 μM, while pretreatment of cells with 1 μM AZE potentiated DEX inhibition, evidenced by a reduction of its pEC50 in one order of magnitude (8.28 ± 0.44 to 9.38 ± 0.2, $p<0,05$). In contrast with our previous results, this suggests that cotreatment might be unsafe in terms of bone impairment. Overall, these discrepancies grant further research to elucidate the composite effect and the molecular mechanisms by which antihistamines modulate the appearance of GC-induced adverse effects.

67. (183) **MOLECULAR PHARMACOLOGY OF *CAENORHABDITIS ELEGANS* SEROTONIN-GATED CHLORIDE CHANNEL MOD-1 AS A NOVEL DRUG TARGET FOR ANTHELMINTIC THERAPY**

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Serotonin-gated ion channels (5-HT_3) belong to the family of Cys-loop receptors, which are pentameric proteins that mediate fast synaptic transmission. In mammals, 5-HT_3 are non-selective cationic channels that can be homomeric (5-HT_3A) or heteromeric. *Caenorhabditis elegans* is a model for the study of the nervous system and for antiparasitic drug discovery. As parasitic nematodes, *C. elegans* contains a homomeric 5HT-gated chloride channel, MOD-1, that modulates locomotory behavior. Due to its absence in vertebrates, MOD-1 emerges as a potential antiparasitic drug target. We deciphered its pharmacological properties and searched for novel ligands by patch clamp recordings from mammalian cells heterologously expressing MOD-1. Macroscopic currents activated by 5-HT showed that MOD-1 does not rectify, desensitizes slowly, and recovers from desensitization with a time constant of 1 s. Dose-response curves revealed an EC_{50} for 5-HT of about 1 μM, similar to that of human 5-HT_3A receptors. However, compared to their actions as partial agonists of human 5-HT_3A receptors, tryptamine showed markedly increased efficacy and 2-Me-5HT showed insignificant agonist activity at MOD-1. The typical anthelmintic drugs ivermectin (IVM), levamisole, and piperazine, which are agonists of GluCl, L-AChR and GABA receptors, respectively, did not activate MOD-1. However, IVM produced a slight and piperazine a profound inhibition of 5-HT activated MOD-1 currents. The analysis revealed that piperazine is a noncompetitive antagonist of MOD-1. To gain further insights into the molecular function of the native MOD-1, we also recorded 5HT-activated chloride channels from *C. elegans* neurons expressing MOD-1 and compared to those heterologously expressed in mammalian cells. The elucidation of the molecular pharmacology of MOD-1 contributes to our knowledge of the function and drug selectivity of Cys-loop receptors and to its potential as a novel target for anthelmintic therapy.

68. (286) **LOCAL EFFECTS OF LOW DOSES OF PTH 1-34 ON EXPERIMENTAL PERIODONTITIS**

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Periodontitis is a highly prevalent, chronic disease that induces a progressive bone resorption. Intermittent PTH administration has anabolic and anti-inflammatory effects, two properties necessary to achieve bone recovery. Periodontal disease can be experimentally induced in rats in few days, via cotton ligatures placement in the gingival sulcus around the molar teeth, that increases biofilm accumulation and disruption of the gingival epithelium, enhancing osteoclastogenesis and bone loss. We investigated whether intermittent administration of a low dose of PTH 1-34 in rats would block the alveolar bone loss observed when the ligature model of periodontitis was used. Periodontitis was induced in 16 female Wistar rats (221±15g) under light anesthesia. Ligature was replaced every 4 days. Rats were randomly divided in two groups and subcutaneously injected every 48 hs for 18 ds. with: G1 saline solution and G2 1.2ug PTH1-34. Eight rats were left unligated as healthy control. After killed hemimandibles were extracted and fixed in formalin buffer for histologic analysis of tibia subchondral bone volume (BV/TV%), alveolar bone BV/TV% and periodontal space height (PSH). Results: tibia BV/TV%: C 38.77±2.59; G1 38.29±3.9 and G2 37.75±1.45; alveolar bone BV/TV% C 50.3±3.6c; G1 35.6±4.3a and G2 42.0±1.4b; PSH (mm): C: 0.196±0.057a; G1 0.809±0.115b and G2: 0.706±0.065c. Different letters show a $p<0.05$. The results evidenced no systemic effect of PTH treatment on the tibia. Alveolar bone composed by trabecular bone showed a significant recovery. The PSH evidenced a little recovery but a greater percentage of osteoid tissue as compared to untreated rats. Conclusion: Intermittent low doses of PTH administration diminishes alveolar bone loss, but increases osteoid formation, suggesting that intermittent PTH administration attenuates periodontitis alveolar bone loss by the induction of tissue regeneration. Grant of Rio Negro National University. PI UNRN 40-A-467.

69. (339) **COMPARATIVE ANALYSIS OF THE SAFETY PROFILE OF EQUINE HYPERIMMUNE SERUMS IN ARGENTINA.**

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In Argentina, 8 hyperimmune sera (F(ab')2 fragments of purified equine immunoglobulins) are distributed free of charge for the treatment of different types of ophidian envenenation (Bivalent Botropic, Tetravalent Botropic, Crothalic and Elapidic antivenoms), and arthropod envenenation (latrodectic, loxoscelico, phoneutria and scorpionic antivenoms) with a pattern of use and particular safety that makes it difficult to compare with other medications. The reports of the envenenation surveillance program (8 years) were compared with the international VigiBase registries (45 years). The reported reactions were classified by MedDRA, severity and seriousness. Frequencies were corrected for time, and average frequencies were compared with international reports. A total of 1,250 envenenation reporting forms using antivenoms were analyzed. A total of 88 adverse reactions were described (7.04% of patients), corresponding to 75 early reactions (mostly nauseas, vomiting, and local reactions), and 13 late reactions (linked to possible cases of serum sickness). A total of 88,98% of cases were non-serious adverse reactions, Reactions were predictable (87%), preventable (94%) and required medical treatment in only 15% of cases. All of them evolved

with ad-integrum restitution. On a VigiAccess search (1975-2020), 572 notifications were found, with an average frequency per year similar to that obtained with local products (11 reports of reactions per year), and similar frequencies of reports per reaction, except for a significantly lower local frequency of serious reactions and a significantly higher percentage incidence of pruritus (67%). The analysis shows that the report of serious reactions at the local level is less than half the worldwide frequency found with similar monitoring techniques, suggesting a good safety profile.

70. (398) **POLOXAMER 188 FUNCTIONALIZED EUDRAGIT RS-NANOCARRIERS AS A NOVEL STRATEGY FOR ENHANCING LOPERAMIDE CENTRAL NERVOUS SYSTEM BIODISTRIBUTION AND REDUCING PROTEOTOXIC STRESS.**

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Brain drug delivery current strategies are of wide scientific interest, mainly for the presence of the blood-brain barrier (BBB). For that matter, we have developed a nanotechnology-based delivery system for brain targeting, after its oral administration. Loperamide was chosen since it does not cross the BBB, but exhibits anti-inflammatory activity, along with its mu-opioid-mediated analgesic effects, and non-mu-receptor associated neuroprotective properties, thus having potential advantages over morphine. Therefore, in this work, we evaluated the capacity of nanocarriers of Eudragit® RS (ERS) covered with poloxamer 188 (P188) and loaded with loperamide (NP-Lop), for crossing the BBB and reduce protein oxidative stress. **Methods:** Central biodistribution was assessed by evaluating NP-Lop supraspinal analgesic effects in rats naive submitted to the Hot Plate Test, after its oral and intraperitoneal administration. Protein oxidative stress was measure in prefrontal cortex (PFC) of rats submitted to traumatic brain injury (TBI), by advance oxidative protein products (AOPP) colorimetric assay, after NP-Lop intravenous administration. Confidence interval was set at 95%; statistical analysis was performed by t-student comparison or ANOVA and Tukey test. **Results:** NP-Lop increased maximum possible effect in the Hot Plate test at 30 min, 2 and 24 h after both, ip and vo NP-Lop administration, in about 20 and 60-fold regarding loperamide in solution (Lop/Sol). In addition, NP-Lop reduce AOPP in about 6.7-fold regarding TBI. **Conclusion:** NP-Lop enhance loperamide CNS biodistribution, and reduces AOPP in a TBI rat model. Finally, the nanocarriers synthesized are potentially a nanopharmaceutical form that may enhance gastrointestinal absorption of drugs like loperamide.

71. (425) **Risk of QT Prolongation related to Drug used in COVID-19: Use of a database mining strategy to detect risk and predict adverse reactions.**

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Introduction: The search for effective drugs in COVID led to an insufficient assessment of adverse reactions. Hydroxychloroquine was has a known risk of arrhytmia and was used without proven efficacy. This situation may have been partially repeated for other drugs. We analyzed the FAERS database in search of signals of new associations. Methods: FAERS reports (2004 to 2020) were analyzed. The Medical Dictionary for Regulatory Activities (MedDRA) was used to identify TdP/QTP cases. We calculated the Reporting Odds Ratios (RORs), Proportional Reporting Ratio (PPR), Yule's Q, and Chi Square with Yate's correction for the association between each Drug used in CoViD treatment, moxifloxacine (positive control) and ceftriaxone (negative control) using MedDRA Standarized Medical Querry for QT Prolongation. Signals were defined as lower limit of the 95%CI greater than 1.0 (for ROR or PPR), greater than 0 (Yule's Q) or a P value less than 0,05 (Chi Square). Results: 17.734.379 reports (including 48.364 arrhytmias) were analyzed. No significant signals were found for Dexamethasone, Remdesivir, ritonavir / lopinavir, and Ceftriaxone. A significant signal was found for prolonged qt prolongation and arrhythmias for: hydroxychlorocine 1.23 (1.10 to 1.56); ivermectin 1.63 (1.09 to 4.51); tocilizumab 1.07 (1.02 to 0.28); ticarcilina clavulanico 3.57 (2.60 to 4.91); piperacillin + tazobactam 2.32 (2.17 to 2.48); ampicillin + sulbactam 1.91 (1.68 to 2.17); clarithromycin 1.46 (1.37 to 1.55); azithromycin 1.54 (1.44 to 1.64); and moxifloxacin 1.84 (1.76 to 1.93). Discussion: There is not only a risk of arrhythmias with drugs such as hydroxychloroquine, but also with antibiotics used in the management of COVID (ampicillin + sulbactam, clarithromycin), and other drugs under study for potential efficacy in COVID (azithromycin, toxilizumab, ivermectin). This information must be taken into account to monitor the ECG and prevent pharmacodynamic interactions that enhance the effect.

72. (492) **PHARMACOLOGICAL INHIBITORS OF THE MOLECULAR CHAPERONE HSP90**

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Hsp90 is a molecular chaperone that stabilizes in an ATP-dependent manner the active conformation of many proteins with stable tertiary structure. Several substrate proteins of this chaperone are related to tumor development and progression, hence making Hsp90 an attractive target for antitumor therapy. The inhibition of Hsp90 activity shows strong anticancer effects, and Hsp90 inhibitors seem to be the only chemotherapeutic agents capable to affect all cancer hallmarks. Unfortunately, one of the most efficient inhibitors, Geldanamycin (GA), cannot be used in clinical trials because of its harmful side-effects.
In the present work we evaluated the capability of synthetized compounds designed by molecular modelling to inhibit the ATPase activity of Hsp90, and their effects on the biological actions mediated by this chaperone, such as PC3 cell viability and migration, as well as GR transport to the nucleus after hormonal stimulation in HEK293T-transfected cells. In all the cases, GA was used as an inhibitory control. Nine compounds named S3, S8, S31, S42, A15, C3, C6, N15 and P1 were tested. All of them confirmed the *in silico* predictions regarding their ability to inhibit the intrinsic ATPase activity of Hsp90. The S-series of dihydroxybenzaldheyde-derived Schiff bases and C-series of pyrazoline-derived drugs (especially C3 and C6) showed a decreased action on cell viability comparable to that shown by GA, but only S3, S8, S31 and S42 decreased cell migration comparable to the positive control. None of the synthetics drugs affected the GR nuclear import.
In summary, in this study we described various synthetic candidates with high pharmacological potential. Importantly, it is also shown that Hsp90 ATPase activity is not an essential requirement for cell viability and GR nuclear import, which opposites the prevailing dogma.

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73. (154) **EFFECTS OF OXIDIZED LOW-DENSITY LIPOPROTEIN (OXLDL) ON PROSTATIC STROMAL CELLS DERIVED FROM PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA**

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Benign Prostatic Hyperplasia (BPH) affects elderly men, being the result of an excessive and uncontrolled cellular proliferative process of both epithelial and stromal prostatic compartments. Novel evidence suggests that dyslipidemias and other factors of the Metabolic Syndrome are associated to BPH progression and aggressiveness. However, little information is available about the pathogenic mechanisms promoting prostatic growth in atherogenic contexts. We therefore aimed to analyze *in* vitro the effects of OxLDL on primary cell cultures. Prostatic stromal cells, surgically harvested from patients with BPH (n=3), were isolated, cultured, and stimulated with OxLDL (20 μM or 100 μM, simulating an atherogenic state) or vehicle for 24h and 48h.
OxLDL induced cell proliferation, as assessed by BrdU incorporation and Ki67 immunocytochemistry, mainly at lower concentrations and in a time-dependent manner ($p<0.001$ vs. vehicle). OxLDL-treated cells also displayed a myofibroblastic phenotype with high metabolic activity, characterized by an increase of cytoplasmic granules, dilated endoplasmic reticulum, and the presence of prominent nucleoli, as evaluated by transmission electron microscopy (TEM). Furthermore, the release and characterization of Extracellular Vesicles (EVs) were determined in supernatants, which were isolated by ultracentrifugation steps and observed by TEM using negative staining. BPH-derived stromal cells showed a very low frequency of secreted EVs, with OxLDL inducing a 10-fold increase, especially in a fraction of 15-20nm ($p<0.001$). At ultrastructural level, these vesicles exhibited an artificial concave shape appearance, compatible with exosomes. Taken together, these findings indicate that OxLDL promotes cell proliferation, stimulation, and EVs release in BPH stromal cells, pointing OxLDL as a strong pathogenic factor in atherogenic contexts supporting uncontrolled prostatic growth.

74. (285) **CHARACTERIZATION OF OUTER MEMBRANE VESICLES FROM MULTIDRUG RESISTANT BACTERIA**
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The outer membrane vesicles (OMV) are vesicles that transport, harbor and are able to deliver its content. Among the content that could be found in OMVs, there are genes associated with antimicrobial resistance. In this study we proposed to characterize the OMVs from two extensively resistant clones with epidemic behavior circulating in Argentina, *E. coli* ST131 (SM5), and *P. aeuriginosa* ST2867 (PAE981). In addition, we looked for the presence of genes associated with antimicrobial resistance in these vesicles. To characterize OMVs, we used an isolation method that was based on the International Society for Extracellular Vesicles guidelines. This procedure recommends: (i) The source of OMVs must be quantitatively defined, for that reason we adjusted the initial culture of bacteria to OD_{600} ~ 0.05, (ii) Total quantification of OMVs, in this case we quantified the proteins with the Micro BCA Protein Assay Kit, (iii) A technique that provides images of individual electric vesicles at high resolution, for this we used transmission electron microscopy (TEM), and (iv) It is needed to have evidence of individual particle analysis technique that estimate biophysical characteristics of EVs, for this we used nanoparticle tracking analysis (NTA). Finally, by PCR assay we looked for genes associated with antimicrobial resistance and confirm the result by PCR and sequencing. NTA results showed a typical distribution of peaks for OMVs with a major peak between 100–300 nm in diameter. These results were confirmed through TEM images, where they showed a heterogeneous distribution and particles of similar size to those found in NTA. PCR and sequencing assays allowed us to detect in the OMVs from *E. coli* ST131 SM5 the $bla_{CTX\text{-}M\text{-}15}$ gene, and from *P. aeruginosa* ST2867 the $bla_{VIM\text{-}2}$ gene. The present study shows an efficient isolation method in different bacterial genera that allows the physical characterization and detection of genes associated with antimicrobial resistance by PCR in OMV.

75. (517) **GLUCOCORTICOID-RECEPTOR MOLECULAR MECHANISMS CONTROLLING PANCREAS DEVELOPMENT AND ENDOCRINE CELL SPECIFICATION**
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The *in vitro* production of functional β cells for transplantation in type 1 diabetic patients is a long-standing goal to achieve. Current protocols derive β cells from human pluripotent stem cells by mimicking cell signaling events that occur during fetal development and manipulate signaling pathways known to perform key and stage-specific functions during pancreas growth and differentiation. Thus, gaining insights into the mechanisms by which novel pathways control this process might significantly impact this field of research. Previous studies have shown that the glucocorticoid receptor (GR) signaling pathway plays an important role in the generation of an appropriate number of β cells in adults, although the underlying molecular mechanisms still remain largely unexplored. We have previously reported the construction of genomic *cis* regulatory maps in human pancreatic islets and in the human embryonic pancreas based on ChIP-seq and RNA-seq experiments. Our analyses led to the discovery that TEAD and YAP are important gene regulators in the embryonic pancreas, in charge of maintaining the pancreatic multipotent progenitor cell phenotype. Further analysis shows that a subset of the genes that are highly expressed in multipotent progenitor cells (MPCs) could be downregulated upon activation of the GR pathway. We used mouse pancreatic explants to assess the transcriptomic effects of GC treatment during early development, finding that endocrine fate-related genes were downregulated. We also profiled by scRNA-seq the transcriptional changes induced by GC treatment of MPCs derived in vitro from hiPS cells. These data suggest new players modulating the MPC cell response to GC in humans. Validation of said results was performed in the murine model. Preliminary results suggest that, despite several similarities between mouse and human pancreatic developmental programs have been reported, there may be substantial differences in the transcriptional response to the GC effects.

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76. (128) **MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) INTERNALIZATION IS DIFFERENTIALLY MODULATED BY PI3K AND MEK-ERK PROTEINS IN ACUTE TUMOR NECROSIS FACTOR ALPHA (TNF)-INDUCED CHOLESTASIS**
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Background: TNF induces internalization of Mrp2. At least, two pathways are involved: one includes NADPH oxidase, reactive oxygen species and MEK-ERK kinases (*Biochem Pharmacol 164:311, 2019*) and the other, PI3K-Akt. The aim of this study is to discern whether these pathways are involved in the initial endocytic internalization of Mrp2 or in the blockage of its subsequent reinsertion toward the canalicular membrane, using the isolated perfused rat liver model (IPRL).

Method: In IPRL, TNF was administered by an intraportal injection (120 pg/100 g b.w.). The roles of PI3K and ERK were evaluated adding their inhibitors wortmannin (W, 200 nM) or PD980589 (PD, 4 μM) to the reservoir 10 min before TNF. Bile was collected over 30 min and bile flow was measured gravimetrically. To evaluate Mrp2 transport activity, 1-chloro-2,4-dinitrobenzene (0.5 mmol/L) was added to the perfusate. Then dinitrophenyl glutathione (DNPG) biliary secretion was determined spectrophotometrically. Finally, Mrp2 localization was analysed by immunofluorescence and confocal microscopy.
Results: (% of control±SE; n=3). Bile flow was decreased by TNF (to about 80% of control values within 20 min) and it did not recover throughout the perfusion period. This impairment of bile flow was restored by inhibitors: PD tended to protect from the beginning whereas W protected from 15 min onwards, suggesting different protection mechanisms. TNF-induced decrease of cumulative biliary DNPG excretion (71±6[a]) was countered by W (100±3[b]) and PD (109±2[b]). Both inhibitors prevented Mrp2 delocalization induced by TNF in confocal images. [a]$p < 0.05$ vs. Control; [b]$p < 0.05$ vs. TNF.
Conclusion: Mrp2 internalization induced by TNF was prevented by W and PD. As W does not protect from the initial bile flow decay induced by TNF, PI3K is probably not implied in the initial transporter desinsertion but in preventing spontaneous reinsertion of the canalicular carrier, previously endocytosed via MEK-ERK.

77. (139) **OXIDATIVE STRESS IS INVOLVED IN THE IMPAIRMENT OF MRP2 ACTIVITY INDUCED BY ESTRADIOL 17ß-D-GLUCURONIDE IN RAT HEPATOCYTES.**

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Estradiol 17β-d-glucuronide (E17G) is an endogenous metabolite of estradiol which mediates the intrahepatic cholestasis of pregnancy. E17G impairs canalicular secretion via several kinase-mediated signaling pathways which leads to endocytosis and intracellular retention of canalicular transporters, contributing the MEK-ERK1/2 pathway to the second process. Oxidative stress has also been shown to trigger these effects on canalicular transporters. We studied the possible role of oxidative stress in E17G-induced impairment of Mrp2 function by assessing the canalicular vacuolar accumulation (cVA) of glutathione-S-methylfluorescein (GS-MF) in rat hepatocyte couplets (RHC). The possible E17G-induced increase in intracellular reactive oxygen species (ROS) was assessed fluorometrically in primary cultured rat hepatocytes (PCH) by the 2?,7?-dichlorofluorescin-diacetate (DCFH-DA) assay. A probable role of ROS in E17G-induced Mrp2 function impairment was evaluated by preincubating RHC for 15 min with the antioxidants vitamin C (VitC), mannitol (Man), N,N'-diphenyl-p-phenylenediamine (DPPD), and also with apocynin (Apo), a specific inhibitor of the ROS-producing enzyme NADPH oxidase (NOX), prior to a 20-min exposure to E17G; a potential role of MEK-ERK1/2 pathway was assessed by its inhibition with the MEK inhibitor PD98059 (PD). E17G increased intracellular ROS by 43±9 %[p<0.05 vs control (C)] after 15 min in PCH and Apo completely prevented this effect. E17G reduced the cVA of GS-MF with respect to C by 45±2%(p<0.05). This was prevented by the antioxidants (VitC: 61±5%, Man: 71±7%, DPPD: 69±6%, p<0.05 vs E17G and C) and by Apo (72±5%, p<0.05 vs E17G and C); PD prevented E17G-induced effect on cVA of GS-MF similarly to Apo and showed no additive effects when added together, suggesting that they share the same pathway. These results propose a role of NOX-generated ROS in the E17G-induced impairment of Mrp2 activity in rat hepatocytes via the MEK-ERK1/2 pathway.

78. (142) **MEK / ERK ARE INVOLVED IN THE DISINSERSION OF THE MRP2 TRANSPORTER IN CHOLESTASIS INDUCED BY IL-BETA.**

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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. Our aim was to study which signaling proteins are involved in the cholestasis model induced by one of these cytokines, IL-1β, and to analyze the location of the Mrp2 transporter using confocal image analysis. In particular, we analyze the role of MEK / ERK proteins using the isolated rat hepatocyte couplet (IRHC) model.
Methodology: IRHCs were pre-incubated for 15 minutes with the MEK1 / 2 inhibitor, PD980589 (PD 5 μM), followed by incubation with IL-1β (10 ng / ml, 20 minutes). Then, they were exposed to chloromethylfluorescein diacetate (2.5 μM, 15 min) converted intracellularly into glutathione methylfluorescein (GMF), an Mrp2 substrate. Finally, Mrp2 activity was estimated by the percentage of IRHC that accumulated GMF in the canalicular vesicle (cVA). At the same time, activation of the MEK / ERK pathway was estimated in isolated hepatocytes treated with IL1β by evaluating ERK phosphorylation by Western Blot. To confirm whether IL-1β produces internalization of Mrp2, IRHCs treated with IL-1β were studied by immunostaining followed by confocal microscopy.
Results: (% of control ± SE): IL-1β significantly reduced Mrp2 activity (cVA: IL-1β = 51 ± 4% a). This was prevented by PD (97 ± 2% b). Treatment with IL1β caused an increase in phosphorylated levels of ERK after 20 min (IL: 210 ± 25% a). a different from control, b different from IL. $P < 0.05$. (n = 3). Confocal images confirmed that Mrp2 associated-fluorescence was internalized in IL-1β-treated IRHCs.
Conclusion: The cytokine IL-1β produces a reduction in Mrp2 function, associated with internalization of the transporter, mediated in part by the activation of the MEK1 / 2 and ERK1 / 2 proteins.

79. (163) **ROLE OF P38 MITOGEN ACTIVATED PROTEIN KINASE (MAPK) IN TAUROLITHOCHOLATE (TLC)-INDUCED IMPAIRMENT OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) ACTIVITY**

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TLC causes cholestasis inducing internalization of canalicular transporters, Mrp2 among them. In part, this effect is due to a sequential activation of sphingosine 1-phosphate receptor 2 (S1PR2), adenylyl cyclase, PKA and PI3K, which blocks the spontaneous reinsertion of Mrp2 but does not affect its initial internalization induced by TLC. Recently, we found that p38 MAPK is also implicated in the actions of TLC on Mrp2 activity independently of S1PR2. Our aim was to evaluate the role of p38 in Mrp2 internalization using the isolated perfused rat liver model (IPRL).
Methodology: In IPRL, TLC (4.5 µmol/liver) was injected in the portal vein. SB203580, p38 inhibitor (SB, 500 nM), was added to reservoir 10 min before TLC. Finally, biliary flow and dinitrophenyl-glutathione (DNPG) excretion were determined. Isolated rat hepatocytes were pretreated with S1PR2 [JTE-013 (JTE 10 μM)] and PI3K [wortmannin (W 100 nM)] inhibitors and exposed to TLC (2.5 μM). Then, cells were lysed and p38 phosphorylation was analyzed by western blot.
Results: (% of control ± SEM; n=3). In IPRL, TLC decreased bile flow to a minimum at min 10 post injection (3.5±0.5[a]) partially recovering at min 30 (19.0±3.0[a]). Similarly, DNPG excretion had a minimum at min 10 (2.1±0.3[a]) and partially recovered at min 30 (10.6±1.2[a]). SB did not prevent the initial decays in bile flow (11.0±2.0[a]) and DNPG excretion (7.2±2.2[a]) but significantly improves recovery at min 30 (bile flow: 40.6±5.4[a,b]; DNPG exc.: 25.0±5.9[a,b]). Activation of p38 induced by TLC (227±26[a]) decreased by W (129±7[b]), whereas, JTE did not affect it (299±45[a]). [a]$p<0.05$ vs control, [b]$p<0.05$ vs TLC.
Conclusion: p38 MAPK mediates TLC-induced cholestasis blocking the spontaneous retargeting of disinserted Mrp2 to the canalicular membrane. Though p38 is not downstream of S1PR2, it is

downstream of PI3K, suggesting that in TLC cholestasis, two different PI3K are involved, one in the pathway initiated in S1PR2 and the other upstream of p38.

80. (241) **TAUROURSODESOXICOLATE (TUDC) PREVENTS THE CHOLESTATIC EFFECTS OF ESTRADIOL 17ß-D-GLUCURONIDE (E17G) VIA ACTIVATION OF Ca^{2+}/ CALMODULIN PROTEIN KINASE II (CaMKII).**

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E17G, the main etiologic agent of pregnancy-induced cholestasis, triggers bile flow drop by inducing endocytosis and intracellular retention of the canalicular transporters involved in bile secretion Mrp2 and Bsep, via activation of cPKC and PI3K, respectively. We showed that TUDC, the active metabolite of ursodeoxycholic acid (the most effective drug used to treat this condition), counteracts all these pathomechanisms. However, the signaling mediators involved in this protection remains unknown. Since TUDC increases cytosolic Ca^{2+} and, by doing so, promotes vesicular exocytosis under cholestatic conditions, we tested in rat hepatocyte couplets (RHC) whether Ca^{2+} and its downstream effector CaMKII mediate TUDC protective effects. E17G (200 μM, 20 min) decreased by 56±1 % and 60±2 % ($p<0.05$) the % of RHC accumulating apically the fluorescent Bsep and Mrp2 substrates cholyl-lysilfluorecein and GSH-S-methylflourescein, respectively, as assessed by inverted fluorescence microscopy. Pretreatment with TUDC (100 μM, 15 min) fully prevented these alterations. Beneficial effects of TUDC were fully blocked by the Ca^{2+} sequestering agent BAPTA (20 μM) and by the CaMKII inhibitor KN62 (10 μM). E17G-induced endocytosis of Mrp2, assessed by immunostaining followed by confocal microscopy and image analysis, was significantly prevented by TUDCA, and this was fully halted by BAPTA and KN62. Western blot analysis revealed that TUDC impeded the translocation of the main cPKC isoform, PKC-α, to plasma membrane and prevented phosphorylation of Akt, two events indicative of activation of these pro-cholestatic signaling molecules, and that these activations were fully blocked by BAPTA and KN62. We conclude that TUDC-induced preservation of Bsep and Mrp2 function and location in E17G-induced cholestasis involves intracellular Ca^{2+} elevations and further CaMKII activation, and that CaMKII crosstalks with the pro-cholestatic signaling pathways cPKC and PI3K/Akt to impede their activation.

81. (446) **STIMULATION OF CELL LINEAGES INVOLVED IN THE REPAIR OF THE GASTRIC MUCOSA (MG) DUE TO THE ULCEROGENIC ACTION OF ACETIC ACID (AC) IN RATS**

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Objective: To stimulate the cellular intrinsic mechanisms of MG repair, by the ulcerogenic action of AC administered in a sub-serous form in the stomach, in rats.
Materials and methods: Two groups (A and B) of 7 Wistar rats of 250 + 30 g were studied. They were anesthetized with ether, and after performing abdominal laparotomy, physiological solution was injected into the subserosal space of the anterior gastric face (union of the gastric body with the antrum). + 0.5 mg 70% AC. The laparotomy was closed. Water was administered ad libitum and they were also fed daily, Group A: for 5 days, Group B: for 21 days. After that time, after anesthesia, the stomach was removed and dissected starting from the greater curvature, quantifying by planimetry the percentage of the macroscopic zone of damage. Sections were made for inclusion in paraffin, and staining with Haematoxylin and Eosin, digested PAS and Alcian Blue at pH 2.50.
Results: Group A: the percentage of macroscopic lesioned area is 4-7 mm ± 3mm. Group B: No macroscopic lesion is observed. Histology: Group A: lymphocytic inflammatory process with reactive vascular proliferation was identified in the submucosa. In the overlying oxyntic (OX) MG, cells with peripheral displacement of the nuclei, with clear, broad cytoplasm, positive with digested PAS staining, and few parietal cells of preserved morphology were observed. No glandular atrophy or deformity of the glandular lumens were found. Group B: There is an area of submucosal thickening due to fibrosis, with an overlying area of partially atrophic mucosa, distended glands, irregularly shaped, lined by high epithelium, not OX, positive with digested PAS staining, constituting a pyloric type metaplasic process (MTP) in the gastric body.
Conclusions: It is proposed that the stimulation of cellular repair mechanisms on the main cells of the gastric body, generated metaplasic cells of the pyloric type (MTP), configuring a new cell lineage in the gastric body.

82. (501) **SPARC (SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE) REGULATES HEPATOCYTE LIPID CONTENT BY MODULATING SREBP1C EXPRESSION AND LOCALIZATION: IMPLICATIONS IN NON-ALCOHOLIC FATTY LIVER DISEASE**

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Nonalcoholic fatty liver disease (NAFLD) is a pathology with epidemic proportions. It characterizes by the accumulation of triglycerides in hepatocytes (steatosis), which could generate hepatic inflammation. Fatty acid accumulation is triggered by an excessive arrival of these from adipose tissue, or a dysregulation between *de novo* lipogenesis and lipid catabolism. SPARC is a widely expressed protein with pleiotropic role. We previously demonstrated that SPARC absence increased hepatic steatosis in a murine diet-induced obesity model. The aim this study was to evaluate the role of SPARC in hepatic lipid deposition and lipogenesis.
Primary hepatocyte cultures from $SPARC^{+/+}$ and $SPARC^{-/-}$ mice or SPARC knockdown HepG2 cells were used to study SPARC effect on lipid droplets and expression of lipogenic genes in free fatty acids (FFA) presence. Hepatocyte survival was assessed by AO/EB staining and MTT. *De novo* triglyceride synthesis was evaluated in hepatocytes. Immunofluorescence for SREBP1 in hepatocyte was performed. In hepatic sections of $SPARC^{+/+}$ and $SPARC^{-/-}$ mice SREBP1 protein localization was evaluated by immunohistochemistry.
$SPARC^{-/-}$ hepatocytes and SPARC knockdown HepG2 cells accumulated higher amounts of lipids in FFA presence. It was demonstrated that the absence of SPARC stimulates *de novo* triglyceride synthesis in hepatocytes. In turn, there is an increase in the expression of genes related to lipid metabolism. In primary hepatocyte cultures it was observed that genes involved in lipid metabolism, transport and lipogenesis were overexpressed in SPARC absence. Srebp1c expression, a key transcription factor in lipogenesis, was increased. Immunofluorescence for SREBP1c showed that, in SPARC absence, this transcription factor is located at the nucleus, while in $SPARC^{+/+}$ hepatocytes it has a cytoplasmic and perinuclear localization.
Our results suggest a key role of SPARC in hepatic lipid deposition and metabolism that could modulate hepatic steatosis development.

83. (182) **PEDIATRIC AND ADULT METABOLIC-ASSOCIATED FATTY LIVER DISEASE (MAFLD): HEPATIC LYMPHOCYTES INVOLVED IN PATHOGENESIS**

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Lipid accumulation, cellular damage and inflammation are involved in MAFLD pathogenesis. Liver inflammation is critical in disease progression, but the cellular infiltrate composition and the role of each lymphocyte population are still up for debate.
Our aim was to characterize the inflammatory infiltrate present in the liver microenvironment both in children and adult MAFLD patients and to evaluate it according to damage severity.
Twenty-six MAFLD pediatric patients [median age: 11.5 years (range 4-17)] and 35 adult patients [median age: 49 years (range 28-72)] were enrolled. Histological parameters as well as localization and frequency of Cytotoxic T Lymphocytes (CD8+), T helper Lymphocytes (CD4+), Regulatory T lymphocytes (Treg, Foxp3+) and Th17 (IL-17A+) were evaluated on formalin-fixed paraffin-embedded liver biopsies by staining and immunohistochemistry, respectively.
In portal/periportal (P/P) tracts, there was a similar proportion of CD8+ and CD4+ lymphocytes, while CD8+ lymphocytes predominated in the intralobular area. IL-17A+ lymphocytes seemed to be nearly exclusive of P/P area. Age-groups comparison demonstrated higher P/P Foxp3+ (p=0.006, M-W test) and intralobular CD8+, CD4+ and Foxp3+ lymphocyte counts (p=0.025, p=0.0004 and p=0.013, respectively, M-W test), but lower P/P IL-17A+/Foxp3+ cell ratio (p=0.041 M-W test) in adults. Severe inflammation was associated with higher intralobular Foxp3+ lymphocytes (p=0.026 M-W test) in children, and lower P/P Foxp3+ and higher IL-17A+ lymphocytes in adults. All cases with fibrosis ≥2 displayed P/P low Foxp3+ and high IL-17A+ lymphocyte counts. Pediatric cases with worse steatosis showed high P/P CD4+ (p=0.023, t test) and intralobular CD8+ (p=0.027M-W test) and CD4+ cells (p=0.012M-W test).
In MAFLD cases, the lymphocyte liver infiltrate composition differs between children and adults. Treg and Th17 balance seems to condition damage progression, denoting their important role in the pathogenesis.

GENÉTICA

84. (95) **BIOINFORMATIC ANALYSES OF KLF1 VARIANTS DETECTED IN ARGENTINEAN POPULATION WITH HEMOGLOBINOPATHIES**

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KLF1 is an erythroid essential transcription factor. Sequence variants (mainly nonsense or substitutions in its Zinc finger domains) lead to distinctive phenotypes. The lack of KLF1 can lead to an inefficient β-globin cluster switch, which can increase the HbF and HbA2 fractions. In consequence, variants mapping in this gene can alter the clinical course of β-thalassemia.
Objectives: Perform structural predictive analyses of the missense variants detected in a group of Argentinean patients and carry out analyses to predict their impact as regulatory targets.
Patients and methods: The DNA from 3 individuals with moderately increased levels of HbA2 and 20 patients with thalassemia intermedia or severe β-thalassemia carriers was obtained and KLF1 was amplified by PCR and sequenced by the Sanger method. Since KLF1 has not been crystallized, predictive models were built with RaptorX contact prediction, their potential as regulatory sites was analyzed with RegulomeDB and their impact on the splicing of the mRNA with ESEfinder.
Results: Only 3 previously described missense variants with no or minor functional consequences were detected: rs112631212, rs2072597 and rs2072596. The first two could affect the structure locally, disrupting α-helixes. However, none affect the Zinc Finger domains. The second variant scored 0.3145 (2a category) in RegulomeDB 2.0. The latter 2 affect exonic splicing enhancers.
Discussion: The structural analysis of the variants matches the lack of effect described. It is unlikely that they could affect the default splicing of the KLF1 mRNA, since these SNPs map far from the exon-exon junctions. rs2072597 was the most frequent variant (11/46 alleles) and it could impact its role as a regulatory target; the ZFX transcription factor motif is disrupted and ChIP assays have demonstrated that this factor interacts with this region in K562 cells. Although this effect may not inhibit KLF1 expression, it could induce changes in its expression levels.

85. (153) **COMPREHENSIVE ANALYSIS OF GENETIC VARIANTS IDENTIFIED BY WHOLE EXOME SEQUENCING IN HEARING IMPAIRED PATIENTS IN ARGENTINA**

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Hereditary hearing loss is the most common sensory disorder affecting 1:500 newborn children. It is a heterogeneous disease and more than 100 genes have been related to the pathology. This complexity led us to design a multistep diagnosis strategy with the use of Whole Exome Sequencing Technique (WES). The objective was identifying genetic variants in deaf patients and analyzed them through in-silico and in-vivo studies.
1250 patients were analyzed for frequent mutations in GJB2 and GJB6 genes by Sanger Sequencing, genotyping 25% of them according to worldwide reports. From undiagnosed patients, 29 families were selected to perform WES. After filtering and analysis, 45% of patients were genotyped, identifying 23 causative mutations (11 novel, 12 reported) classified according to ACMG/Hearing Loss-Expert Panel Standards.
Some of the novel variants were further studied in silico by structural, stability and motifs studies of the mutated proteins. In addition datasets from deafness and population databases were interrelated with protein motifs in order to predict the theoretical pathogenicity effect of the amino-acid changes. The pathogenic prediction of most of the variants was reinforced after analysis, and surprisingly in one case diminished the predictive deleterious effect.
On the other hand, knock down phenotype rescue assay in zebrafish is underway to accomplish in-vivo validation.
Preliminary results in zebrafish confirmed the pathogenicity of one novel variant in MYO6 gene which affected the hair cell function and hence, auditory system physiology.
This study shows that our algorithm is successful for deafness genetic diagnosis. Comprehensive analysis is crucial to strengthen the pathogenicity effect of variants and discard some of them. These findings highlight the importance of genetic studies followed by in silico and in vivo validation to better understand the genetic basis of hereditary hearing loss.

86. (187) **EVALUATION OF MITOCHONDRIAL DNA MASS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

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Objective
Polycystic ovary syndrome (PCOS) is characterized by insulin resistance (RI) which can influence the content of mitochondrial DNA (mtDNA). Our objective was to evaluate the content of mtDNA in women with PCOS compared with control women and the relation with metabolic parameters.
Materials and methods:
We studied fifty women with PCOS and thirty-four control women aged 17 to 45 years. The determination of the number of copies of mtDNA was carried out in peripheral blood leukocytes by Quantitative real-time PCR. The results were calculated using the comparative-cycle threshold (ΔΔCtq) method. Statistical analysis were carried out by Student's t-test, correlation and linear regressions with a significance level of 0.05 (SPSS 25).
Results:
Compared to controls, PCOS patients have higher weight, body mass index (BMI), waist circumference (WC), also higher levels of TG, LDL, total cholesterol, fasting plasma glucose and lower levels of HDL cholesterol. By linear regression we observed that mtDNA was significantly lower in the presence of PCOS (135.57 ± 81.34 vs 190.37 ± 135.19; p = 0.023). A significant negative correlation was observed between the mtDNA content and telomere length (p = 0.034). Within PCOS patients, women with insulin resistance according to a HOMA index ≥ 2.5 have a lower mtDNA content compared to PCOS women with HOMA <2.5 (169.89 ± 105.3 vs 116.45 ± 53.2; p = 0.03).
Conclusion:
A decrease in mtDNA content occurs in the presence of PCOS and RI, which can be explained by the damage to mitochondrial DNA (mtDNA), proteins and lipids due to the oxidative stress associated with PCOS. More studies are required to determine the scope of the results obtained.

87. (231) **MYD88 AND CXCR4 MUTATIONS IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA AND IGM-MGUS**

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Waldenström Macroglobulinemia (WM) is a lymphoplasmacytic lymphoma with involvement of the bone marrow (BM) and the presence of a monoclonal IgM gammopathy. It is usually preceded by an IgM monoclonal gammopathy of undetermined significance (MGUS). WM and IgM-MGUS are associated to *MYD88* gene mutations, particularly $MYD88^{L265}$, and *CXCR4* gene mutations, being $CXCR4^{S338X}$ the most common variant. These mutations are of importance in diagnosis, treatment selection and response evaluation. We have analyzed *MYD88* and *CXCR4* mutations in patients with WM and IgM-MGUS in order to established their frequency and distribution in our cohort. BM or peripheral blood genomic DNA was used; ASO-PCR and bidirectional Sanger sequencing were performed. The study was approved by the local Ethic Committee; all individuals provided their informed consent. Thirty-one patients with WM: 22 at diagnosis, 4 in relapse, 5 during post treatment control (13 males; mean age 67.5 years) and 12 with IgM-MGUS (5 males; mean age 76.9 years) were evaluated. The activating mutation $MYD88^{L265P}$ was found in 81.8% WM patients at diagnosis, 100% at relapse, 0% in post treatment control and in 41.6% IgM-MGUS. *CXCR4* mutations were found in 2/22 (9%) cases with WM: one patient showed $CXCR4^{S338}$ C>G transversion at nucleotide 1013 and the other $CXCR4^{R334X}$ C>T, c.1000C<T variant, both resulting in the generation of a stop codon. Analysis of data showed the following distribution: $MYD88^{MUT}/CXCR4^{WT}$ (85.7% cases), $MYD88^{MUT}/CXCR4^{MUT}$ (9.5%) and $MYD88^{WT}/CXCR4^{WT}$ (4.8%). No *CXCR4* mutations in the IgM-MGUS patients were found. Our cohort showed *MYD88* positivity within reported values, instead we found a low frequency of *CXCR4* mutations. Although ASO-PCR is highly sensitive, it is advisable to analyze BM samples in relapse evaluation. To our knowledge, this is the first analysis of both mutations in patients with WM and IgM-MGUS from our country, being of significance in the way of a personalized medicine.

88. (234) **IMPACT OF HYPERGLYCAEMIA AND THE THERAPEUTIC ACTION OF METFORMIN ON THE LENGTH OF THE TELOMERES.**

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Objectives: To analyze the absolute telomere length (LTa) in individuals with altered fasting glucose levels compared to individuals with normal fasting glucose levels. Also, we performed a prospective controlled study to evaluate the variation in LTa in patients with decompensated type 2 Diabetes (T2D) before and after a treatment metabolic compensation.
Materials and Methods: The study included 246 individuals of both genders, which were divided according to normal fasting glucose levels <110 mg/dl (NFG group) and alterated fasting glucose levels ≥110 mg/dl (AFG group). In addition, they were divided by age groups into: under 25 years (<25Y), between 25 and 50 years (25-50Y) and over 50 years (>50Y). A subgroup of 30 patients with newly diagnosed T2D was studied, at the beginning (T0), and at 6 months (T6) of a pharmacological treatment and hygienic-dietary measures. Biochemical and clinical variables were analyzed for all the individuals. The LTa were determined by qPCR. Statistical analyzes were performed with GraphPad Prism and SPSS.
Results: LTa significantly correlated with age (r=-0.21, p=0.009) and the increase in blood glucose ranges (r=-0.32, p<0.001). The NFG group showed a significantly higher LTa than the AFG group (p<0.001), also when comparing the same age group: <25Y (p=0.013); 25-50Y (p=0.002) and> 50Y (p=0.002).
The T2D subgroup showed a negative association between the variation in LTa and age (r=-0.12, p=0.02) after 6 months of treatment. The most relevant result was the positive and significant association found between the variation of LTa and the treatment of dose of Metformin (r=0.003, p=0.007).
Conclusion: Glycemic control could prevent accelerated telomere shortening and reduce the risk of developing age-related diseases. The increase in LTa after treatment in T2D was associated with younger individuals and the use of higher doses of Metformin. LTa can be an effective marker for early intervention in hyperglycemia.

89. (274) **GENETIC VARIANTS OF CYP2E1 AND ITS RELATIONSHIP WITH PORPHYRIA CUTANEA TARDA DEVELOPMENT**

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Porphyria Cutanea Tarda (PCT) is due to a partial deficiency in uroporphyrinogen decarboxylase (URO-D); there are two main types: hereditary (H-PCT) or acquired (A-PCT). The cytochrome variants P-450, CYP1A1 and CYP1A2 alter their drug metabolizing capacity generating metabolites that can inhibit URO-D, increasing susceptibility to trigger Porphyria. The product of the CYP2E1 variant metabolizes ethanol, known as a porphyrinogenic agent. The objective was to investigate the role of CYP2E1*5B (NG_008383.1:g.3979C>T; rs2031920) and CYP2E1*7B (NG_008383.1:g.4963G>T;

rs6413420) variants in PCT development. H-PCT (30), A-PCT (31) and control (33) groups were genotyped by RFLP-PCR and sequenced when the band pattern was unclear. When we analized CYP2E1*5B, the frequencies of the reference homozygote were similar to those of the heterozygote, the alternative homozygote were not present and C allele was the most common. There was no significant risk association between this variant and PCT. Studying CYP2E1*7B, the reference homozygotes genotypes were more frequent than heterozygotes and both have higher frequencies than alternative homozygotes; the frequency of G/T was significantly higher in H-PCT individuals compared to A-PCT (p=0.045), being the reference allele the most frequent. Comparing H-PCT vs A-PCT, G/T vs G/G gave a significant risk association (OR=4.11; 1.01<CI<17.2; p=0.044), being T allele for these same groups of not significant risk. The study of risk haplotypes for CYP2E1*5B/*7B in both types of PCT vs control gave T-T (non-significant differences). Since both variants are associated with an increase in transcriptional activity of CYP2E1 gene, it is suggested that they could be a risk factor to trigger PCT. These studies are valuable for personalized medical advice in order to prevent carriers from being exposed to porphyrinogenic agents.

90. (305) **DESIGN OF A CRISPR CAS9-BASED METHOD TO ASSESS THE FUNCTIONAL PATHOGENICITY OF LDL RECEPTOR GENE VARIANTS IN FAMILIAL HYPERCHOLESTEROLEMIA**

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Introduction: More than 2200 *LDLR* variants have been described in clinical Familial Hypercholesterolemia. Since reporting variants without full knowledge of their pathogenicity represents a risk for patients and their family group, establishment of functional studies for them is of utmost importance for Familial Hypercholesterolemia diagnosis. Somatic genome editing, using CRISPR-Cas9 technology, has a tremendous potential for human gene therapy of lipid disorders.

Objective: we aimed at designing an *in silico* strategy for generating a knockout cell line in *LDLR* gene, using a CRISPR-Cas9 system, to set the basis for a future knock-in stage for variants whose functional pathogenicity must be demonstrated.

Material and Methods: Guide crRNAs for *LDLR* gene were designed using the Chop-Chop and CRISPOR platforms. We investigated the structure of *LDLR* gene and protein by analysing their functional domains using bioinformatics tools like ELM and SMART. The efficiency scores were calculated by Doench JG, *et al* (2016) and Moreno-Mateos M, *et al* (2015).

Results: We obtained a battery of 6 crRNAs which were ranked by their genomic position, efficiency, number and localization of off targets and frameshift frequency. Although several crRNAs were obtained, we selected those ones targeted at exons 1 and 2 of the *LDLR* gene in order to have higher performance in the knockout process. The efficiency score ranged from 46 to 66%, with a maximum of 7 off targets with 3 missmatches. The off targets did not interact or participate in the *LDLR* gene metabolic pathway.

Conclusion: Our strategy provides a battery of 6 crRNAs, targeted at exon 1 and 2 of the *LDLR* gene affecting the translation start and ligand binding domain, respectively. Given these results, we will try to prove these crRNAs in HepG-2 cell line in order to resemble the *in vivo* lipid metabolism to set the basis for functional categorization of *LDLR* gene variants associated with Familial Hypercholesterolemia.

91. (322) **MOLECULAR CHARACTERIZATION OF THYROGLOBULIN VARIANTS IDENTIFIED IN PATIENTS WITH GOITROUS HYPOTHYROIDISM. ANALYSIS OF THE SPLICING MECHANISM.**

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Thyroglobulin (TG) is a homodimeric glycoprotein synthesized by the thyroid gland. To date, two hundred twenty-seven variations of the TG gene have 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. Splicing mutations represent a major cause of human disease, between 15–50% of all human disease. Variants at the level of the splice site lminate in important defects at the level of the pre-mRNA splicing process. The splicing process is quite complex whose molecular bases and interactions with underlying elements are still not entirely clear, resulting as we show in the present work, a rare phenotype involving mechanisms of such processing of those pre-RNAS from a variant founded for our group in a hypothyroid patient.Thepurpose of the present studywas to identifyand characterizenew variants in the TG gene. We report an Argentine patient with congenital hypothyroidism, enlarged thyroid gland and low levels of serum TG. Sequencing of DNA, expression of chimeric minigenes as well as bioinformatics analysis were performed.

DNA sequencing identified the presence of compound heterozygous variant in the TG gene: the maternal mutation consists of a c.3001+5G>A, whereas the paternal mutation consists of p.R296*. Minigen analysis of the variant c.3001+5G>A performed in HeLa, CV1 and Hek93T cell lines, shows a total miss of transcript expression. So, in order to validate that the lack of expressionwas caused by such variation, site-directed mutagenesis was performed on the mutated clone,who had a pSPL3 vector change, to give rise to a wild-type clone c.3001+5G and to indorsing that the mutation c.3001+5G>A is the cause of the total lack of expression. These results open up new perspectives in the knowledge of the mechanism of splicingforthe TG pre-mRNA.

92. (385) **A RARE POU1F1 SPLICING VARIANT AS A CAUSE FOR ANTERIOR HYPOPITUITARISM**

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POU1F1 is a signature pituitary transcription factor that directly regulates the transcription of growth hormone (*Gh)*, prolactin (*Prl)*, and both the alpha (*Cga*) and beta subunits of thyroid stimulating hormone (*Tshb*). Multiple missense mutations in *POU1F1* have been reported to cause combined pituitary hormone deficiency and/or isolated growth hormone deficiency (IGHD). Alternative splicing in this gene results in two isoforms: the predominant transcriptional activator alpha and the minor isoform beta that acts as a transcriptional re-

pressor. The POU1F1 beta isoform transcript is created by utilization of an upstream splice acceptor sequence in exon 2 which results in a protein with insertion of 26 amino acids that encode an ETS1 binding domain inserted in the transactivation domain. All the reported mutations are in domains shared by the alpha and beta isoforms of POU1F1 and were functionally tested using the alpha isoform only. We performed whole exome sequencing (WES) in a familial case with IGHD and found a heterozygous and synonymous variant (c.150T>G, p.Ser50Ser50) in *POU1F1* presented on the affected father and son. Interestedly, this variant affects POU1F1 splicing without changing the amino acid sequence. By a high throughput reporter assay we found that this variant shifts splicing to favor the POU1F1 beta isoform almost exclusively, while retaining its transcriptional repressor activity on the *POU1F1* enhancer. Therefore, we conclude that this mutation is causative of the patient's phenotype, highlighting the importance of a detailed analysis of sequencing results, particularly of synonymous mutations near splicing sites, which are often overlooked.

93. (405) **VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) MODEL SHOW**

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Muscular Dystrophies (MD) are a group of rare inherited diseases that cause weakness and progressive degeneration of muscle tissue. The clinical symptoms of these pathologies overlap, hindering differential diagnosis, which is of paramount importance to establish the standard of care. Among them, Dystrophinopathies are the most prevalent type of MD and are caused by mutations in the DMD gene. 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). One of the major challenges of the Next Generation Sequencing (NGS) data interpretation is the occurrence of Variants of Uncertain Significance (VUS). The present work aims to provide a thorough strategy to analyze the effect of VUS, applying different predictive software, conservation/evolutionary and protein modeling tools. A cohort of 141 patients with presumptive clinical diagnosis of dystrophinopathy and negative MLPA result was analyzed by WES. We deepened the screening to all the MD associated genes included in the Gene Table of Neuromuscular Disorders. In a subset of 6 individuals, we detected VUS in the following genes: DMD (2/6), FKRP (2/6) and POMT2 (2/6). We implemented several predictive software to analyze the effect of VUS, and UCSF ChimeraX for protein modeling. Also, in one case, we could do a segregation analysis of the variants. The implemented strategy provided new insights to predict more accurately the effect of the identified sequence variants and even reclassified them. Finally, this work provides alternative approaches for the analysis of sequence variants, especially when functional studies are not possible to be carried out, to determine the effect of VUS.

94. (406) **BEYOND CLASSIC MOLECULAR ALTERATIONS: NON-CONTIGUOUS MUTATIONS IN DMD GENE**

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Introduction: Dystrophinopathies are neuromuscular X-linked recessive diseases caused by DMD mutations. Molecular alterations in this gene are large deletions/duplications in 80% of cases and small variants in the remaining. Several authors reported the occurrence of non-contiguous rearrangements within the same DMD allele, with frequencies up to 4%. The present work aims to characterize the incidence of complex rearrangements in an Argentinian dystrophinopathy cohort and unravel the causing molecular mechanisms.
Materials and Methods: We analyzed 437 boys with clinical diagnosis of Dystrophinopathy. The following techniques were implemented: MLPA, WES, WGS, PCR-Sanger Sequencing, CGH Array and HUMARA assay. In 2 cases, breakpoints were precisely determined, so we performed a bioinformatic screening of microhomologies, interspersed repeats, secondary structures and recombinogenic motifs 50pb surrounding each breakpoint.
Results: We detected 6 patients carrying complex rearrangements in DMD: 2 deletions-duplications, 3 non-contiguous duplications and 1 large deletion plus a 20pb insertion. These accounted for 1.4% of our cohort. In a deletion-duplication case, familial segregation and bioinformatics analysis suggested that the duplication was the first mutagenic event caused by Fork Stalling and Template Switching (FoSTeS), while the deletion occurred secondly by Non-homologous end joining. Furthermore, bioinformatic screening of the deletion plus insertion propose that the deletion was due to Microhomology-mediated end joining, while the insertion arose by FoSTeS.
Conclusions: Our findings widen the understanding of the molecular events that may take place in DMD and characterize the occurrence of complex rearrangements in our dystrophinopathy cohort.

95. (407) **IDENTIFICATION OF LIKELY PATHOGENIC VARIANTS IN NOVEL CANDIDATE GENES FOR HYPOPITUITARISM IN ARGENTINIAN CHILDREN**

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Congenital hypopituitarism(CH) comprises of a spectrum of disorders that range in severity from isolated growth hormone deficiency(IGHD) to combined pituitary hormone deficiency(CPHD) when two or more pituitary hormones are deficient. The clinical spectrum varies widely and can present in isolation or with other birth defects. We conducted target panel genetic screening using single-molecule molecular inversion probes sequencing to assess the frequency of mutations in known hypopituitarism genes and new candidates. We captured genomic DNA from 170 pediatric patients with CH, either alone or with other abnormalities. We identified novel pathogenic, likely pathogenic(LP) or variants with uncertain significance in 26 cases. Interestingly, we found that the prevalence of known variants in transcription factor genes involved in pituitary development like PROP1, and POU1F1 was quite low in our cohort. A significant number of disease-causing variants in known causative genes(*LHX3*, *LHX4, GLI2, OTX2 and HESX1)* were found, and for LHX3 and

LHX4 variants, both in silico and functional in vitro testing using luciferase assays were performed. One important novelty from our study is the identification of pathogenic variants in novel genes recently discovered in the etiology of CPHD. We found two heterozygous variants in *FOXA2*(p.R228S and p.R229*) which may affect the DNA binding ability of the coding protein in patients with IGHD and CPHD, respectively, and a missense *PNPLA6* variant(p.T1115P) in a patient with CPHD, retinitis pigmentosa and neurodevelopmental delay. In this work we were able to expand our knowledge of pituitary target genes for genetic diagnosis for CH. Identifying population-specific pathogenic variants will improve the capacity of genetic data to predict eventual clinical outcomes for better diagnosis and treatment for the patients.

96. (412) **IMPLEMENTATION OF MOLECULAR DIAGNOSIS FOR PAH RELATED DISORDERS IN A PUBLIC HOSPITAL**

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Introduction: Hyperphenylalaninemia (HPA) is a biochemical phenotype mainly due to variants in PAH. Its spectrum ranges from classical phenylketonuria (PKU) to persistent benign hyperphenylalaninemia (PHPA). Genotyping has become a useful tool to either design the diet accurately or to consider other treatment options now available. Aim: to efficiently implement the molecular diagnosis for PAH related disorders in our patients. Methods: 27 patients (9 female) with clinical and biochemical diagnosis of HPA were included. According to their tolerance to phenylalanine they were classified as classic PKU (n:6), moderate (n: 8), mild (n:7) or PHPA (n:6). Six patients underwent molecular diagnosis by NGS (TSO Illumina, NextSeq500) and 21 were studied by Sanger sequencing of PAH exons and intronic flanking regions. Variants were classified according to ACMG criteria and information available in BIOPKU database. Parents and siblings were studied to assess segregation for all prioritized variants. Results: 25 different already reported variants were found. 26 patients were homozygous or compound heterozygous. In one patient, NGS found only one heterozygous variant but bioinformatic CNV-analysis predicted a PAH exon 3 deletion (confirmation pending). Most frequent variants were c.1066-11G>A (intron 10:21%), p.V388M (exon 11:7,7%), p.R261Q (exon 7:7,7 %), p.R408W (exon 12:5,8%) and p.R158Q (exon 5, 5,8%). 35% of all variants found were in exon 11 and its intronic regions, 17% in exon 12, 13% in exon 7, 7% in exons 2 and 3, 5,5% in exons 5, 6 and 10 and 2% in exon 9. No variants were found in exons 1, 4, 8 or 13. Conclusions: We were able to fully characterize our cohort confirming the allelic heterogeneity of PKU patients and the prevalent occurrence of variants in 6 exons, accounting for 83% of the variants found. This suggests that PAH molecular characterization in our media should start with these exons and be followed by the other ones only if negative or incomplete.

97. (430) **DETECTION AND CHARACTERIZATION OF *FLT3* GENE VARIANTS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA**

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FLT3 gene is altered in 30% of patients with Acute Myeloid Leukemia AML) associated with a worse prognosis. They are missense variants affecting residues D835-I836 in the tyrosin-kinase domain 2 (TKD) and internal tandem duplications (ITD) within the juxtamembrane domain.
Our aim was to detect the presence of these variants and to describe their characteristics in patients with newly diagnosed AML.
Samples from 215 patients with AML diagnosed between May-18 to Jan-20 were analyzed. DNA extraction was carried out with commercial columns from mononuclear isolated cells. Detection of ITD (spanning exons 13-14) was performed by PCR amplification, agarose gel visualization, and capillary electrophoresis to calculate the allelic ratio, while TKD (exon 20) by PCR-RFLP and agarose gel visualization. Both variants were characterized by automatic sequencing.
FLT3(+) were detected in 61 (28%) patients (43 ITD, 13 TKD and 5 ITD/TKD), presenting medians (Mdn) of age of 53 years, blasts 75%, and higher white blood cell counts 93396/μL (vs 34149/μL FLT3-wt, T test, $p<0.00001$). ITDs were single in 41 (85%) patients and double in 7 (15%) with a Mdn allelic ratio of 0.4 being ≥0.5 in 23 (48%) patients. ITD covered 15-192 bp, residues M578-G613 were duplicated in 78% patients, G583-A659 in 8% and 14% of them were accompanied by insertions of 3-17 bp.
TKD(+) identified were 16 single nucleotide variants and 2 indels: 14 c.2503G>T (p.Asp835Tyr), 1 c.2503G>C (p.Asp835His), 1 c.2505T>C (Asp835=), 1 c.2504_2508delATATCinsTT (p.Asp835_Ile836delinsVal) and 1 c.2502_2504delAGA, (p.Asp835del). These last 2 variants were novels.
The results obtained show a frequency of mutations in FLT3 of 28%, consistent with the literature, and a predominance of ITD vs TKD. The detection by PCR and capillary electrophoresis allowed highly sensitive and specific results. The characterization and range of the sequence variants found in FLT3 gene correspond to what was published in the query databases.

98. (438) **CHARACTERIZATION OF MOLECULAR BIOMARKERS ASSOCIATED WITH INFLAMMATORY BOWEL DISEASES IN THE ARGENTINE POPULATION.**

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Inflammatory bowel disease (IBD) represents a complex chronic disorder comprising two main types: Ulcerative Colitis (UC) and Crohn's Disease (CD). The objective of our study was to identify and characterize molecular biomarkers of IBD in the Argentine population. For this, the circulating expression levels within the Peripheral-Blood-Cell-Fraction (PBCF) and the intestinal expression levels contained in the Fecal-Matter-Cell-Fraction (FMCF) of three miRNAs were studied: hsa-miR-146a-5p, hsa -miR-155-5p and hsa-miR-223-3p. 57 individuals were recruited from the Posadas Hospital Gastroenterology Service, from which 51 blood samples (13 CT, 20 CU and 18 CD) and 38 stool samples (12 controls, 15 CU and 11 CD) were obtained. Total RNA was extracted and retro-transcribed using the MMLV reverse enzyme and Stem Loop Primers designed for this purpose. The quantification was carried out by qPCR by means of Absolute Quantification by Standard Curve using synthetic plasmids. The data obtained were statistically analyzed with the GraphPad Prism 6.01 program using non-parametric tests. The results ($p<0.05$) showed that in PBCF miR-155-5p is overexpressed in patients with IBD compared to controls, and that miR-223-3p differentiates controls and patients in FMCF. Breaking down each disease according to treatments, it is observed that the case-control differentiation is maintained in the mildest treatments. In CD, the ile-

ocolonic location presents higher levels of expression of miR-223-3p than the colonic one in FMCF and greater expression of miR-155-5p in active patients than in inactive patients in PBCF. In UC, miR-223-3p was shown to have a lower expression in patients with high endoscopic activity in PBCF. Our results show the biomarker potential of miR-155-5p and miR-223-3p in IBD as well as the potential of different non invasive samples used for their analysis.

99. (443) **IDENTIFICATION OF GENETIC VARIANTS IN MYELOID MALIGNANCIES BY TARGETED SEQUENCING**

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Next Generation Sequencing (NGS) technology has provided powerful tools to identify genetic variants with a high sensitivity in myeloid malignancies. However, it has not been widely adopted due to difficulties with health insurance coverage.

Our aim was to describe pathogenic variants associated with myeloid malignancies detected by targeted sequencing approaches in two institutions of our media.

Sequencing was performed in an Illumina platform using amplicon-based targeted sequencing (Illumina Ampliseq Myeloid Panel-Hospital El Cruce, Florencio Varela) and hybrid capture-based sequencing (Customized Panel, SOPHiA Genetics- Laboratorio de Especialidades Bioquímicas, Bahía Blanca), to screen 40 and 30 genes, respectively.

A total of 43 patients were evaluated (16-MDS, 7-CMML, 20-AML) and 99 sequence variants were found (37 in epigenetic regulators, 22 in transcription factors, 22 in signaling pathways, 10 in splicing factors, 6 in damage repair and 2 in cohesins). Seven MDS patients presented 10 affected genes accumulating 17 nucleotide variants with a media of 2 (1-5) mutated genes and 2 (1-7) sequence variants/patient. CMML patients presented 27 variants in 14 genes with a media of 3 (2-4) affected genes and 4 (2-5) variants/patient. The AML cohort had a media of 2 (1-5) mutated genes and 3 (1-6) variants/patient, adding 55 variants in 20 genes. TET2 was the most compromised in MDS (4/17) and CMML (11/27), while in AML it was DNMT3A (7/55). The median VAF was 0.48 in CMML, 0.44 in AML and 0.30 in MDS.

Both panels comprised the most relevant genes and allowed the identification of pathogenic variants in 84% of patients with myeloid malignancies. CMML displayed a higher media of variants with a higher VAF and a prevalence of TET2, while the number of affected genes was increased in AML. Sequence-based genetic tests provide useful information, not only at clinical level, but to improve the description of altered genes and pathways in myeloid diseases.

100. (447) **ANALYSIS OF COOPERATING PATHOGENIC GENE VARIANTS IN PATIENTS WITH MYELOFIBROSIS**

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The myelofibrosis (MF) is a myeloproliferative neoplasm derived from a clonal hematopoietic stem-cell associated with bone marrow fibrosis. The Dynamic International Prognostic Scoring System (DIPSS) enables prognosis assessment at any point during clinical disease follow-up. This model considers the age, hemoglobin level, leukocyte count, circulating blasts and constitutional symptoms to predict survival. Most of patient present one driver mutations in JAK2, CALR or MPL genes, and the majority acquire others affecting epigenetic (ASXL1, IDH1/2) and splicing (SRSF2) genes, often in multiple combinations.

In the current study, we screened hot-spot regions of ASXL1, IDH1/2 and SRSF2 to identify pathogenic variants and to describe their prevalence in the context of the DIPSS classification.

The series included 67 patients (61% females) with MF diagnosed according to the 2016 WHO criteria. At the time of testing, the median age was 65 years old (range 20–88) and laboratory characteristics included a median hemoglobin level of 10 g/dL (3-16), leukocyte counts of $11x10^9$/L (16-124) and circulating blast of 0% (0-15). The distribution according to the DIPSS was: 18% low, 18% intermediate-1, 30% intermediate-2, and 34% high risk. Driver mutational status revealed 49% JAK2, 30% CALR, 9% MPL and 12% triple-negatives.

Genomic DNA samples was amplified 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). Fifteen patients (22%) presented pathogenic variants in ASXL1, 2 (3%) in IDH2, 1 (1.5%) in SRSF2 and 6 (9%) combined two of them. Overall mutational frequencies according to the DIPSS were 3% for low, 6% intermediate-1, 9% intermediate-2 and 18% high risk patients.

Of all the subclonal cooperating pathogenic variants found, 50% were identified in the DIPSS high risk patients associated with a more aggressive disease with clinical and therapeutic implications.

101. (482) **NEXT GENERATION SEQUENCING TECHNOLOGIES APPLIED TO THE MOLECULAR DIAGNOSIS OF CONGENITAL HYPOTHYROIDISM**

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Congenital hypothyroidism is the most frequent endocrine disorder in pediatric patients. Over thirty monogenic forms of the disease have been reported. A meta-analysis demonstrated that only 5-10% of patients with thyroid dysgenesis and 45-88% of patients with thyroid dyshormonogenesis are diagnosed using single-gene sequencing. Here, we used single-gene and next generation sequencing to investigate the etiology of the disease.

Ten patients (p1-10) with dyshormonogenesis showing a defined iodide transport defect phenotype were studied by single-gene analysis. Nine patients (p11-19) with thyroid dysgenesis (n=2) or dyshormonogenesis (n=7) were explored by targeted next generation sequencing. Patients 1 and 17 were studied by trio whole-exome sequencing.

SLC5A5 gene sequencing analysis (p1-10) revealed four compound heterozygous variants (c.970-3C>A/p.D369V; p.G543K/p.L562M) and three in homozygous state (c.1973C> T; c.1673A> C; p.G561E). Multiple gene sequence analysis (p11-19) revealed three heterozygous variants (p.F1542V*fs**20; p.Y2563C; p.S523P) and two compound heterozygous variants (p.Q29*/c.177-2A>C) in *TG* gene. Moreover, the analysis revealed heterozygous variants in *DUOX2* (p.E1496D*fs**51) and *FOXE1* (p.P203R) genes. Finally, we deepened the study of two patients (p1 and p17), without mutations in putative candidate genes and, in the case of p17, with an unusual autosomal dominant inheritance pattern using trio whole-exome sequencing. Heterozygous variants were identified in TG (p.G653D) and *PFKFB2* [c.741-60_741-61insG(2)TG(8)] genes, the latter has not previously been associated with the disease. All identified variants were predicted pathogenic.

Next-generation sequencing constitutes an attractive alternative to systematically explore congenital hypothyroidism. However, we evidenced that a considerable proportion of patients remain undiag-

nosed. Trio whole-exome sequencing revealed *PFKFB2* as a novel candidate gene in congenital hypothyroidism.

102. (506) **A NOVEL DEEP INTRONIC *DMD* VARIANT CAUSE DUCHENNE MUSCULAR DYSTROPHY BY PSEUDOEXON ACTIVATION ENCODING A NONSENSE CODON**

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Dystrophinopathies are a group of X-linked recessive neuromuscular disorders caused by pathogenic variants in the *DMD* gene, which include Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, X-linked dilated cardiomyopathy and mild forms of the disease. The spectrum of dystrophin gene pathogenic variants includes large deletions (60%), large duplications (5-10%) and small variants (30%) (missense, nonsense, indels and splicing variants) that are detected by standard diagnostic methods; namely, MLPA and sequencing of the coding regions of the *DMD* gene. However, in a minority group of patients (<1%) deep intronic variants are detected by mRNA analysis from muscle biopsies. The aim of this study is to present the molecular findings in a patient with clinical suspicion of DMD, absence of dystrophin in muscle biopsy and negative molecular studies for deletions, duplications and small variants. In order to search for deep intronic variants, RT-PCR of the mRNA isolated from muscle biopsy was performed and the cDNA of the entire *DMD* gene was amplified into 14 overlapping fragments. Sanger sequencing of these fragments revealed an insertion of 141 bp between exon 8 and 9. This pseudoexon inclusion introduced a premature stop codon at the mRNA level. Sequencing of the pseudoexon and its flanking regions of gDNA was performed to investigate the underlying mechanism causing the insertion. The variant NG_012232.1 (NM_004006.3): c.832-186T>G, which creates a cryptic 5' splicing donor site (T>G substitution at the +1 position) and the pseudoexon activation, was detected. Carrier status was confirmed in the proband's mother. In conclusion, RNA analysis followed by gDNA sequencing allowed us to confirm the genetic cause of the disease. The introduction of a premature stop codon due to the pseudoexon activation correlates with the absence of dystrophin in muscle biopsy. Besides, this study allowed to provide an adequate and timely genetic counselling to the family.

103. (508) **A CUSTOMIZED NEXT-GENERATION SEQUENCING-BASED PANEL APPROACH FOR THE MOLECULAR DIAGNOSIS OF EARLY-ONSET NEUROMUSCULAR DISORDERS IN REFERRAL CENTRE IN ARGENTINA**

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Neuromuscular disorders (NMD) are phenotypically and genetically heterogeneous diseases. To date, 587 genes and 1042 different diseases have been described. Congenital myopathies (CM), congenital muscular dystrophies (CMD), early-onset forms of limb-girdle muscular dystrophy (LGMD) and congenital myasthenic syndromes (SMC) present in the neonatal or childhood period. NGS offers a value tool for the molecular diagnosis of NMD due to the large number of candidate genes, phenotype heterogeneity and overlapping clinical features. **Objective:** to perform the molecular characterization in a pediatric patient cohort with clinical and pathological features of CM, CMD, LGMD and SMC. **Methods:** we included 49 patients divided in four groups according to the initial clinical suspicion: CM (n=27), CMD (n=11), LGMD (n=1), SMC (n=7) and SMC vs MC (n=3). Phenotypes groups were classified according to the clinical signs and symptoms in the neurologic examination, pathological features in muscle biopsy and/or electrophysiological studies. We designed two customized NGS panels, TruSeq Amplicon (Illumina) (n=11) and SureSelect (Agilent) (n=44) to study 28 and 80 related genes respectively. Six patients with TruSeq Amplicon negative results were restudied with the SureSelect panel. **Results:** pathogenic variants were detected in 29 patients (59%); 16 cases in genes associates with CM (9 *RYR1*, 3 *NEB*, 2 *ACTA1*, 1 *TPM2* and 1 *TTN*), 6 cases with CMD (2 *COL6A1*, 1 *COL6A3*, 2 *LAMA2*, 1 *LMNA*), 5 individuals with SMC (2 *RAPSN*, 1 *CHRNB1*, 1 *COLQ*, 1 *DOK7*) and 2 cases with LGMD (1 *CAPN3,* 1 *SGCG*). SureSelect technology gave a diagnostic yield of 59% compared to 27% for TruSeq Amplicon. **Conclusion:** the diagnostic sensibility obtained in this study highlights the advantages in applying an NGS-based panel approach for genetically and phenotypically heterogeneous diseases such as NMD. Besides, it provides information for therapeutic options for treatment conditions and can contribute to the genetic counselling.

104. (526) **THE ROLE OF NM_004827.3:c.421C>A VARIANT OF ABCG2 GENE IN THE TRIGGERING OF PORPHYRIA CUTANEA TARDA IN HIV-INFECTED INDIVIDUALS**

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Genetic variants affect the expression of the ABCG2 transporter, altering the efflux of drugs and heme; NM_004827.3:c.34G>A, NM_004827.3:c.376C>T and NM_004827.3:c.421C>A variants are present in a high frequency. Porphyria Cutanea Tarda (PCT) is caused by a deficiency in Uroporphyrinogen decarboxylase; there are 2 main types of PCT: hereditary and acquired. Xenobiotics, alcohol, abuse drugs and hepatotropic viruses are the main triggering factors of the disease. In our country, 16% of PCT patients are HIV infected individuals. Previously, the influence of *ABCB1* genetic variants, a transporter of the same family as ABCG2, in the onset of PCT in HIV carriers was reported. The aim was to evaluate the role of the NM_004827.3:c.421C>A (rs2231142) variant of ABCG2 gene in the association PCT-HIV. A population of control, HIV, PCT and PCT-HIV individuals was studied. Genotyping was done by PCR-RFLP. The non-wild type allele A was in a very low frequency in all the groups. In PCT-HIV, the frequency of A (0.21) was higher than PCT and HIV values (0.05; $p<0.001$). When analyzed the genotypic frequency, SNV was in a very low frequency in heterozygosis in all the groups with higher values for PCT-HIV group (36%, $p<0.01$) than PCT (10%) and HIV (9%). The AA genotype (3%) was only found in PCT-HIV group. These results, although preliminary, suggest that NM_004827.3:c.421C>A variant in the *ABCG2* gene could be related to the manifestation of this porphyria only in HIV patients. The analysis of the other SNVs (NM_004827.3:c.34G>A, NM_004827.3:c.376C>T) will allow us to establish the presence or absence of risk haplotypes in the manifestation of PCT associated or not with HIV infection. The results of this analysis, together with those previously obtained for *ABCB1* drug transporter gene variants, will enable us to further conclude about the risk haplotype for PCT triggering.

105. (551) **CHEMERIN GENE VARIANTS ASSOCIATION WITH METABOLIC PARAMETERS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

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Objective: To evaluate *Chemerin* gene (RARRES2) SNVs rs4721 and rs17173608 association with clinical and biochemical characteristics, also with the metabolic and androgenic condition in women with Polycystic Ovary syndrome (PCOS).
Materials and methods: We analyzed 107 women with PCOS

according to the Rotterdam criteria (17-38 years). PCOS were divided into subgroups by the presence or absence of metabolic syndrome (MS) and hyperandrogenism (HA). Peripheral blood genomic DNA was purified and genotyped by T-ARMS PCR (Tetraamplification refractory mutation system for rs 17173608 (RARRES2 NC_000007.14(NM_002889.4):c.280-494A>C) and PCR-RFLP for rs4721 (RARRES2 NM_002889.4):c.*13A>C). Statistical analyzes were performed with GraphPad Prism and SPSS (t-Student test, ANCOVA with p-values adjusted for age and χ2 analysis).
Results: The PCOS patients showed a genotype distribution of TT genotype (44.9%), followed by TG genotype (43%) and GG (12.1%) for the rs4721; similar to the frequency found in Caucasians (HapMapProject). The population was in Hardy Weinberg equilibrium (x2 = 0.147; p = 0.70). The rs17173608 could not be analyzed because of the low presence of the minor allele (4%). Through ANCOVA analysis age adjusted, it was shown that the presence of rs4721 G allele was associated with higher levels of total cholesterol/HDL (p=0.04), LDL-c (p=0,02) triglycerides (p=0.03), insulin (p=0.02), HOMA (p=0.04), LAP index (Lipid accumulation product, p=0.03), testosterone (p= 0.08), LH/FSH (p=0.03). Also, rs4721 G allele was associated with larger telomere length (p=0.04) and lesser mitochondrial DNA mass (p=0.08).
Conclusion: In women with PCOS, rs4721G allele was associated with worse metabolic and hormonal parameters. In this preliminary study, no association was found between SNV rs4721 *Chemerin* gene and susceptibility to MS and HA in women with PCOS.

HEMATOLOGÍA

106. (62) **ERYTHROPOIETIN AND IRON AVAILABILITY IN THE REGULATION OF HEPCIDIN IN HEPATIC CELLS**
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Maintenance of systemic iron (Fe) levels undergoes regulation by the erythropoietic demand, inflammation and Fe status. Liver hepcidin (Hep), a small peptide which binds the Fe exporter ferroportin thus inducing its internalization and degradation, is a key regulatory target for Fe homeostasis.
We examined the ability of erythropoietin (Epo) to regulate Hep mRNA expression (real-time PCR) in the human hepatic cell line HepG2. Control (C) Hep levels were significantly reduced by Epo (160 ng/mL, 6 h), while its non-erythropoietic, carbamylated derivative cEpo failed to exert this effect (C: 1, *Epo: 0.4±0.2, cEpo: 0.9±0.1; *P<0.05 vs C). Abrogation of the Epo receptor with a specific siRNA or a blocking antibody prevented Hep downregulation in Epo-treated cultures (C: 1; *Epo: 0.4±0.2, siEpoR+Epo: 1.0±0.1; antiEpoR+Epo: 1.0±0.1; *P<0.05 vs C), showing EpoR is required for Epo signalling in this context. The observed decrease in Hep mRNA was followed (24 h) by lower intracellular Fe levels and higher Fe release to the culture media in Epo-exposed cells (ferrozine method).
Regarding the simultaneous regulation of Hep by Epo and different extracellular Fe conditions, Fe chelation by 100 μM deferoxamine reduced Hep mRNA by half compared with untreated cells, while 3 μM Fe-citrate almost doubled it. However, higher Fe-citrate concentrations caused lower Hep levels than in untreated cultures, and only in this condition was Epo unable to suppress Hep (C: 1; *Fe100μM: 0.3±0.1; Fe100μM+Epo: 0.7±0.2, *P<0.05 vs C). Protein levels of transferrin receptor 1 were not affected by Epo, but were decreased by Fe addition. However, no differences were observed between 3 and 100 μM Fe-citrate.
Our findings show Epo can increase Fe availability through Hep downregulation in hepatic cells, though there seems to be a complex interplay between Epo and Fe status. Further research is needed to clarify the role of Epo on Fe availability in different pathological and therapeutical scenarios.

107. (94) **SELECTIVE RESPONSE OF IRON CYCLE PROTEINS THROUGH IRON AND ERYTHROPOIETIN SIGNALS IN MOUSE KIDNEY.**
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Iron overload can be regulated by different mechanisms related to erythropoiesis and kidney tissue.Taking into account the labile nature of iron,its circulation and storage are strictly controlled.Approaches to addressing iron trafficking through the hepcidin regulator and importer(DMT1,ZIP14) and exporter(FPN) proteins may offer new insights.The presence of the erythropoietin receptor(EPO-R)in the kidney suggests various non-erythropoietic functions of EPO.This study was designed to extend our previous studies on the relationship between iron overload and iron protein regulation to another important organ,the kidney.The non-erythropoietic functions of EPO will also be analysed.CF1mice(25±5g;3m)split in(n=4/group):1*)Iron-adequate*(IA);2)*Iron-overload*(IO)(ironsaccharate;-*days0,4,8,12*ip;1800mg/kg);3)*EPO(days17-19ip;20000UI/kg)*;4)*Iron-overload+EPO(IO+EPO)*.Immunohistochemistry:anti-DMT1(divalent-metal-transporter1),anti-ZIP14(Zrt-Irt-likeProtein14),anti-prohepcidin.Iron levels Wiener kit.The Protocol was approved by CICUAE-UNS.
Iron levels showed an increase in *IO/IO+EPO* respect to IA/EPO. Abundant hemosiderin was observed in IO in the proximal tubule S2(PTS2),glomerulus and medulla;it was moderate in *IO+EPO* and scarce in EPO/IA.The DMT1 expression was evident in the PTS2 and medulla in IA/EPO and slight in *IO/IO+EPO*.In IO the ZIP14 expression was intense in PTS2 and medulla and slight in *EPO* being this the predominant signal.The prohepcidin expression was intense in *IO/IO+EPO* and slight in IA/EPO.We can conclude that in iron overload,a coordinated regulation of the iron cycle proteins occurs in the kidney,suggesting a protective mechanism against iron excess due to the reduction of iron uptake according to the following modifications:decrease in both the uptake of DMT1 and the release of FPN,also showing a negative regulation of kidney-DMT1 by hepcidin.The cytoprotective role of EPO in controlling iron storage could be explained by the reduced expression of ZIP14 observed.

108. (207) **INFLAMMATORY RESPONSE MEDIATED BY REACTIVE OXYGEN SPECIES IN NEUTROPHILS OF INMUNOCOMPROMISED NO-HIV INFECTED PATIENTS**
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Immunocompromised patients (IP) with neutropenia (moderate (MN), less than 1500 neutrophils PMN)/mm³ and severe (SN), less than 500/mm³) generate susceptibility to infections. Neutropenia is prevalent in Leukemias (Le) and Lymphomas (Li). In order to analyze the inflammatory response the study was made on non-HIV IP with Le, Li and renal transplanted IP (RT), infected (I, no-HIV) and without infection (WI); it was determined in PMN venous blood from IP (n=49, 48±17 years, hospitalized), and healthy volunteer donors (C, n=30, 35±12 years): oxygen consumption (ΔO_2, Clark electrode, indicates generation of superoxide anion, O_2^-), production of hydrogen peroxide (H_20_2, fluorometry), spontaneous chemiluminescence of PMN (CL, photon counter, measures light emission of singlet oxygen ($^1O_2$)), and C-reactive protein (CRP). Increases were observed in IP with respect to C: ΔO_2 9 fold in WI and 3 in I ($p<0.01$); H_2O_2 3 fold ($p<0.001$) and CRP 33 ($p<0.05$) both in I; CL 73 fold in WI ($p<0.0001$). When analyzing results in MN and SN, ΔO_2 increased

in SN: 7 fold in WI and 9 in I ($p<0.001$) with respect to C and MN, without differences between WI and I; H_20_2 increased in SN 10 fold ($p<0.01$) in I; 70 fold CRP in I with SN ($p<0.001$). In IP with Le: ΔO_2 increased 14 fold in WI ($p <0.001$) and 17 in I ($p <0.05$), H_20_2 1.5 ($p<0.05$) and CRP 17 ($p <0.001$) in I; in RT and Li, ΔO_2 increased 4 fold in WI ($p<0.001$) and 2 in I ($p<0.05$); H_20_2 3 fold in WI with RT ($p<0.001$). In IP with Li, H_20_2 and CRP increased 10 fold with chemotherapy in WI ($p<0.001$ and 0.01) and not in IP. The inflammatory state is much higher in IP with SN; in IP with Li or Le, chronic inflammation would be associated with O_2^- production in WI; only IP PMNs with Le respond with H_20_2 generation. The inflammatory state of IP decreases response of PMN mediated by O_2^- and H_20_2, and generate oxidative damage, mediated by PMN $^1O_2$. Chemotherapy increases H_2O_2 production in PMN of WI with Li, effect decreased in infected IP.

109. (210) **OPTIMAL CONDITIONS FOR THE TREATMENT OF SAMPLES FOR THE STORAGE OF RED BLOOD CELLS STORED IN A BLOOD BANK**

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Red blood cell (RBC) storage injury is the deterioration caused during its conservation in the blood bank. One of its causes is the autoxidation of hemoglobin (Hb), which accumulates peroxides (XOOH), oxidized Hb species (EOHb) and fluorescent degradation products (FHP). The bibliography presents different protocols for the same method. The objective was to define the optimal conditions to evaluate EOHb, FHP and XOOH in 10 units of GR stored in the blood bank (DRG). The EOHb were evaluated with a spectral sweep between 500 and 700 nm at different concentrations and pH. The most influential factor was the Hb concentration of the sample, adjusted to 0.7 mg/mL, followed by the pH, adjusted to 7.5. The FHPs represent the intracellular content of H_2O_2 as a fluorescence peak at 485 nm under excitation at 321 nm. The emission spectrum between 400 and 600 nm was analyzed at different concentrations and storage conditions. A peak was not observed at 485 nm, but the emission average between 470 and 490 nm was correlated with it and was more precise (CV 5.5% vs 11.4%, $p <0.003$). The Hb concentration was adjusted to 50 uM to standardize the quenching. The results were stable for 18 days in samples stored at -80 °C. The estimation of XOOH uses cells or plasma, optionally adds Fe(II) and its measurement range oscillates between micro and millimolar. GRDs, their supernatants and plasmas with and without Fe (II), and H_2O_2 calibrators between 0.1 uM and 100 mM were assayed. The DRGs did not produce a reaction, the quantification range in acellular media was 15 to 100 mM and Fe(II) only acted as a catalyst. The best working conditions for EOHb are to adjust the Hb concentration to 0.7 mg/mL at pH 7.5, for FHP the Hb concentration must be adjusted to 50 uM, and use acellular solutions to estimate XOOH without Fe(II) for end-point reactions.

110. (244) **REVERSION OF THE HEMATOLOGICAL AND HEMATOPOIETIC ALTERATIONS PRODUCED BY 4T1 MURINE BREAST TUMOR THROUGH DOCETAXEL.**

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Docetaxel (D) is a chemotherapeutic agent widely used in several types of malignancies including breast cancer. Here we studied the impact of D on hematopoietic populations in the bone marrow (BM) and the hematological parameters in peripheral blood in both healthy and 4T1 tumor bearing mice, as a model of triple negative breast cancer. We also studied the impact of D on the proliferation, migration and survival of 4T1 cells in vitro. 4T1 cells were injected subcutaneously in female BALB/c mice and, when tumors were established they were treated i.p with vehicle or D 10 mg/kg twice a week for 3 weeks. Healthy mice were injected with vehicle (control) or D. After treatment, we determined the hemogram and identified hematopoietic precursors by immunofluorescence and flow cytometry. Proliferation and survival assays were performed by Trypan Blue count, and migration by wound healing assay. Mice with 4T1 tumor developed a myeloid leukemoid reaction with a high granulocytic count ($p<0.0001$), a decrease in haemoglobin ($p<0.01$) and an increase in white blood cells (WBC) ($p<0.0001$) vs. control. D treatment diminished WBC ($p<0.001$) and spleen size ($p<0.001$) in tumor bearing mice. 4T1 tumor induced a macrocytic anemia with extremely diminished immature erythroid populations in the BM. Common myeloid progenitors (CMP) were increased in BM, but not granulocytic-monocytic progenitors (GMP). Proliferation, survival and migration diminished when 4T1 cells were treated with D in vitro ($p<0.01$). In conclusion, the 4T1 tumor induces a drastic decrease in different maturation stages of the erythroid populations in the BM, which is not reversed by D treatment. CMPs probably rise due to the large amount of G-CSF released by the tumor. D treatment elicits an antineoplastic effect not only by reducing proliferation, survival and migration of 4T1 cells, but also by decreasing the marked splenomegaly and leukocytosis caused by extramedullary hematopoiesis produced by 4T1 tumor.

111. (463) **TREATMENT FOR 10 DAYS WITH THE EXTRACT ENRICHED IN PROANTOCIANIDINS FROM *LIGARIA CUNEIFOLIA* ON THE CELLULAR FACTORS THAT INTERACT WITH THE KINETICS OF ERYTHROCITARY AGGREGATION IN BLOOD OF HIGH FAT DIET WISTAR RATS**

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In folk medicine, *Ligaria cuneifolia* (*Lc*) is used to increase blood fluidity by lowering plasma (Cho) cholesterol. A fraction enriched in proanthocyanidin (P*Lc*) was obtained that led to a decrease in Cho and Triglycerides in rats fed a high fat diet (HFD). So far, the effect of treatment with P*Lc* on blood fluidity at low flow rates, estimated by the kinetics of erythrocyte aggregation (EA), has not been studied. We evaluate the effect of treatment with P*Lc* on blood fluidity at low flow rates, estimated by the kinetics of erythrocyte aggregation (EA) in HFD rats, characterizing cellular factors. Wistar rats fed a standard diet added with 40% of first bovine juice for 28 days, then were injected via i.p. every 24 hours for 10 days with: physiological solution (HFD; n=6) or with P*Lc* 3mg/100g body weight (T10; n=6). The fourth day, the rats were anesthetized with Ketamine/Xylazine (100mg/kg /3mg/kg, i.p.), obtaining blood by cardiac puncture. In Plasma Cho and triglycerides (TG) were determined by enzymatic methods. In whole blood, the kinetics of EA by an optical method was assessed obtaining two parameters that estimate: size of aggregates (T) and aggregation speed (V). Distinction of forms by optic microscopy, and the morphological index (MI) was calculated. Plasma: Cho (mg %): HFD: 191.7 ± 3.8; T10: 105.8 ± 4.2 *; TG (mg %): HFD: 333.5 ± 20.2; T10: 148.8 ± 9.7*; IM: HFD: -1.460 ± 0.14; T10: -2.063 ± 0.84; Blood: T: HFD: 2.042 ± 0.02, T10: 1.667 ± 0.133 *; V: HFD 0.110 ± 0.003, T10: 0.006 ± 0.002; * (mean ± EE; * $p <0.05$ vs. HFD; Student's t-test for unpaired data). P*Lc*-treatment show a lipid-lowering effect, with a significant decrease in the AE and had not changes in MI. We have obtained a fraction of *Lc*, which decreases total Cho and TG in plasma without causing improving blood fluidity at low flow rates.

INFECTOLOGÍA Y PARASITOLOGÍA

112. (5) **CORRELATION BETWEEN THE VOLUME OF VAGINAL CERVICAL FLUID AND THE GROWTH OF TRITRICHOMONAS FETUS**

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Bovine trichomonosis is a venereal disease caused by Tritrichomonas foetus. The protozoan can be isolated from the cervical mucosa 2-3 months after intercourse and can reach the uterine mucosa where it is presumed to cause abortion. Cell growth dynamics, vaginal environmental factors, and pathogenicity mechanisms are not fully understood at this time. For the reasons stated, the objective of this study was to characterize the growth of T. foetus in vaginal cervical fluid (FCV) corresponding to different stages of the bovine estrous cycle in vitro. For this, the following methodology was applied, the FCV was extracted from 3 animals with synchronized heat every 3 days during 3 successive estrous cycles. T. foetus was inoculated in FCV aliquots of different stages and growth was evaluated, it was analyzed by ANOVA, using Graph Pad Prism version 5.01 software. The data show that the growth of the microorganism was clearly higher in oestrous fluids, also observing a stationary behavior and correlated with the volume of FCV extracted ($p < 0.05$). In conclusion, the increased secretion of fluid in the estrous stage and its evident association with the proliferation of T. foetus allows us to understand the cell growth dynamics that the parasite could develop in vivo.

113. (6) **USE OF A TAGUCHI DESIGN FOR THE STUDY OF DIFFERENT EXPERIMENTAL CONDITIONS IN LOOP-MEDIATED ISOTHERMAL AMPLIFICATION REACTIONS (LAMP)**

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Loop-mediated isothermal DNA amplification (LAMP) has been used for the diagnosis of a wide variety of diseases. This technique has great advantages over other molecular techniques such as PCR. It can be carried out with little equipment, with minimal training of personnel and achieve high sensitivity and specificity. However, it is not without its difficulties. The occurrence of false positives or false negatives is not unusual. Using a suitable experimental design it is possible to identify and optimize the components of the reaction that most influence the result of the amplification. A Taguchi design allows this objective to be achieved quickly, minimizing the number of experiments and efforts. For the reasons stated, the objective of this work was to evaluate the performance of a LAMP reaction under different experimental conditions using a Taguchi design. For this, the work methodology consisted in the amplification of a fragment of the ef1a1 gene of *Tritrichomonas foetus*. This was carried out varying the surfactant (Tween20 or Triton X-100) and the concentration of dNTPs (0.06-0.24mM), betaine (0.4-1.8M) and MgSO4 (2-9mM). The data were analyzed by means of ANOVA, using the program, Graph Pad Prism version 5.01 software. Preliminary results showed that the surfactant had a notable influence on the kinetics of the reactions, those containing Tween 20 being faster. The influence on the kinetics of magnesium and betaine ions was greater in reactions with Tween 20. The occurrence of false positives was minimized in reactions containing Tween 20 as a surfactant. In conclusion, it can be suggested that the use of an appropriate experimental design allows not only optimizing a reaction but also studying the influence of each of the factors on amplification.

114. (7) **STUDY OF THE VIABILITY OF TRITRICHOMONAS FETUS IN BOVINE CERVICAL FLUID**

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Bovine trichomonosis is a venereal disease caused by the protozoan *Tritrichomonas foetus* and is very common in countries where extensive husbandry and natural service are practiced. In the bull, the presence of the parasite is asymptomatic and tends to become chronic in the adult animal. Infection in females occurs naturally during heat and usually manifests as failed services and repetition of heat. In pregnant females, the protozoan can reach the uterus and invade the embryo tissues. The multiplication of *T. foetus* must guarantee its persistence until a new estrus and the infection of the male. For the reasons mentioned, in this work the ability of *T. foetus* to grow in vitro in cervical mucus (MCV) of females at different stages of the estrous cycle was evaluated. For this, the degree of crystallization (fern formation) and the cellularity of each cervical fluid were used in the work methodology in order to infer the moment of the estrous cycle that allows the multiplication of the protozoan. The data were analyzed by ANOVA, using the Graph Pad Prism version 5.01 program. The preliminary data obtained show that *T. foetus* showed positive growth during estrus. In contrast, no growth was observed in the right-hander. The results allow us to conclude that it is hypothesized that the protozoan in a natural infection must modify the environment to persist in the female reproductive tract throughout the entire estrous cycle.

115. (390) **GENE EXPRESSION INVOLVED IN THE CYTOPROTECTIVE AND INFLAMMATORY RESPONSE IN ACUTE LEUKEMIA**

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The generation of reactive oxygen species and cytoprotective response play an important role in perpetuity of acute leukemia (AL). The aim of this work was to study the behavior of antioxidant enzymes and pro-inflammatory cytokines at transcriptional level, and relate it with the transcription factor *nuclear factor erythroid 2-related factor 2* (Nrf2) expression in AL. A descriptive study was conducted between September 2016 - July 2019. Ninety-three subjects (54 with AL and 39 apparently healthy individuals) were evaluated at the Universidad Nacional de Tucumán. The leukemia characterization was performed by: blood count, cytochemistry and flow cytometry. Malondialdehyde (MDA) levels and catalase (CAT) serum activity were determined. Transcription factor *forkhead homeobox type O* (FoxO3a) and Nrf2, antioxidant enzymes CAT, *peroxiredoxin-2* (PRX-2) and *superoxide dismutase* (SOD), and the cytokines *tumor necrosis factor-alpha* (TNF-α) and *interleukine-6* (IL-6) gene expression were analyzed with real-time PCR in peripheral mononuclear blood cells. Statistical analyses were performed by SPSS V.25 statistical software. The comparative study was performed using the Kolmogorov-Smirnov non-parametric test. All statistical analyses were considered significant at $p<0,05$. We detected 41% of acute lymphoid leukemia, 39% acute myeloid and 20% acute promyelocytic leukemia. MDA concentration was similar in the groups analyzed. However, CAT activity was significantly increased in AL patients respect to controls (CAT nmol/mg prot AL=0,35 (0,01-1,41); controls=0,25 (0,08-0,53). SOD, IL-6 and TNF-α transcriptional expression were significantly downregulated in AL group. Nrf2 gene expression showed moderate association with PRX-2 (R^2=0,44) and CAT (R^2=0,60) gene expression. These findings show the transcriptional modifications of some redox regulators in patients with LA and shed new light on the redox imbalance and inflammatory status un-

derlying in this neoplasm.

116. (463) **TREATMENT FOR 10 DAYS WITH THE EXTRACT ENRICHED IN PROANTOCIANIDINS FROM *LIGARIA CUNEIFOLIA* ON THE CELLULAR FACTORS THAT INTERACT WITH THE KINETICS OF ERYTHROCITARY AGGREGATION IN BLOOD OF HIGH FAT DIET WISTAR RATS**

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In folk medicine, *Ligaria cuneifolia* (*Lc*) is used to increase blood fluidity by lowering plasma (Cho) cholesterol. A fraction enriched in proanthocyanidin (P*Lc*) was obtained that led to a decrease in Cho and Triglycerides in rats fed a high fat diet (HFD). So far, the effect of treatment with P*Lc* on blood fluidity at low flow rates, estimated by the kinetics of erythrocyte aggregation (EA), has not been studied. We evaluate the effect of treatment with P*Lc* on blood fluidity at low flow rates, estimated by the kinetics of erythrocyte aggregation (EA) in HFD rats, characterizing cellular factors. Wistar rats fed a standard diet added with 40% of first bovine juice for 28 days, then were injected via i.p. every 24 hours for 10 days with: physiological solution (HFD; n=6) or with P*Lc* 3mg/100g body weight (T10; n=6). The fourth day, the rats were anesthetized with Ketamine/Xylazine (100mg/kg /3mg/kg, i.p.), obtaining blood by cardiac puncture. In Plasma Cho and triglycerides (TG) were determined by enzymatic methods. In whole blood, the kinetics of EA by an optical method was assessed obtaining two parameters that estimate: size of aggregates (T) and aggregation speed (V). Distinction of forms by optic microscopy, and the morphological index (MI) was calculated. Plasma: Cho (mg %): HFD: 191.7 ± 3.8; T10: 105.8 ± 4.2 *; TG (mg %): HFD: 333.5 ± 20.2; T10: 148.8 ± 9.7*; IM: HFD: -1.460 ± 0.14; T10: -2.063 ± 0.84; Blood: T: HFD: 2.042 ± 0.02, T10: 1.667 ± 0.133 *; V: HFD 0.110 ± 0.003, T10: 0.006 ± 0.002 (mean ± EE; * p <0.05 vs. HFD; Student's t-test for unpaired data). P*Lc*-treatment show a lipid-lowering effect, without changes in the cellular factors estimated by EA, and had not changes in MI. We have obtained a fraction of *Lc*, which decreases total Cho and TG in plasma without causing alteration on blood fluidity at low flow rates.

117. (16) **DEVELOPMENT OF A NOVEL NS1 COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE EARLY DETECTION OF ZIKA VIRUS INFECTION**

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Zika virus (ZIKV) is a flavivirus that has emerged as a global health threat after the 2015 outbreak in the Americas, where devastating congenital defects were documented. There are currently no vaccines to prevent ZIKV infection nor commercially available diagnostic tests demonstrated to identify ZIKV without cross-reactive interference of related flaviviruses. Early diagnosis is critical when treating symptomatic patients and in preventing ZIKV transmission. In this context, the development of sensitive and accurate diagnostic methods is urgently needed for the detection of ZIKV acute infection. Recent studies have demonstrated that heat dissociation of the NS1 homo-hexamer, a useful diagnostic marker for flavivirus early detection, is a convenient alternative to enhance the sensibility of commercially available ELISAs by an increase in the antigen available monomeric forms. With this strategy in mind, we aimed to obtain monoclonal antibodies (mAbs) against denatured monomeric ZIKV NS1 (ZNS1) protein in order to develop a highly specific and sensitive ZNS1 indirect competitive ELISA (icELISA). The production of hybridomas secreting ZNS1 mAbs was carried out through immunizations with denatured monomeric ZNS1. We selected two specific hybridoma clones, 1F5 and 6E2, which recognized the heat-denatured ZNS1 form by indirect ELISA. In addition, cross-reaction studies indicated that these mAbs specifically recognize a ZNS1 linear epitope, and that they do not cross-react with the NS1 protein from other related flaviviruses. The1F5 mAb enabled the development of a sensitive, reliable and reproducible icELISA to detect and quantify ZNS1 disease marker in heat-denatured human sera. We established a valid 1F5 based-icELISA that allows the detection and quantification of small amounts of ZNS1 (156 ng/ml), and constitutes a promising bioanalytical method for control strategies and the prevention of ZIKV propagation.

118. (20) **EFFECTS OF EXTRACTS OF LARREA DIVARICATA AGAINST THE STORED PRODUCT PESTS TRIBOLIUM CASTANEUM (COLEOPTERA: TENEBRIONIDAE).**

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Several shrubs belonging to genus *Larrea* (Zygophyllaceae) occur in semi-arid region from Argentina and are used in traditional medicine to treat fungal and bacterial infections. The most common species is *Larrea divaricata*, known as female jarilla. The ethanolic extract of *L. divaricata* leaves has reported as antifungal when tested against *S. cervisiae*, C. albicans y *A. niger*. Nordihydroguaiaretic acid (NDGA), found in the resin of leaves and stems, has inhibitory effects on the ecdysis of Rhodnius prolixus; and extends the life cycle of Aedes Aegypti. Members of the Zygophyllaceae family have demonstrated larvicidal activity against *Cx. Pipiens*, antifeeding and repellent activity against *Sitophilus oryzae*. However, no studies were found on the bioactivity of *L. divaricta* against the cereal pest *Tribolium castaneum* (Tenebrionidae). The storage pest *T. castaneum* is a major pest of both raw and processed commodities. This study was designed to evaluate the bioactivity of *L. divaricata*, against the *T castaneum*. Samples were collected, in the province of La Pampa. The phytochemical extraction was performed using organic solvent extraction as well as aqueous extraction, according to conventional techniques. *T. castaneum*, two stages, larvae and adults were used (10 insects per well), four doses (2.5 to 50 mg) were evaluated, in triplicate. The solid resin was tested on the one hand, and the methanol extract on the other. The number of dead insects was recorded every 24 hours for 7 days post treatment. Comparisons among groups of data were done using one-way ANOVA, an associated probability of $P<5\%$ was considered significant. The result showed that, extracts from *L. divaricata* lack insecticidal activity under the conditions tested. In conclusion, *L. divaricata* does not present insecticidal and repellent activity against the *T. castaneum*. However, these preliminary results are of interest, since there are no previous works in this regard.

119. (24) **IN SEARCH OF A RECEPTOR FOR ALPHA HEMOLYSIN OF *E.COLI* IN HUMAN ERYTHROCYTES**

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INTRODUCTION Alpha-hemolysin (HlyA) is a hemolytic protein secreted by uropathogenic strains of *E. coli*. The binding of HlyA to a putative toxin-specific receptor produced contradictory results. Glycophorins (GPs) were characterized as presumed receptors in horse red blood cells (RBCs), though other studies indicated HlyA did not interact with a specific protein receptor in rabbitRBCs. Conversely, we previously demonstrated that HlyA induces a decrease in humanRBCs (hRBCs) deformability and the release of ATP, effects usually associated to the interaction of ligands with GPs. **OBJECTIVES** Study the contribution of GPs and alternative membrane proteins in mediating the hemolytic effects of HlyA on hRBCs.

METHODS We tested the lytic activity of HlyA on hRBCs pretreated with different antibodies to block the binding of HlyA to GPs (anti-GPA/GPB, anti-GPA and nanoantibody iH4), and also on the rare clinical mutant GPA/GPB null hRBCs. We measured the dissociation constant between GPA and HlyA, ProHlyA (inactive protoxin) and HlyA$^{\Delta 914\text{-}936}$ (HlyA mutant lacking the binding domain to GPA) by Surface Plasmon Resonance (SPR). Finally, we performed Far Western Blot assays plus mass spectroscopy analysis, to explore whether HlyA binds to a specific hRBC membrane protein other than GPs. **RESULTS AND CONCLUSIONS** The hemolytic activity was slightly inhibited by high concentration of anti-GPA/GPB. Surprisingly, the hemolytic activity of the toxin on the rare clinical mutant GPA/GPB null was similar to control RBCs, indicating that GPs are not necessary for HlyA activity. SPR measurements indicated that the three proteins variants bind with similar strength to GPA, and to human seroalbumin, showing that the binding of the proteins to GPA is unspecific. Far Western Blot results showed that HlyA interacts with two hRBCs membrane proteins. The next step is to study the specific interaction between HlyA and membrane proteins on hRBCs in a more biological environment using a Pull Down assay.

120. (28) **TRICHOMONAS SPP. AN ETIOLOGICAL FACTOR IN THE PATHOGENESIS OF ORAL DISORDERS IN IMMUNOSUPPRESSED PATIENTS**

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Mucosal ulcerations are an oral complication that can often affect immunosupressed patients and adequate treatment depends on the correct determining of its etiology.

The diagnosis covers drug-induced ulcers, fungal infections, viruses, as well as opportunistic infection by other microorganisms. Parasitic infection in the oral cavity has not been well studied and it is thought to be underreported.

Here we present a case report of an oral lesion present in a kidney transplanted patient. The presumptive diagnosis: drug-induced ulcers, CMV or EBV lesions or ulcers caused by mycosis. Toxic effect of medication was discarded due to the minimal dose administered. The biopsy showed non-specific ulcers with a fibrinoleukocyte layer, lymphoplasmacytic infiltration with diffused neutrophils and eosinophils. Ziehl Neelsen, Giemsa and immunostaining for CMV did not show positive results for any specific microorganisms. EBV was also discarded. The sample from the swab test showed *Streptococcus viridians* and normal oral microbiota. Direct microscopic observation of moist sample showed a motile pyriform protozoon with flagellar movements compatible with *Trichomonas* spp. *Trichomonas tenax* was a variant of *T. vaginalis* that developed genotypic changes and acquired a phenotype suitable to the ecosystem of the oral cavity. It was isolated from periodontal pockets and expressed proteinases that could disrupt the host tissue integrity promoting oral tissue destruction.

Although it is not possible to identify the strain of *Trichomonas* nor to prove causality between the presence of the protozoan and the development of oral ulceration in our patient, the clinical response to treatment and the absence of other pathogens indicates that this parasite was likely acting as a pathogen.

In conclusion, this is the first case that highlights the possibility that *Trichomonas spp.* should be considered as a potential etiological factor in the pathogenesis of oral disorders in immunosuppressed patients.

121. (55) **rRNA OPERON COPY-NUMBER IMPACT ON GROWTH RATE IN *Brucella* AND *Bradyrhizobium*.**

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Growth rate (GR) varies widely among bacterial species. Comparative genomics suggest that the structure of the chromosome contributes to determine GR. In particular, bacteria bearing a high number of ribosomal operons (*rrn*) display higher GRs. The *rrn* number varies widely across bacteria ranging from 1 to 11 copies, with an average of 6 copies per genome. Alpha proteobacteria from the genera *Bradyrhizobia* and *Brucella* are capable of interacting, either symbiotically or pathogenically, with hosts. These bacterial groups show a low GR which makes its study difficult. Close examination of complete genomes within these group of slow growers, shows that these clades bear 1 to 3 *rrn*. To test the links between their slow growth and ploidy of ribosomal RNA genes, we compared GRs of different isolates and we modified the *rrn* content within the studied groups.

On one hand, we compared growth through manual and automated growth curves, of completely sequenced strains of the *Bradyrhizobium* genus: *B. diazoeficiens* USDA110 and *B. diazoefficiens* USDA122, with one *rrn* and *B. japonicum* E109 and *B. japonicum* USDA6T that have 2 *rrn*s. We observed that strains bearing 2 *rrn* grew faster, displayed a shorter lag phase and outcompeted strains with 1 rrn when co-cultured. On the other hand, the *Brucella* genus, that groups microorganisms causing brucellosis, bear 3 rrn. We observed that deletion of one *rrn* by allelic replacement, caused a 20% decrease in GR of *Brucella suis 1330*. In sum, *rrn* ploidy seems to impact GR in slow growers from Alpha proteobacteria. This may have a large impact on how these bacterial groups interact in host, particularly in *Brucella* pathogenesis causing brucellosis. Further, reducing rrn ploidy in *Brucella suis* might have a greater impact in its physiology without altering gene content. Such changes could reduce their pathogenicity making this strategy attractive to develop a strain to test as a vaccine.

122. (198) **BESNOITIA SP. INFECTION IN A NATURAL POPULATION OF VIZCACHAS (LAGOSTOMUS MAXIMUS, RODENTIA) IN BUENOS AIRES, ARGENTINA**

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CEBBAD, Universidad Maimonides.

Our laboratory uses vizcachas as a model for reproductive endocrinology studies and we have often found abnormal cyst-like structures in ovaries. Our specimens come from a natural population at La Plata. A coccidian parasite that affected several domestic rabbits from a rabbit breeder in La Plata was the first report of a *Besnoitia* infection in Argentina. To evaluate whether vizcachas could also be host of this parasite, we examined histological sections of various female organs. Pinhead-sized tissue cysts were found in ovaries, adrenals, mammary glands, uterus, pituitary, brain, spleen and fascia. Either superficial or deep tissue cysts were simultaneously seen in several organs of the same animal. Cysts were found in both pregnant and non-pregnant females. Histologically, a thick wall made up of an outer layer of collagen fibers and an inner granular-looking layer surrounded each tissue cyst, which was packed with numerous bradyzoites and some host nucleated cells. Outside the cysts, host cellular structures showed normal appearance. Mammary gland acini of infected pregnant females showed normal morphology and the ovaries exhibited follicles in different stages despite the presence of cysts. These results indicate that infection with this parasite would not affect the reproductive process. The presence of cysts in brain areas could be indicative of the parasite ability to cross the blood-brain barrier whereas cysts in the embryonic organs suggest the same for the placental barrier. Based on a comparative morphological analysis with the tissue cysts exhibited by the naturally-infected rabbits of La Plata we identified ours as *Besnoitia sp.* This is the first report of *L. maximus* as a host for *Besnoitia*. Considering that bovine besnoitiosis is a serious problem affecting Middle East, Asia, Africa and Europe with significant economic losses, our report is highly relevant since the distribution map of *L. maximus* largely overlaps with that of Argentinian cattle.

123. (259) **EFFECTS OF ALPHA HEMOLYSIN OF *E.coli* ON HUMAN PLATELETS**

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Alpha hemolysin (HlyA) is the major virulence factor of uropathogenic *Escherichia coli* (UPEC) pathology, causing 50% of all extraintestinal disease in humans — such as urinary-tract infection, pyelonephritis and septicemia — and the expression of HlyA was found to correlate with the severity of the infections produced. During sepsis, HlyA is released in the vasculature where it can directly interact with cell membranes of red blood cells (RBCs). Sublytic concentrations of the toxin caused changes in RBC morphology, activation of endogenous sphingomyelinases and externalization of ceramide. All these events induced the delivery of microvescicles containing the toxin in the membranes. In this work we studied the interaction of HlyA with isolated platelets. Results show that a relatively low toxin concentration (10.36 nM) induced the release of lactate dehydrogenate, thus indicating a cytotoxic effect. Morphological changes of HlyA-treated platelets were observed by Scanning Electron Microscopy (SEM), evidencing a disruption in the external platelet structure. We also measured the rise in intracellular calcium concentration, by flow cytometry, finding increments in the concentration of the cation directly correlated with toxin concentration. Overall results show that pure HlyA isolated from *E.coli*, caused cytotoxicity and morphological changes in human platelets, providing the basis for futher studies on the signaling pathways activated by this toxin in bloodstream cells.

124. (265) **EVALUATION OF VEGETABLE EXTRACTS INHIBITORY EFFECT'S ON THE BIOFILM FORMATION IN METICILIN-RESISTANT *Staphylococcus aureus* ISOLATES CAUSING INVASIVE INFECTIONS IN PARAGUAYAN CHILDREN**

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In recent years there has been an increase in the incidence of infections caused by methicillin-resistant *S. aureus* (MRSA) strains, both in Paraguay and in the world. Bacterial biofilms constitute a resistance mechanism, which can cause treatment failure and the chronicity of infections. In this context, the study of the antimicrobial activity of plants has acquired renewed interest due to a large number of compounds with biological activity that they can produce. This experimental study was carried out to evaluate the inhibitory effect of plant extracts on the formation of biofilm in MRSA isolates that cause invasive infections in Paraguayan children, representative of the most frequent clones in Paraguay and the region. The inhibitory effect of the biofilm formation of 20 methanolic plant extracts, of genera for which there are bibliographic references of some antimicrobial activity, was analyzed on the mentioned SARM isolates. The test was carried out using the method described by Stepanovic et al. (2000), which is based on the formation and fixation of the biofilm in the wells of the flat-bottom polystyrene microplate and staining it with crystal violet. The optical density (OD) reading was performed at 570 nm in an ELISA plate reader (Multiskan GO, ThermoFisher Scientific, Finland). The inhibition percentage of each extract was calculated mathematically, and the cut-off point criterion for the interpretation of the plants extracts effect on the biofilm as described by Famuyide et al. (2019). Excellent inhibitory activity was observed for five of the 20 extracts studied, with high inhibitory activity against most of the SARM isolates used. These findings constitute a starting point for the exhaustive study of these plants, to elucidate the possible components involved in the observed inhibitory effect and to determine the mechanisms of action involved in it.

125. (266) **PRESERVATION OF PROTECTIVE EFFECTS OF HYPERIMMUNE ANTI STX2 BOVINE COLOSTRUM AGAINST EHEC O157:H7 PATHOGENICITY AFTER PASTEURIZATION AND SPRAY-DRYING PROCEDURES.**

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Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is a major etiologic agent responsible for bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Shiga toxin (Stx) is the main virulence factor of EHEC and the main responsible for the onset of HUS. Although many efforts have been made to develop an effective treatment for Stx-mediated HUS, a specific therapy has not been found yet. It is well known that human consumption of bovine colostrum has therapeutic effects against several gastrointestinal infections, as it contains a range of peptides and proteins (including antibodies) with antimicrobial and endotoxin-neutralizing effects. Consistent with these findings, we have previously demonstrated that colostrum from Stx type 2 (Stx2)-immunized pregnant cows effectively prevents Stx2 cytotoxicity and EHEC O157:H7 pathogenicity. This study evaluated different pasteurization (72°C for 15 s vs 60°C for 60 min) and spray drying paramenters (Inlet temperature 110°C vs 120°C and outlet temperature lower or higher than 55°C) in order to optimize the preservation of the protective properties of hyperimmune colostrum (HIC-Stx2) against Stx2 after processing. Characterization of neutralizing properties of colostrum were assayed *in vitro* and *in vivo*. Our results showed that pasteurization at 60°C for 60 min, combined with spray-drying at inlet and outlet temperatures of 120°C and >55°C, respectively, showed the highest yield and lowest humidity on final samples ($p<0.05$). Reconstituted HIC-Stx2 colostrum after processing under optimized conditions preserved specific IgG quantity and effectively neutralized Stx2 cytotoxicity on Vero cells. Furthermore, this pasteurized/dehydrated and reconstituted HIC-Stx2 preserved the protective properties against EHEC infection in a weaned mice model. In this regard we propose that hyperimmune bovine colostrum has the potential to be administered to patients with the aim of protecting children against EHEC infection.

126. (288) **ROLE OF ESPF FROM ENTEROHAEMORRAGIC *ESCHERICHIA COLI* O157:H7 ON THE MECHANISMS RELATED TO PRODUCTION OF SHIGA TOXIN TYPE 2.**

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Enterohaemorrhagic *Escherichia coli* O157:H7 (EHEC) strains are responsible for multiple clinical syndromes including bloody diarrhea, hemolytic uremic syndrome (HUS). HUS is a systemic disease

caused mainly by Shiga toxin type 2 (Stx2). EHEC employs a type III secretion system to colonize the bowel and to inoculate effector proteins such as EspF. This protein is responsible to disrupt tight junctions, inhibit phagocytosis and induce effacement of microvilli and apoptosis. However, the role of EspF on the mechanisms related to Stx2 production across human intestine are not well known. The EHEC*ΔespF* mutant was constructed by Chengsong Wan and co-workers (Zhao et al. PlosOne 2013). We have evaluated the equivalent amount of Stx2 on Vero cells (ATCC CCL-81) cultured with the supernatants of EHEC wild type (SNwt) and EHEC *ΔespF* (SN*ΔespF*).

Both strains, EHEC O157:H7 wt and EHEC O157:H7 Δ*espF* were grown in LB medium for 18 h at 37 °C in LB with shaking at 150 rpm and then diluted 1:10 in DMEM/F12 medium with the addition of 10 mM of HEPES and grown to exponential phase (optical density at 630 nm of 0.3-0.4). Bacterial supernatants were collected after centrifugation at 10,000 *g* for 5 min and sterilized by filtration through a 0.22-μm-pore-size filter. The titers of Stx2 of filter-sterilized supernatants were determined on Vero cells.

A significant cytotoxic effect was observed when monolayers of Vero cells were exposed to different concentrations of purified commercial Stx2 under growth-arrested conditions. The CD_{50} was maximal after 72 h of incubation. Both, SN wt and SN *ΔespF* showed similar degrees of cytotoxicity on Vero cells corresponding to equivalent amount of Stx2 calculated by a non-parametric Mann-Whitney test.

This initial trial shows that the absence of the gene *EspF* might not significantly affect secretion of Stx2 in the bacterial culture. More studies are needed to evaluate the effect of EspF on Stx2 production of EHEC.

127. (290) **CONSIDERING PRIORITIES FOR THE APPLICATION OF A FUTURE COVID-19 VACCINE USING A MODEL BASED ON SUBPOPULATIONS WITH DIFFERING CONTACT RATES**

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Although many attempts have been made to mathematically model the SARS-CoV-2 epidemic, few models have been conceived as interactive tools for users of diverse backgrounds. The goal of this study was to develop a model that incorporated heterogeneity in contact rates and to implement it in a user-friendly application for the estimation of the impacts of possible interventions. An extension of the Susceptible-Exposed-Infected-Resistant (SEIR) model was developed, called SEIR-HL which assuming two subpopulations with different contact rates. A formula for the calculation of the basic reproduction number (Ro) for n subpopulations was derived. SEIR-HL was compared to SEIR showing that the incorporation of contact rate heterogeneity results in predictions with earlier but smaller infected peaks and lower total numbers of infected. SEIR-HL was used to show that transferring individuals from high-contact (H) to low-contact (L) positions delays and reduces infected peaks and lowers the total number of infected. Fitting SEIRL-HL to SARS-CoV-2 epidemic data in Argentina shows that the data is consistent with an increase in contact rates around May. Finally, since a future vaccine supply is unlikely to suffice for massive vaccination, SEIR-HL and the Ro formula were used to estimate the impact of the selective vaccination of age groups, the 20-39 year population being the best target for the reduction of Ro (and therefore of the spread of the infection) and the population over 70 being the best target for the reduction of hospitalization and mortality. The model also predicts that the younger population with high contact rate is more likely to have a high fraction of recovered individuals as the epidemic progresses meaning that vaccination of this population might be to a great extent redundant. The SEIR-HL model, an extended version for more than two subpopulations and a Ro calculator were implemented informatically and made available to the community (ecm.famaf.unc.edu.ar).

128. (311) **STUDY OF THE FREQUENCY OF TEMPORARY APPEARANCE OF COVID-19 CASES IN ARGENTINA, SANTA FE AND ROSARIO, FOR PROGNOSTIC PURPOSES**

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The COVID19 pandemic was generated by the new beta coronavirus SARS type 2. The viral behaviour recreates a fractal rhythm,property of dynamic and non-linear systems, feasible to be analyzed by mathematical algorithms. The frequency of temporal appearance and the state of adaptation to the environment of COVID-19 can be determined through the Fractal Dimension (DF) and the predictive determination coefficient (R^2). R^2 ranges from 0 to 1, values below 0.5 would indicate a decrease of the COVID-19 system response to the environment demands. It was proposed to investigate the predictive behaviour of COVID-19 according to its frequency of temporal appearance by the Higuchi algorithm (AH) in Argentine Republic (RA), Santa Fe Province (PSF) and Rosario City (CR). Observational, longitudinal and prospective study. Positive cases (CP) of COVID-19 were considered by testing (PCR swabbing) according to daily reports from the Ministry of Health of RA and PSF, from the epidemiological week (SE) in which the first CP appeared in each territory until the SE-36. AH was applied by SE. DF and R^2 were expressed as median (M) and Standard deviation (±). Outcomes: DF (RA): M = 1.40 ± 0.3, R^2 (RA): M = 0.84 ± 0.13; DF (PSF): M = 0.62 ± 0.16, R^2 (PSF): M = 0.49 ± 0.15 and DF (CR): M = 0.43 ± 0.13, R^2 (CR): M = 0.42 ± 0.14. Pearson correlation coefficient obtained between DF and R^2 for RA: r = 0.91 ($p<0.0001$); PSF: r = 0.74 ($p<0.0008$) and CR: r = 0.82 ($p<0.0001$). It is concluded that COVID-19 behaviour in RA shows growing and sustained temporal manifestation and interaction of the system with the environment, while in PSF and CR it has found limitations to interact with the environment. COVID-19 could hold for longer in RA compared to PFS and CR if the actual conditions sustain. This behaviour requires studying the impact of sanitary measures in analyzed territories.

129. (318) **PREDICTIVE ANALYSIS OF THE TERRITORIAL DISTRIBUTION OF COVID19, BY BOX COUNTING, IN THE ARGENTINE REPUBLIC AND THE PROVINCE OF SANTA FE**

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Coronavirus 2, from the severe acute respiratory syndrome (SARS-CoV-2), a disease called COVID19; resulted in a pandemic. COVID19 would have a nonlinear and deterministic behaviour, with a finitude that could be predicted with mathematical models through the determination of the Fractal Dimension (DF) and the coefficient of determination (R^2). DF would express the territorial occupation of COVID19 and R^2 the ability to respond to the environment demands. DF surpasses and fractionates the topological dimension. R^2 ranges from 0 to 1, and values greater than 0.5 would indicate persistence of

the system. It was proposed to study the spatial distribution of Positive Cases (CP) for COVID19, through the Box Counting algorithm (ABC), in Argentine Republic (RA) and Santa Fe Province (PSF) by epidemiological week (SE) with prognostic purposes. CP were considered by testing (PCR swabbing). Digitized maps of RA and PSF were created with the Flourish Studio software since the SE when the first CP was registered until the SE-33. Weekly CP geolocation was carried out, per province in the RA maps and per city in PSF; the representative point was proportional to a reference point established according to the number of total PC. The data were obtained from daily reports from the RA and PSF Ministry of Health. Frackout! software was used to apply ABC. Median (M) and standard deviation (±) of DF and R^2 were obtained. Pearson's correlation coefficient (r) between both variables was considered. Outcomes: DF (RA): M = 0.94 ± 0.21; R^2 (RA): M = 0.97 ± 0.02; DF (PSF): M = 0.77 ± 0.25; R^2 (PSF): M = 0.96 ± 0.06. The Pearson coefficient was for: DF vs R^2 in RA: r = 0.92 (p = <0.0001), and for PSF: r = 0.76 (p = <0.0055). It is concluded that ABC reveals that COVID-19 in RA and PSF is adapted to environmental stressors; Until SE-33, no COVID-19 finitude was shown in the studied territories. This raises continuation and deepening of the study and of the external conditioning factors.

130. (330) **MOLECULAR CHARACTERIZATION OF A NEW SEROTONERGIC G-PROTEIN COUPLED RECEPTOR FROM CESTODES: NEW POTENTIAL TARGET FOR DRUGS AGAINST NEGLECTED TROPICAL DISEASES**

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Introduction: *Echinococcus canadensis* is a platyhelminth parasite that belongs to the class Cestoda and is the etiological agent of Hydatid disease, a neglected disease that affects public health and economy in Argentina and worldwide. Currently, the treatment for echinococcosis in humans relies on benzimidazoles. However, the emergence of resistant parasites, makes the discovery of new anthelmintic drugs an imperative need. To tackle this problem, we propose to characterize G-protein coupled receptors from cestodes as new pharmacological

targets. In our previous work1, we found that serotonergic GPCRs (5-HT GPCRs) are of major importance in cestode movement and showed distinctive pharmacology. **Objective**: the aim of this work was the bioinformatical characterization, function and localization of a new 5-HT GPCR from *Echinococcus canadensis*. **Material and methods:** Bioinformatics analyses suggest the existence of genes encoding 5-HT GPCRs. Using this information, a novel cDNA coding for a new 5-HT GPCR was cloned, sequenced and expressed in HEK293 cells. Intracellular levels of calcium were measured. Hyperimmune antiserum was generated against the receptor protein and confocal laser microscopy was used to study the localization of the receptor. **Results:** When the cell line was transfected with a gene encoding for the receptor, the calcium levels increased only in the presence of serotonin but not with of other biogenic amines. Whole mount immunofluorescence revealed branched fibers corresponding to the nervous system of the worm. **Conclusion:** The dataset confirms the bioinformatic analyses showing that the cloned gene codes for a new 5-HT GPCR conserved in cestodes with major roles in the nervous system of the parasite. The molecule analyzed here could be exploited at the pharmacological level to design or repurpose drugs to treat neglected diseases caused by cestode parasites.

References

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131. (331) **ELIGLUSTAT AS A POSSIBLE STRATEGY TO PREVENT THE DETRIMENTAL EFFECTS OF SHIGA TOXIN TYPE 2: PRELIMINARY RESULTS *IN VIVO***

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Typical Hemolytic Uremic Syndrome (HUS) is a complication of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) infection and the most frequent cause of acute renal failure in children in Argentina. Stx2 binds to globotriaosylceramide (Gb3) receptor and causes direct damage on human renal microvascular endothelial cells (HGEC). Previously, we found that Eliglustat (EG), a Gb3 synthesis inhibitor, prevents the cytotoxic effects of Stx2 on HGEC. In this work, we evaluated the action of EG against the effects of Stx2 *in vivo*. Male BALB/c mice at weaning (17-21 days) received 3 doses of EG (0.6 mg/g body weight (bwt) administered intraperitoneally (i.p.) every 24 h. After a rest period of 5 days, mice were i.p. injected with a lethal (1ng/g bwt) or sublethal (0.1 ng/g bwt) dose of Stx2 (EG+Stx2) or PBS (EG). Two additional groups of mice without EG pre-treatment were injected one with PBS (Ctrl) and another with Stx2 (Stx2). Survival, body weight (Δ weight= body weight after Stx2 or PBS injection-body weight at a day before injection) and food intake were registered daily. EG did not affect body weight gain (Δ weight: EG: 0.61 ± 0.07 g *vs.* Ctrl: 0.76 ± 0.09 g; n=3, ns). After Stx2 lethal dose treatment, EG+Stx2 mice showed a body weight decrease and a survival time (48-72 h) similar to Stx2 mice. On the contrary, after 3 days of Stx2 sublethal dose injection, while Stx2 mice exhibited piloerection and inactivity and body weight loss, EG+Stx2 mice did not show signs of illness and gained weight (Δ weight: EG+Stx2: 0.81 ± 0.09 *vs.* Stx2: -0.65 ± 0.05 g; n=3, p<0.05). Body weight loss in Stx2 mice was associated with a significant decrease (70%) in food intake, unlike EG pre-treated mice that reduced intake by only 15% (n=3, p<0.05). These results suggest that EG may reduce the disease symptoms caused by Stx2, such as poor appetite and the resulting body weight loss. Future studies will analyze if EG prevents the renal damage and will improve EG treatment to avoid mortality.

132. (364) **BENZHYDROXAMATE DERIVATIVES ARE POTENTIAL ANTHELMINTIC DRUG AGAINST NEGLECTED TROPICAL DISEASES CAUSES BY CESTODES**

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Neglected tropical diseases (NTDs) caused by cestodes, such as echinococcosis and cysticercosis, represent a serious public health problem in many countries; including Argentine. These diseases have a reduced number of safe and efficacious approved anthelmintic drugs; therefore, the identification of novel drug candidates is urgently required. In this work, we present the anthelmintic profile of several series of recently developed selective histone deacetylase (HDAC) inhibitors (benzhydroxamate derivatives) against the isotype 8, using the model cestode *Mesocestoides vogae* (syn. *M. corti*). Phenotypic screenings were performed measuring parasite

motility together with optical microscope observations. Several compounds showed potent anthelmintic activities, producing a significant reduction on parasite viability and inducing extensive alterations on general morphology. Two of these compounds, TH65 and TH92, showed the most powerful anthelmintic effects, displaying anthelmintic effects in a dose-dependent manner, with micromolar range activities, and inducing drastic alterations on general morphology and ultrastructural features. These effects were irreversible and with higher potencies than the current anthelmintic drug albendazole. Additionally, binary combinations of HDAC8 inhibitors and albendazole showed potent anthelmintic effects in a time-dependent manner at very low concentrations, suggesting an apparent synergistic effect. However, the effects determined for some compounds were not comparable in concentration and/or incubation time to those previously reported on *Schistosoma mansoni* viability and human cell lines, suggesting differences in the affinity for HDAC8 from *M. vogae* compared to other HDAC8s. We expect that this work contributes to understand the role of HDAC enzymes on cestodes, in order to aid in developing of new anti-parasitic treatments against NTDs caused by these parasites.

133. (373) ***IN VITRO* ANTHELMINTIC ACTIVITY OF *STEVIA MULTIARISTATA* DICHLOROMETHANE EXTRACT ON *ECHINOCOCCUS GRANULOSUS* PROTOSCOLECES**

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Cystic echinococcosis (CE) is a worldwide zoonotic disease caused by the larval stage of the parasite *Echinococcus granulosus,* which causes long-term infections in humans and animals, being a serious public health problem. Albendazole, the main drug used against CE, has undesirable side effects and their efficacy is about 50%. Thus, n*ew* treatment alternatives are urgently needed. In the last few decades, there *has* been *an increased interest* in studying the anthelmintic activity of natural products. Plants from *Stevia* genus (Asteraceae) are a potential source of antiprotozoal and antimicrobial compounds. The antiparasitic activity of extracts from different *Stevia* species has recently been demonstrated on *Trypanosoma cruzi* and *Leishmania braziliensis*. In the current study, we demonstrated the *in vitro* efficacy of the *Stevia multiaristata* dichloromethane extract against protoscoleces of *E. granulosus*. Viable and free protoscoleces were treated with 100, 50, 10 and 5 µg/ml of the extract. Culture tubes were followed microscopically every day. Viability assessment using the methylene blue exclusion test and ultrastructural studies with scanning electron microscope were performed. Control protoscoleces remained viable throughout the experimental period and no morphological changes were observed. The greatest protoscolicidal effect was observed with the concentrations of 100 and 50 µg/ml, where viability decreased rapidly to 0 % at days 6 and 9, respectively. The concentrations of 10 and 5 µg/ml reduced the viability to 50% between days 5 and 6. At day 1, the protoscoleces treated with 100 µg/ml showed soma contraction and at day 3 a total loss of morphology, total loss of microtriches, rostellar disorganization and presence of blebs in the tegument. In conclusion, *S. multiaristata* extract demonstrated a marked *in vitro* effect against *E. granulosus* larval stage.

134. (432) **ANTI-HBS SEROCONVERSION IN ANTIVIRAL TREATED HEPATITIS B VIRUS CHRONIC PATIENTS OF MAR DEL PLATA**

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HBsAg clearance is considered the primary goal of the antiviral treatment in HBV chronic patients. This event may be followed by the seroconversion or the appearance of anti-HBs antibodies, indicating recovery and a life-long immunity against HBV. The aim of this study was to describe the characteristics and the evolution of a cohort of patients who achieved anti-HBs seroconversion after antiviral treatment. All 6 patients included in the study were male, with a median age of 48.33 years (34-64 years). They have been previously diagnosed with chronic HBV infection and treated with nucleos(t)ides analogues (entecavir or tenofovir). All patients were HBeAg (+), indicating no long term chronic infections. The biochemical and virological responses were followed during treatment evaluating different parameters. At diagnosis transaminases were elevated in all cases, but normalized in half of the cases at week(wk)-12 and in the rest half at wk-48. Elevated total and direct bilirubin levels were detected in 2/6 patients and one of them normalized at wk-48. All patients showed high pretreatment viral loads (>108copies/mL), resulting over treatment in undetectable loads in 1/6 patients at wk-24 and in 5/6 at wk-48. Regarding seroconversion, all patients achieved HBsAg loss in a mean time of 32 months (12-72 months) and anti-HBs detectable titles in 40 months (15-96 months) since the undetectable viral load. To indeep in the characterization, genomic sequences were obtained from subgenotye F1b samples (the most prevalent in the city). No mutations at BCP and pC regions were detected and neither mutations associated with antiviral resistance were found at the POL gene. In conclusion, the analysis of these cases provide evidence that the treatment with nucleos(t)ides analogues can produce the functional cure of HBV by the seroclearance of HBsAg, but also can be associated with anti-HBsAg seroconversion, indicating a clinical recovery and a life-long immunity from HBV infection.

135. (531) **EFFICACY OF TOPICAL RISEDRONATE AND RISEDRONATE - EUDRAGIT E COMPLEX IN A MODEL OF CUTANEOUS LEISHMANIASIS INDUCED BY *LEISHMANIA (L.) AMAZONENSIS***

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An efficacious topical treatment for cutaneous leishmaniasis (CL) is highly desirable but still an ongoing challenge. Systemic risedronate (Ris) has been reported to have anti-leishmania properties and Eudragit EPO (EuE) has shown in vitro activity against *L. (L.) amazonensis*. The aim of this work was to investigate the in vivo efficacy of topical Ris and EuE-Ris complexes on CL. Surface charge and Ris release kinetics from the different dispersions were analyzed. BALB/c mice were infected intradermally with promastigotes of *L. (L.) amazonensis*. Ulcers were treated with 100 µl of Ris or EuE-Ris hydrogels, both containing 2 mg of Ris, once a day from Monday to Friday during three weeks. All the lesions that received topical Ris or EuE-Ris showed an improvement with respect to control: reduction of ulcer average size, cicatrisation, flattened edges and no signs of necrosis. In addition, a marked parasitic inhibition of 69.5 and 73.7% was observed in the groups treated with Ris and EuE-Ris, respectively. The IgG2a/IgG1 ratio in mice treated with Ris (0.53) or EuE-Ris (0.4) showed higher levels than in the control group without

treatment (0.29), indicating a tendency towards cure. The results are promising and the system should now be enhanced to achieve total parasite elimination.

136. (563) **INTEGRACIÓN DE HERRAMIENTAS DIAGNÓSTICAS PARA EL ESTUDIO DE LA ENFERMEDAD DE CHAGAS CONGÉNITO EN NIÑOS NACIDOS EN EL HOSPITAL PÚBLICO MATERNO INFANTIL (HPMI) DE SALTA CAPITAL**

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La Enfermedad de Chagas congénita es de naturaleza multifactorial (parásito, madre, placenta y feto) donde la probabilidad de transmisión vertical depende de interacciones complejas entre agente causal, respuestas inmunes maternas/fetales y factores placentarios. Objetivo: integrar herramientas diagnósticas para el estudio de Chagas Congénito. Métodos: Se reclutaron 82 madres al control prenatal (3° trim) en HPMI, positivas para la infección por T.cruzi por serología convencional. Obtuvimos muestras de sangre de madres, tejido placentario y sangre de cordón al parto y de los recién nacidos (RN) sangre periférica (SP) al día 1, 30 y/o 90. Todas se analizaron por PCR convencional (ADN parasitario), qPCR (carga parasitaria), en los RN se efectúo microstrout (MS) y serología convencional a los 9 meses (protocolo de OPS/OMS). Las placentas se evaluaron histólogicamente (H&E, Gomori) e IHQ. Resultados: 13.067 partos se asistieron en HPMI entre Agosto 2018 a Junio 2020; 184 madres fueron seropositivas (1.4%) y 7 niños tuvieron infección congénita de T.cruzi. Dos RN se diagnosticaron por MS al nacer, ningún MS de cordón resultó positivo. Las PCR resultaron positivas en 3 (42%) cordones y en el 100% de SP de RN. Las madres seropositivas+PCR positiva tuvieron más probabilidad de transmisión vertical que las de PCR negativa, pero sin alta carga parasitaria (resultados preliminares de qPCR). Al análisis histopatológico las muestras placentarias de madres transmisoras presentaron parásitos en forma libre y en nidos a nivel de decidua, corion, cordón umbilical y membranas, pero con escasa reacción inflamatoria. A pesar de los intensos esfuerzos, sólo el 57,7% de los bebés en riesgo regresaron al 9° mes para diagnóstico. Conclusiones: Dada baja sensibilidad del microstrout y la alta tasa de pérdida de seguimiento, los programas de detección actuales no alcanzan al 43% de los RN. Las técnicas moleculares o evaluación histopatológica placentaria podrían mejorar la detección precoz.

INMUNIDAD ANTINFECCIOSA

137. (292) **NEUTRALIZING PROPERTIES OF HUMAN MILK AGAINST SHIGA TOXIN TYPE 2**

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Shiga toxin (Stx) producing *Escherichia coli* (STEC) is a foodborne pathogen responsible for Hemolytic Uremic Sindrome (HUS). Stx is the main virulence factor responsible for this disease and Stx type 2 (Stx2) has been associated with more severe cases affecting mainly children under 5 years of age. Breastfeeding is one of the least cost-effective public health tools to protect the newborn from diarrhea and its effectiveness can be attributed to different bioactive compounds transmitted through milk. We have previously demonstrated that rats immunized against Stx2 can transfer through lactation protection against a lethal dose of Stx2 to their offspring. In this work we aim to evaluate whether human milk from human healthy donors may have protective properties against Stx2. Human milks (n=107) were collected under informed consent at the National Hospital Prof. Alejandro Posadas by the manual method from healthy women (18-45 years). After collection, milks were stored at 20°C until used. Milks were delipidated by centrifugation at 3000 rpm for 45 min. Supernatant was collected from samples and used for the evaluation of: 1) Neutralizing capacity of Stx2 *in vitro* on Vero cells, 2) Total protein content by the BCA kit 3) Total IgA content by radial immunodiffusion (RID) and 4) Ig Anti STEC titer by ELISA. Collected milk samples showed a mean of 229 mg/dL of total IgA levels (range: 10-675 mg/dL). Total protein content of samples was heterogeneous and ranged from 5.5 to 166.5 mg/ml. Fourteen milk samples (14/107; 13%) showed neutralizing properties against Stx2 *in vitro*. When comparing IgA levels and protein content of positive and negative samples for Stx2 neutralization, no significative differences were observed. Neutralizing capacity of Stx2 has no correlation with high Ig titer against STEC. These results indicate that some human milk may have neutralizing properties against Stx2. These data may be important for HUS prevention of newborn by promoting breastfeeding.

138. (304) **CORRELATION BETWEEN PROINFLAMMATORY MEDIATORS AND VENTRICULAR DYSFUNCTION IN AN EXPERIMENTAL MODEL OF CHAGAS DISEASE IN IL10 KO MICE**

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Chagas disease, caused by Trypanosoma cruzi (Tc) infection, is conditioned by parasite persistence and the development of an inflammatory response. Interleukin 10 (IL10) is a pleiotropic cytokine involved in the regulation of inflammatory processes. In previous works we showed that Fenofibrate (Fen), a PPARα synthetic ligand, modulates inflammation and restores cardiac function in T. cruzi -infected wild type mice. Furthermore, infection of IL10 knockout mice (IL10 KO) with the K98 clone of CA-I Tc strain triggered ventricular dysfunction and the increase of expression and concentration of proinflammatory mediators. Fen treatment inhibited the expression and release of inflammatory mediators and restored the ventricular function. Taking this into account, we considered to analyze the correlation between various proinflammatory mediators and ventricular function. Using Spearman correlation test, we observed that there is a strong correlation between the increased mRNA expression of IL6, NOS2, TNFα and TGFβ in cardiac tissue, as well as with the increase in plasma concentration of IL6, TNFα, and IL17 with the decrease in the percentage of both Ejection Fraction (EF) and Shortening Fraction (SF) in Tc-infected IL10 KO mice. The strongest correlations were observed between the concentration of IL6 (r=-1) and TNFα (r=-0.94), and expression of TGFβ mRNA (r=-0.95) with both EF and SF. Treatment with fen inhibits the expression and release of these mediators, thus improving ventricular function. This is evidenced by the decrease in the correlation between these proinflammatory mediators, and both EF and SF. Meanwhile, no asso-

ciations were found between the studied parameters in WT mice, since infection did not cause ventricular dysfunction. These results suggest a strong correlation between expression and release of proinflammatory mediators and ventricular dysfunction caused by K98 infection in IL10 KO mice.

139. (348) **CHARACTERIZATION OF SUBPOPULATIONS OF MONOCYTES ACCORDING TO THE STAGE OF CHRONIC CHAGAS DISEASE. MODULATORY ROLE OF FENOFIBRATE**

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Chronic cardiomyopathy is the most important clinical manifestation of Chagas disease. We previously demonstrated in a murine infection model that fenofibrate (Fen), PPARα agonist, controls inflammation, prevents fibrosis and improves cardiac function. Considering that different subpopulations of monocytes (mono) are involved in the progression of cardiomyopathy, we decided to characterize these subpopulations according to the stage of the disease and to evaluate if in vitro treatment with Fen drives mono towards a tissue repair profile. Seventeen healthy individuals (HI) and 16 patients with positive serology for Chagas, without (E0) and with chronic Chagas cardiomyopathy (CCC) were included. Peripheral blood mononuclear cells were purified, cultured and stimulated or not with T. cruzi (Tc) lysates (10µg / ml), and treated with Fen (100µM). Under basal conditions, CCC showed a higher percentage of classical mono than E0 ($p < 0.05$) and E0 a higher percentage of non-classical mono than HI ($p < 0.05$). Stimulation with Tc increased the percentage of classical mono in E0 and Fen inhibited this effect ($p < 0.05$). We also found that Fen increases non-classical mono in HI and E0 ($p < 0.05$). To decipher the ability of mono recruitment to the site of infection or their repair functions, we evaluated CCR2 expression. Tc decreased both the expression of CCR2 as well as the percentage of CCR2+ mono ($p < 0.05$) in HI, E0 and CCC, and Fen tended to restore it. The same effect was observed in mono subpopulations. Altogether, these preliminary results suggest that E0 patients remain in a ?more alert? state, with a higher percentage of basal non-classical mono that increases with Fen. Signs of inflammation or damage might mobilize them for rapid endothelial transmigration. We also believe that Tc stimulation triggers the internalization of CCR2, and Fen might reverse this effect.

140. (196) **NITRIC OXIDE INHIBITION IMPROVES CYTOTOXIC IMMUNE RESPONSE IN A MURINE INVASIVE BLADDER CANCER MODEL**

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Bladder cancer (BC) is a common malignancy of male urinary tract. According to the invasion degree, tumors are classified as non-muscle invasive (NMI) and invasive (MI). The constitutive expression of the inducible isoform of nitric oxide synthase (iNOS), which produces high levels of nitric oxide (NO), is a poor prognostic marker in human BC, associated with invasion and early recurrence. We previously showed that its pharmacological inhibition with L-NAME in NMI tumor-bearing mice (TBM) reversed tumor immunosuppression and improved the response to gold standard BCG treatment.
Objectives: To evaluate the variations on cytotoxic ($CD8^+$ and NK cells) and immunosuppressive (Treg) immune populations on iNOS-expressing MI murine BC model under L-NAME treatment and to analyze its combination with BCG.
Methods: *Ex vivo*: Normal splenocytes were stimulated *in vitro* with MB49-I cells in presence of L-NAME (2mM) +/- BCG (2mg/ml). *In vivo:* Splenic and tumor leucocytes of normal mice and MI MB49-I TBM treated with 0.5 g/L L-NAME in drinking water were analyzed. The percentage of $CD8^+$, NK cells and Treg were quantified by flow cytometry.
Results: The *ex vivo* stimulation of splenocytes with MB49-I cells, was able to increase $CD8^+$ ($p \leq 0,01$) and reduce Treg ($p \leq 0,05$), respect to unstimulated splenocytes. The combination of L-NAME with BCG increased NK cells ($p \leq 0,01$) and reduced Treg ($p \leq 0,05$), inducing the highest relation $CD8^+$/Treg ($p \leq 0,001$). MB49-I TBM presented a decrease in $CD8^+$ and NK cells ($p \leq 0,001$) and an increase in Treg ($p \leq 0,05$) compared to normal mice. L-NAME treatment was able to decrease Treg and increase $CD8^+$ ($p \leq 0,001$).
Conclusion: NO inhibition with L-NAME reverses an immunosuppressive state in MB49-I TBM. Its combination with BCG improves the relation $CD8^+$/Treg, suggesting an increased anti-tumor immunity. Inhibitors of NO production may be potential immunomodulators for patients with iNOS positive BC.

141. (256) **MODULATION OF T CELLS IN A MURINE BREAST CANCER MODEL DEVELOPED IN HISTAMINE H4 RECEPTOR DEFICIENT MICE**

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The histamine H4 receptor (H4R) is preferentially expressed in immune cells and is a potential therapeutic target for inflammatory and autoimmune diseases. This study aimed at further exploring the role of H4R in the immunobiology of breast cancer.
We used wild type (WT) and H4R deficient (H4R-KO) Balb/c mice to evaluate whether H4R genotypes show different distribution of T cell subsets in spleens, tumours and tumour draining lymph nodes (TDLN) in a syngeneic ErbB2-positive breast cancer model developed orthotopically with LM3 cells and its impact on tumour growth. In addition, the migration capacity of TDLN cells from WT and KO animals was analyzed.
The presence of tumours had a differential impact on the distribution of T cells in TDLN from H4R-KO mice compared to WT ones. At day 21 post inoculation (p.i.) of cells, despite no significant changes in the tumour weight, TDLN from H4R-KO mice showed a significantly increased proportion of $CD8^+$ T cells compared to WT mice ($P=0.0052$). At day 38 p.i. of cells a reduced tumour weight was evident in H4R-KO mice ($P=0.0431$). This was accompanied by a decreased proportion of $CD4^+CD25^+FoxP3^+$ regulatory T cells in TDLN of KO compared to WT mice ($P=0.0104$). In agreement with the tumour weights, the spleen weight was significantly reduced in tumour-bearing KO mice compared with WT counterparts ($P=0.0280$). Tumour-bearing KO mice showed a better survival compared to WT mice ($P=0.0511$). Furthermore, H4R deficient TDLN lymphocytes showed impaired chemotaxis compared with WT mice ($P=0.0054$).
We conclude that H4R-mediated mechanisms may modulate the immune tumour microenvironment, promoting an immunosuppressive milieu. Results suggest that H4R could be explored as an immunotherapeutic target with potential benefit in combination with immunotherapy.

142. (272) **IMMUNOTHERAPEUTIC STRATEGIES WITH META TYROSINE AND INHIBITORS OF P38 PATHWAY IN LOCAL RECURRENCES AND METASTATIC MURINE TUMOR MODELS**

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Immune-checkpoints inhibitors (ICI) have been considered the most promising anti-tumor immunotherapy in the last years. However, ICI were effective to restrain only incipient experimental tumors. Failures of immunotherapy may be associated with the fact it would produce weak immune responses that would promote rather than inhibit tumor growth upon activation of TLR4 and p38 signaling path-

ways. Herein, we compare the effect of ICI on the growth of both incipient and residual tumors. Then, we have combined ICI with meta-tyrosine (m-tyr) - which, according to previous results, might restrain immune checkpoints not counteracted by classical ICI - and SB 202190, a selective inhibitor of p38, to counteract the tumor-immune-stimulation. We have used two murine tumor models: a local recurrence after subcutaneous (s.c.) MC-C fibrosarcoma (about 800 mm3) was surgically excised leaving intact the underlying skin; and a metastatic model after s.c. LMM3 mammary carcinoma (LMM3) was radically removed when spontaneous lung metastases were already established. One day after surgery, mice were treated with ICI (anti-CTLA-4 for MC-C, or anti-PD-L1 for LMM3) or a combination of ICI with m-tyr and SB 202190. Local recurrences and metastases were not inhibited by the very same treatments that strongly inhibited incipient tumors ($p < 0.001$). In fact, growth of local recurrences upon treatment with ICI was enhanced similarly that mid-sized tumors from which residual tumors derived ($p<0.01$). The combined treatment not only produced tumor-inhibitory effects ($p<0.001$ for MC-C and $p<0.01$ for LMM3) but even it managed to cure about 80% of mice bearing local recurrences and about 40% of mice bearing lung metastases while in controls 100% of mice bearing residual tumors reached the humane endpoint in a month after surgery. The use of tumor models that closely resemble the real clinical situation might be useful to understand both the scope and the limitations of anti-tumor immunotherapies.

INMUNIDAD INNATA

143. (66) **CHARACTERIZATION OF NK CELLS PRESENT IN PEDIATRIC PATIENTS' TONSILS IN THE CONTEXT OF EBV INFECTION: AN IMMATURE POPULATION?**

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EBV is a member of the *Herpesviridae* family, that infects 90% of the word population. In children, NK cells, part of the innate immune system, play a fundamental role in the asymptomatic infection by EBV. It was described that the $NKG2A^+$ subpopulation can successfully control both primary infection and EBV-mediated transformation in children from developed countries.

In this study we characterized the NK cells in the tonsils of a cohort of 47 pediatric patients infected or not with EBV. We defined EBV status by serological assay, the presence of CD56, IFNγ and Granzyme B (GzB) by immunohistochemistry (IHC), and the expression of CD56. CD16. NKG2D, NKG2A, CD57, CD34 and IFNγ production by flow cytometry (FC).

We characterized 9 primary infected patients (PI); 27 healthy carriers (HC), 7 patients going through viral reactivation (R) and 4 non-infected patients (NI). The IHC assay did not show differences between groups in the mean counts of CD56, IFNg or GzB ($p> 0.05$ in all cases; Kruskal-Wallis test). By FC, we did not observe a particular increase in the IFNg production ($p>0.05$**;** Kruskal-Wallis test). The two major subpopulations of NK cells in the tonsils were $CD56^{Bright}$ (40.5%) and $CD56^{Dim}CD16^-$ (42.2%), without difference among EBV-infection groups ($p>0.05$; Kruskal- Wallis test). $CD56^{Bright}$ $NKG2D^-NKG2A^-$ subpopulation prevailed in all groups, particularly in older HC ($r=0.6173$, $p=0.0325$, Spearman correlation test). $CD56^{Dim}CD16^-$ cells displayed less proportion of $NKG2D^-NKG2A^+$ y $NKG2D^+NKG2A^+$ subpopulations ($p= 0.0369$ and $p= 0.0011$ respectively, Kruskal-Wallis test). Preliminary assays targeting the expression of markers of maturity showed high levels of $CD43^+$ cells ($28.04\% \pm 17.40$) and low proportion of $CD57^+$ cells ($13.38\% \pm 9.43$). This study reveals the presence of a less developed NK subpopulation, which could be related with a deficient control of the EBV infection. Further assays are required to determine the functionality of this potentially immature population.

Key words: NK cells, NK development, tonsils, pediatric tonsils, EBV, asymptomatic infection.

MEDICINA REGENERATIVA Y TERAPIA CELULAR

144. (121) **ANTI-INFLAMMATORY SMALL EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS IMPROVE DONOR LUNG PRESERVATION**

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Infusion of human mesenchymal stromal cells (MSCs) during donor lung ablation improves organ preservation by reducing oxidative damage and inflammation. Here, we studied whether the secretion of small extracellular vesicles (sEVs) mediates these beneficial effects. The rat lung preservation model used here consisted of: cardiac arrest, warm ischemia (2h), cold ischemia (1.5h), and normothermic ex-vivo lung perfusion (EVLP, 1h). Human umbilical cord-derived MSCs (1x10^6 cells) or their secreted sEVs were administered via the pulmonary artery after 1h of warm ischemia. A decrease in lung compliance indicates poor graft quality, and it was determined from pressure-volume curves at baseline and after warm ischemia or EVLP. Small-EVs were purified from MSC-conditioned medium by anion-exchange chromatography. They were positive for CD63- and CD81-antigen, and showed the typical size (mean 137 nm, range 90-190 nm) and morphology by nanoparticle tracking analysis and electron microscopy, respectively. Moreover, sEVs significantly suppressed the M1 phenotype acquisition in LPS-stimulated RAW264.7 macrophages (53% respect to control). Interestingly, sEVs inhibited tissue damage quickly. After warm ischemia, lung compliance dropped by 42% in the sEVs-treated group, while vehicle and MSCs groups showed a stronger reduction (73% and 76%, respectively). Warm ischemia also produced edema in all groups. Noteworthy, the benefit of EVLP on recovering edema and lung function was more evident in treated groups. Lung compliance significantly dropped by 62% between baseline and EVLP in the vehicle group, whereas MSCs and sEVs protected lung function as evidenced by a smaller decrease (35% and 27%, respectively). These data indicate that MSC-derived sEVs play a key role in preserving lung function. Compared to cell therapy, sEVs provide rapid protection of the organ and have a superior safety profile. Thus, sEVs may represent an innovative cell-free therapy for organ preservation.

145. (146) **DEVELOPMENT OF TWO *IN VITRO* ANGIOGENIC MODELS TO EVALUATE THE PRO-ANGIOGENIC CAPACITY OF DERMAL PAPILLA SPHEROIDS**

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Tissue-engineered skin represents a useful treatment of deep skin injuries. The neovascularization, essential to supply oxygen and nutrients to the cells in grafted constructs, remains a major challenge. We have shown that the presence of dermal papilla cells (DPCs) in these constructs favors the vascularization process, resulting in a better wound healing and graft take. We also have seen that 3D culture increases the expression of angiogenic factors as VEGF, angiogenin and FGF. Our objective was to fit two models to evaluate the DPC's culture influence over the ability of endothelial cells (HUVEC) to migrate and form tubules *in vitro*.

For the migration assay, isolated DPC or fibroblasts (Fbs) were cultured in a transwell and maintained at 37°C for 72 hours. HUVEC were then seeded on the insert and placed into wells. After 8 hours, we observed 12% more migrated cells in the coculture with DPCs than in the fibroblast and control.
For tubulogenic assay, DPC conditioned medium was concentrated 10 times and diluted by half in fresh medium. HUVEC were resuspended in the mixed medium and seeded on Matrigel. We observed an increase in the total tubule's length and in the number of segments and joints, compared to control. The higher differences were obtained at 5 hours.
To compare the inductive molecules secretion between 2D and 3D cultures, we looked for the condition in which both systems contained approximately the same amount of metabolically active cells. We obtained that 55 spheres of 5,000 cells each with 72 formation hours are metabolically equivalent to 10,000 cells/cm2 monolayer seeded cells, cultured during the same period.
Progress was made in the development of two angiogenic models that would allow us to study the influence of 3D culture of DPCs on the capacity of endothelial cells to migrate and to form tubules *in vitro*. In this way, its use in skin substitutes could favor the vascularization of the grafts, favoring the closure of the wound and the graft take.

146. (271) **NOVEL INTERPLAY BETWEEN P53 AND HO-1 TO MAINTAIN PLURIPOTENCY IN EMBRYONIC STEM CELLS**

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Embryonic stem (ES) cells have the ability to differentiate into all the adult cell lineages and are an invaluable resource due to its regenerative-medicine potential in the clinic. Stress mitigation in stem cells is critical both attributable to their essential role in development and the risk for malignant transformation. In these cells, redox status regulates the balance between self-renewal and differentiation, making crucial oxidative stress control. Heme Oxygenase 1 (HO-1) is a highly relevant component of the antioxidant system. Besides its cytoprotective function, HO-1 is involved in embryo development and in ES cells differentiation. At the same time, p53 controls cellular response to multiple types of harmful stimulus, including oxidative stress. Particularly, the primary role of p53 in ES cells is to promote differentiation in response to DNA damage or developmental signals. Despite the role of both proteins has been extensively studied, little is known about their relationship. The aim of this work is to explore the HO-1 - p53 interplay in ES cells. We found that p53 knockout (KO) ES cells have higher HO-1 levels but similar HO-1 mRNA levels than the wild type (WT) ES cell line. Furthermore, evaluation of NRF2, the main HO-1 transcription factor, showed no significant differences in both its total levels and its active form with phosphorylation at Ser40 between p53 KO and WT cells. Cycloheximide treatment revealed increased HO-1 abundance in p53 KO cells suggesting that p53 modulates HO-1 protein stability. Notoriously, despite higher HO-1 basal levels in KO cells, both hemin treatment and differentiation induced HO-1 expression. Finally, H_2O_2 treatment did not increase HO-1 levels in p53 KO ES cells evidencing an altered response to oxidative stress. Our findings demonstrate the existence of a connection between p53 and HO-1 in ES cells highlighting the relationship between these stress defense pathways in the stem cells context.

147. (281) **HUMAN AMNIOTIC EPITHELIAL STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS REDUCE CCL_4-INDUCED LIVER FIBROSIS IN MICE**

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The placenta and fetal membranes have been proposed as an important stem cell source for regenerative medicine. Amniotic epithelial cells (hAECs) can be isolated from the amnion of the human placenta at term. They are pluripotent and they have immunomodulatory, anti-inflammatory, and regenerative properties. These features position hAECs as an ideal candidate for cell therapy. Hepatic failure is one of the major causes of morbidity and mortality worldwide. The available treatments have several obstacles. Recently, hAECs have been spotlighted as an alternative hepatocytes source because of their potential for hepatogenic differentiation. The aim of this work was to study the regenerative capacity of the hepatocyte differentiated hAECs (HD) in a CCl_4-induced liver fibrosis mouse model. Previously, we have demonstrated that hAECs efficiently differentiate to hepatic-like cells. We demonstrated that these hepatocyte-like cells are functional by determining collagen and albumin expression. HD cells were transplanted into CCl_4-induced hepatic fibrosis BALB/c mice. After 4 weeks of injection, liver fibrosis characteristic parameters such as necrosis, collagen deposition, and liver enzyme levels were evaluated. We found that the fibrotic nodules decreased in livers transplanted with HD cells. Moreover, the HD cell transplantation reduced the necrosis area and the proinflammatory cell infiltration, evaluated by hematoxylin-eosin staining. We also observed that the collagen deposition, significantly diminished by 1.68-fold in the HD group, analyzed by Masson's trichrome staining. In addition, the ALT, AST, and AF liver enzyme levels decreased up to 1.18-fold, 1.25-fold, and 1.16-fold respectively, after HD cell treatment, compared with the CCl_4 group. Altogether, our results demonstrate the potential application of hAECs derived hepatocyte-like cells for liver disease treatment.

148. (381) **DEVELOPMENT OF CHITOSAN-BASED MICROSPHERES WITH POTENTIAL AS A MICROCARRIER IN TISSUE REGENERATION**

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The aim of this work was to explore a new biomaterial in the form of microspheres to create microcarriers for tissue regeneration. The microspheres were developed from a mixture of chitosan (CHI) and a copolymer of vinyl acetate and hydrolyzed diisopropyl fumarate (PVFH). For the synthesis of the microspheres, the mixture of PVFH and CHI solutions in a 50:50 ratio of the polymers was made, using borax as a crosslinking agent. The aqueous polymeric mixture was sonicated for 5 min and was dripped above olive oil with a controlled flow under 1500 rpm magnetic agitation at 28 °C. The emulsion obtained was left under stirring for 24 hours. Finally, the microspheres were isolated by centrifugation. The oil phase was discarded and successive washes were performed with distilled water until neutrality. The mean diameter and size distribution of microspheres were studied using a Nikon Eclipse TS100 inverted optical microscope and pictures were taken with a Nikon Coolpix 4500 digital camera. The biocompatibility of microspheres was assessed *in vitro* using murine macrophage RAW 264.7 cells and mesenchymal stem cells (MSC), in the presence and absence of microspheres. In both cases, proliferation was studied using the MTT bioassay at different times. Also, we evaluated the production of nitric oxide (NO) with RAW 264.7 as an immunogenic marker, using the Griess test. The mean diameter of the microspheres was 96.6 ± 2.8 μm. The results with RAW 264.7 cells on proliferation and NO levels did not show significant differences between cultures with and without microspheres at 24 and 48 h. In the assay using MSC, it was observed that proliferation at 48 h was favored in the presence of the microspheres. In conclusion, microspheres with a suitable size as a microcarrier were

obtained, which proved to have good biocompatibility and potential for use in tissue regeneration.

149. (299) **IDENTIFICATION OF MIRNAS REGULATED BY E2F TRANSCRIPTION FACTORS IN HUMAN EMBRYONIC STEM CELLS**

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Human embryonic stem cells (hESCs) exhibit an unusual cell cycle structure with an abbreviated G1 phase. MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in many key processes; including cell cycle regulation and cell differentiation, as post-transcriptional regulators of gene expression. E2F transcription factors (E2Fs) regulate G1/S transition. G1 duration contributes to hESCs fate determination and miRNAs play a key role in achieving this cell cycle regulation. Due to this, we aimed to identify miRNAs that are regulated by E2Fs in hESCs. Importantly, we had previously reported high mRNA expression levels of the canonical E2Fs in hESCs line H9 in comparation to somatic cells (human fibroblasts). First, we treated H9 hESCs with the general inhibitor of E2Fs (pan-E2F inhibitor) HLM006474. Concentration and incubation time used for HLM006474 treatment was fine-tuned by studying the cell cycle profile of hESCs-treated cells determined by measuring DNA content by propidium iodide staining followed by flow cytometry analysis. A 20mM HLM006474 concentration and 24 hours treatment was chosen for further experiments as it induced an increase in G1 cell population in H9 hESCs. Next, we performed a RNA-seq analysis of small RNAs of H9 hESCs treated or not with HLM006474 inhibitor (20mM, 24h). We found that 52 miRNAs were differentially expressed upon E2Fs inhibition (FDR: 0.1), some of which were already related with E2Fs family and others whose relationship with these factors or with hESCs-cell cycle has not yet been reported. Finally, upon validation of the expression levels of 20 selected miRNAs candidates by RT-qPCR with specific stem loop primers, we concluded that miR-19a-3p, miR-19b-3p, miR-4454, miR-1260a, miR-1260b, miR-454-3p and miR-301a-3p would be transcriptionally regulated by canonical E2Fs.

150. (381) **DEVELOPMENT OF A FIJI PLUGIN FOR THE ANALYSIS OF PREVIOUSLY SEGMENTED IMAGES WITH DEEP LEARNING: APPLICATION IN A SKELETAL MUSCLE REGENERATION MODEL.**

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The quantitative analysis of fluorescence microscopy images has a fundamental role in biology and medicine. One of the biggest challenges in the field is to detect cells stained for different markers automatically. The recent development of deep learning algorithms such as Cellpose is revolutionizing the field and bringing us closer to the wide adoption of image cytometry techniques. However, this algorithm returns its results in labelled image format, and its use is therefore limited to people with programming skills. The objective of this work was to develop a plugin for the FIJI / ImageJ software that would allow the use of segmentations generated in Cellpose by scientists with basic knowledge in image analysis. Our plugin extracts the objects from the labelled images and returns them in the form of regions of interest or ROIs. We checked its performance in cross-sectional images of mouse skeletal muscle labeled for laminin or stained with phalloidin or WGA. Cellpose allowed thousands of muscle fibers to be identified in each image. However, the cross-sectional area (CSA) measurements of the fibers were strongly biased by the type of staining used, being up to 30% higher than those obtained by manual segmentation. The incorporation of a segmentation erosion tool into our plugin allowed us to correct this bias, making them practically indistinguishable from those generated manually by experts. We tested the use of Cellpose together with our plugin in muscle damage and regeneration tests, where there is an overall change of the average CSA. Taking into account that manual segmentation of each image can take days, the use of Cellpose and our plugin allows a very significant saving of time and wide adoption by users without programming knowledge.

151. (426) **GENERATION OF KNOCKOUT STEM CELL LINES TO STUDY THE FUNTIONAL ASSOCIATION OF lncRNAs AND CIS-REGULATORY REGIONS DURING CARDIAC DIFFERENTIATION**

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Long non-coding RNA genes (lncRNA) were identified more than a decade ago, and since, they have been shown to fulfill a diverse range of functions. Particularly, their interaction with neighbouring regulatory regions has been proposed to modulate protein coding genes within the constraints of higher-order chromatin organizations during cell differentiation. However, many aspects of their regulatory functions are still unclear. Therefore, we set to identify lncRNAs differentially expressed (DE) during cardiac differentiation, which may be potentially involved in modulating gene expression through cis-regulatory regions (super-enhancers, SE). To this end, we first differentiated human pluripotent stem cells (hiPSC) into cardiomyocytes (CM) and performed RNA sequencing. Data analysis revealed 2,208 DE-lncRNAs between the cell populations (FDR<0.05), of which 729 co-occurred with an active SE (in respect to cell identity) within the boundaries of a Topologically Associating Domain (TAD). The co-occurrences were statistically significant when compared to a random distribution (p-value<0.001), indicating a possible functional association between the two features. Next, we implemented a protocol to modulate the expression levels of 8 selected candidates among the 729 DE-lncRNAs (4 expressed in hiPSC and 4 in CM) for the evaluation of their functional implication in gene regulation. To do so, we used a CRISPR/Cas9-based strategy to generate knockout (KO) hPSC lines for each candidate lncRNA. We designed two CRISPR RNA guides flanking the region which includes the promoter and the first exon to efficiently disrupt expression. Successful excision of target candidates was evaluated by PCR directly on genomic DNA from transfected pools of cells. In conclusion, we identified a set of lncRNAs that may be implicated in regulating gene expression through cis-acting regulatory regions and designed a KO strategy to functionally assess their role during cardiac differentiation.

152. (431) C**19MC MICRORNA CLUSTER REGULATES THE TRANSITION TO A GASTRULATION-LIKE STAGE IN A MODEL OF HUMAN EARLY DEVELOPMENT USING HUMAN PLURIPOTENT STEM CELLS**

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Human pluripotent stem cells (hPSC) have the capacity to self-renew and differentiate *in vitro* into all the cell types of the organism. We previously described the miRNome during the *in vitro* cardiac differentiation of hPSCs. It revealed a yet unexplored group of 56 microRNAs transcribed during pluripotency and whose expression decayed during the early mesoderm stage. These microRNAs are clustered in a primate-specific 100 kb sequence at chromosome 19, hence known as C19MC. To ascertain a possible role of this cluster

during early human embryogenesis, we generated a C19MC-deficient human induced pluripotent stem cell line using CRISPR/Cas9. When maintained under pluripotency conditions, these cells displayed no evident changes in the cell cycle, apoptosis, or differentiation markers compared to control cells. However, this line was drastically impaired to differentiate into cardiac and endothelial lineages, evaluated both by RT-qPCR, flow cytometry and a total lack of contractile cardiomyocytes. Since cardiac and endothelial lineages share a common mesodermal progenitor, we evaluated different markers, and found that early mesodermal genes MESP1/2, TBX6 and EOMES were downregulated compared to wild type cells, while the early ectoderm marker SOX2 was upregulated. Since the balance between TBX6 and SOX2 has been reported as critical for gastrulation, we next explored the genome-wide transcriptional changes by performing an RNA-seq at 0h and 24h of cardiac induction in the parental and C19MC line, a period that in vitro recapitulates gastrulation. Gene ontology analysis showed a global dysregulation of the differentiation pathways ERK/FGF, VEGF, NODAL, as well as focal adhesion related genes. We then performed an Ago-IP RNAseq experiment in pluripotency conditions with miR-520a over-expression and found an enrichment of WNT, FGF, and NOTCH signaling pathway genes. Thus, we show that the C19MC miRNA cluster has a critical role during early mesodermal specification.

153. (469) **GENERATION AND CHARACTERIZATION OF DESMINOPATHY PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS FOR PERSONALIZED THERAPY DEVELOPMENT**

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Desmin is a type III intermediate filament encoded by the *DES* gene,-mainly expressed in striated muscle,and is involved in contraction, organelle location and gene expression.Mutations in *DES* generate protein absence or abnormal protein folding disrupting cytoskeletal organization,mitochondrial function and the ubiquitin-proteasome pathway,among others.These phenotypes are known as desminopathies and have an incidence of 1 in 2000 patients.Obtention of patient-specific induced pluripotent stem cells(iPSC) allows the modeling of a desminopathy *in vitro*.In this work,we aim to generate and characterize a stable iPSC of a male patient with a congenital desminopathy (DES-J).DES-J has nucleotide triplet duplication in *DES* exon 6 which leads to a Glu353 incorporation.*DES* mutation was confirmed by Sanger sequencing and *in silico* analysis revealed the aminoacid incorporation alters desmin folding.To generate DES-J iPSC,a blood sample was taken from the patient and erythroblasts were reprogrammed by infecting with STEMCCA lentiviral vector.A clonal iPSC DES-J colony was amplified for further characterization.STEMCCA silencing was confirmed by RT-qPCR and normal karyotype of DES-J iPSC line was ensured through G-banding.For pluripotency validation,alkaline phosphatase activity was confirmed and flow cytometry analysis revealed positive staining for the pluripotency markers CD326 and TRA-1-81.Expression of *NANOG*,*OCT4* and *SOX2* was assessed by immunofluorescence and RT-qPCR.Finally,we proved that DES-J iPSC was capable of differentiating into the three germ layers by embryoid body formation and their characterization.These results enable to conclude that DES-J erythroblasts could be reprogrammed and developed pluripotent characteristics successfully.Our next step is to differentiate validated patient-derived DES-J iPSC into cardiac and skeletal myocytes in order to unravel the desminopathy.Our final purpose is to develop a personalized therapy that reverses the diseased phenotype.

154. (493) **THE PLURIPOTENCY TRANSCRIPTION FACTOR OCT4 REPRESSES HEME OXYGENASE 1 GENE IN EMBRYONIC STEM CELLS**

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Several mechanisms ensure genomic stability avoiding the propagation of genetic damage in embryonic stem (ES) cells, which can differentiate into all the adult cell lineages including the germ line. In these cells, redox status regulates the balance between self-renewal and differentiation, making crucial oxidative stress control. Heme oxygenase (HO) is the limiting enzyme in heme catabolism, and the inducible isoform HO-1 is known by its antioxidant and antiapoptotic functions. In the last years, new functions of HO-1 in the context of pluripotency were reported. Interestingly, it is required for efficient induced pluripotent stem cells generation and also, for specific differentiation processes, making its role in such different processes an enigma. In these contexts, the modulation of its expression remained unexplored. The purpose of this work was to study HO-1 gene regulation in ES cells. In this work we found that HO-1 is expressed in ES cells, localize both in nucleus and cytoplasm and is induced during differentiation. Since *in silico* analysis of HO-1 gene promoter region displayed multiple putative binding sites for pluripotency transcription factors (TFs) we analyzed whether they were involved in HO-1 gene regulation by a shRNA approach. We found that Oct4 downregulation increased HO-1 mRNA levels in ES cells. In agreement, by analyzing available data from ChIP-seq experiments, we found that this TF binds to HO-1 promoter region in ES cells. Moreover, Oct4 ectopic expression in heterologous systems repressed both HO-1 gene promoter reporter and endogenous HO-1 gene, further supporting that this pluripotency TF represses HO-1 gene expression. We have previously found pluripotency TFs regulate the expression of other relevant genes involved in oxidative stress defense in ES cells. The results presented in this work provide further evidence to the connection between pluripotency and redox homeostasis and could contribute to unveil HO-1 functions.

155. (557) **STRATEGY TO IMPROVE TROPISM AND TRANSDUCTION EFFICIENCY OF BACULOVIRUSES IN MAMMALIAN CELLS.**

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Autographa californica nucleopolyhedrovirus (AcMNPV) belongs to Baculoviridae, a family of arthropod-specific viruses. Although AcMNPV does not replicate in vertebrates, it can enter mammalian cells and express heterologous genes with mammalian promoters (gene transduction). The aim of this work was to obtain pseudotyped baculovirus to increase the transduction efficiency. To this end, we generated a transgenic insect cell line that constitutively expresses the G glycoprotein of vesicular stomatitis virus (VSV-G). The rationale is that baculoviruses propagated in these cells would acquire VSV-G inserted in the envelope of budding virions.
The ORF encoding VSV-G was cloned into the expression plasmid pIP-vsvG. High five cells (Hi5) were transfected with pIP-vsvG and selected with puromycin to obtain a Hi5-vsvG cell line. For the transduction assays, AcMNPV-dtomato was amplified in Hi5 wild type (control) or Hi5-vsvG cells. Then, infection supernatants were cleared by centrifugation and incubated with mammalian cells. Finally, viral plaques were counted by fluorescence microscopy.
Initially, we verified the presence of G protein in Hi5-vsvG cells by immunofluorescence. Then, we used the virus propagated in these cells to transduce human liver hepatocellular cells (HepG2) and human lung adenocarcinoma cells (A549). Transfection efficiency assesed by red fluorescence showed a higher performance of AcMVPV amplified in Hi5-vsvG, compared to the control. The same conditions were tested in HEK 293T and in Vero cells, but no significant differences in transduction efficiency were found ($p<0,05$ unpaired *t*-test).

In conclusion, we generated a pseudotyped baculovirus with VSV-G that showed to be more efficient that virus propagated in Hi5 wt in certain mammalian cells. This platform will allow us to explore alternatives to increase the tropism and transduction efficiency of baculoviruses for their application as vectors for gene therapy.

METABOLISMO Y NUTRICIÓN

156. (245) **EVOLUTIONARY AND STRUCTURAL CONSTRAINTS INFLUENCING APOLIPOPROTEIN A-I AMYLOID BEHAVIOR**

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Apolipoprotein A-I has a key function in the reverse cholesterol transport mediated by the high density lipoprotein (HDL) particle. However, aggregation of apoA-I single point mutants can lead to hereditary amyloid pathology. In this work, we combined evolutionary studies, in silico saturation mutagenesis and molecular dynamics (MD) simulations to provide a comprehensive analysis of the conservation and pathogenic role of the aggregation prone regions (APRs) present in apoA-I.

ApoA-I sequences analysis demonstrated the pervasive conservation of an APR, named APR1, within the N-terminal α-helix bundle. Moreover, stability analysis carried out with the FoldX engine showed that this region contributes to the marginal stability of apoA-I. When the thermodynamic

and pathogenic impact of different groups of apoA-I point variants was compared, we found that mutations associated with amyloid pathologies showed a destabilizing effect when compared against HDL-deficiency or natural variants extracted from the gnomAD database (p-value 0.05, Mann-Whitney-Wilcoxon Test).

MD simulations of the amyloid variant G26R evidenced an increase in the exposure of APR1 with respect to the wild type protein (p-value 0.1, Tukey's Test) and the occurrence of β-strand secondary elements at the C-terminus of apoA-I. Our findings highlight APR1 as a relevant component for apoA-I structural integrity and emphasize a destabilizing effect of amyloid variants that leads to the exposure of this region. This information contributes to our understanding of how apoA-I, with its high degree of structural flexibility, maintains a delicate equilibrium between its lipid-binding function and its intrinsic tendency to form amyloid aggregates. In addition, our stability measurements could be used as a proxy to interpret

the structural impact of new mutations affecting apoA-I.

157. (40) **EFFECT OF DIET WITH HIGH OLEIC SUNFLOWER OIL ON LIPIDS´ PROFILE: STUDY IN AN EXPERIMENTAL MODEL.**

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The importance of diet in maintaining health is widely accepted and recognized. The aim is to analyze the effect of diets with high oleic sunflower oil(HO) in adequate and high concentration on serum, thymus, brain and liver´s lipids profiles of growing rats. Weanling Wistar rats were fed during 40 days with F%15 and 40 (F%=Kcal lipids/100total Kcal) dietary fat provided by HO(F15 and F40 groups). Control group(C) received normocaloric diet(soy oil, F%15) according to AIN´93. Thymus, brain and liver were removed. Fatty acid profiles were determined by gas chromatography (GC). Lipid peroxidation in liver was determined by TBARS assay in F% 40 group. The statistical analysis was Student Test (*p<0.01). Results: FATTY ACID PROFILE(%Area): OLEIC serum F15=24,10±6,37*; F40=26,96±3,71*; C=11,29±2,27; Thymus F15=34,85±7,22*; F40=36,13±10,72*; C=16,84±5,20; Brain F15=14,69±3,00; F40=15,53±1,26; C=13,00±1,76; Liver F15=26,12±2,16*; F40=39,03±7,45*; C=11,11±1,25; LINOLEIC serum: F15=5,26±1,48*; F40=7,41±1,89*;C=18,50±3,31; Thymus F15=2,45±0,26*; F40=4,02±0,65*; C=10,22±3,10; Brain F15=0,43±0,10*; F40=0,40±0,04*; C=1,00±0,20; Liver F15=4,96±0,69*; F40=5,89±1,36*; C=17,15±3,18; LINOLENIC serum: F15=0,30±0,16*; F40=0,32±0,13*; C=0,81±0,22; Thymus F15=0,31±0,09*; F40=0,25±0,07*;C=0,75±0,11; Brain F15=0,23±0,16; F40=0,24±0,02; C=0,34±0,09; Liver F15=0,12±0,04*; F40=0,14±0,03*; C=0,69±0,15. These results suggest that it was exacerbated ω9 family with diminution of essential fatty acids on serum, thymus and liver. Only linoleic acid is lower in brain. TBARS liver(ugMDA/g tissue) were: F40:0,06±0,02; C 40D:0,06±0,02.The administration of this experimental diet did not show an increase in lipid peroxidation in liver, due the antioxidants provided by the diet might be playing a protective role. These findings demonstrate an increase in oleic acid and decrease of the essential fatty acids when a diet rich in oleic acid is administered independent of F% of the diet. UBACyT20020150100011BA

158. (54) **EPICARDIAL ADIPOSE TISSUE LIPIDOME AND LIPID METABOLISM: DO CIRCULATING MARKERS REFLECT ITS BEHAVIOR IN CORONARY ARTERY DISEASE?**

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Epicardial adipose tissue (EAT), a visceral AT surrounding myocardium and coronary arteries, emerged as an important actor in coronary artery disease (CAD). The increase in its volume is an independent risk factor for CAD. We have previously demonstrated that lipoprotein lipase (LPL) activity is increased in EAT from CAD patients, potentially contributing to the increase in the tissue volume. EAT composition could contribute to CAD as well. Whether circulating markers reflect EAT behavior is still unknown. Our aim was to evaluate LPL and glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein-1 (GPIHBP1) levels in serum and EAT from CAD patients, as well as tissue and lipoproteins lipidomic profiles, searching for possible parallelisms.

Materials and methods: in serum, EAT and subcutaneous AT (SAT) from patients undergoing coronary artery bypass graft (CAD,n=25) or valve replacement (No CAD,n=25), LPL and GPIHBP1 levels were evaluated by immunoassays and western blot, respectively. In serum, lipoprotein profile was assessed, and very low and high density lipoproteins (VLDL and HDL) were isolated by ultracentrifugation. Tissue and isolated lipoproteins lipidomes were evaluated by UHPLC-MS using a LC C18 column and a Q-Exactive plus mass spectrometer, in positive and negative ionization modes.

Results: Insulin-resistance markers were higher in CAD ($p<0.05$). Serum LPL levels were decreased ($p=0.045$) in CAD, without differences in EAT or SAT. Circulating GPIHBP1 levels were decreased in CAD ($p=0.047$), while EAT GPIHBP1 expression was increased ($p<0.001$). EAT lipidome was enriched in bioactive lipids such as Ceramides in CAD, and 51 species were found in common between

EAT and lipoproteins. A positive correlation between EAT and lipoproteins was found in 5 lipid species.
Conclusion: EAT lipidome would contribute to CAD risk. The tissue unique characteristics should be evaluated locally, given that circulating factors would not reflect EAT behavior.

159. (57) **SLEEVE GASTRECTOMY MIGHT IMPROVE METABOLIC OUTCOMES AND OXIDATIVE STRESS INDICATORS IN AN ACCESIBLE AND REPRODUCIBLE RAT MODEL**

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Backround: Fluctuations of glucose over a daily period of intermittent hyperglycemia and obesity might activate oxidative stress and inflammatory pathways playing an important role in cardiovascular diseases. Animal models might be an essential tool for the understanding of the regulation of body weight and metabolic pathways after bariatric interventions.
Methods: An experimental analytic study was performed using male Wistar rats. Animals were divided in 6 groups of 12 rats each: Control (C), Metabolic syndrome induction (MS), Sleeve Gastrectomy (SG), MS + SG for 6 (SG6), 12 (SG12) and 24 (SG24) weeks. Oxidative (Nitric Oxide, Fibrinogen, Superoxide Dismutase, Miloperoxidase), metabolic (Blood glucose, Triglycerides, Total Cholesterol, LDL, HDL and TC/HDL-C index) variables, weight loss and food intake were assessed.
Results: The survival of the animals was satisfactory. MS showed a statistical significant increment of oxidative and metabolic variables and weight. SG induced an improvement of the affected variables that tended progressively towards control. The induced weight loss was associated to the intake.
Conclusions: The proposed model might be reproducible and secure. Sleeve gastrectomy in rats has shown to improve the impaired oxidative and metabolic assessment.

160. (61) **EFFECT OF ANTIOXIDANT HORTICULTURAL EXTRACTS ON ACETOMINOPHEN HEPATOTOXICITY IN HEP-G2**

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We have demonstrated that antioxidant N-Acetylcysteine (NAC) inhibits lipids accumulation in 3T3-L1 adipocytes (AD) (Soto et al. 2017 and 2020). NAC is also an antidote for acetaminophen (AP) hepatotoxicity *in vivo*, decreasing to 50% the levels of hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities after 24 h NAC treatment. Our aim is to evaluate horticulture extracts (HE) with antioxidant capacity (AO) as hepatic protectors and compare their effects with NAC. Methods: Assays were conducted with HE from *Citrus sinensis* (orange), *Silybum marianum* (thistle), *Quassia amara* (quassia), *Baccharis articulata* (carqueja), *Peumus boldus* (boldo), *Cynara scolymus* (artichoke), *Verbena bonariensis* (verbena), *Schinopsis balansae* (quebracho, rich in polyphenols) and *Ribes nigrum* (cassis). We determined AO by DPPH assay. Hep-G2 cells were treated with 15 mM AP and HE for 24h. After these treatments, we evaluated cytotoxicity by MTT assay, ALT and AST activities. In HE-treated AD, we assessed cholesterol (Chol) and triglyceride (Tg) levels. Results: The highest HE AO/mg were 88 $\pm$ 5% (orange, OE), 74 $\pm$ 1% (carqueja, CE), 70 $\pm$ 2% (quebracho, QE) and 68 $\pm$ 4% (cassis, CsE). CsE was toxic to Hep-G2 cells, but CsE treatment on AD resulted in a significant decrease in Chol (by 31%) and Tg (by 62%) levels, without toxic effects. AP treatment was toxic to Hep-G2 cells and increased by 3 folds AST and ALT activities compared to non-treated control cells ($p<0.01$). OE, CE and QE treatments produced a significant percentage reduction in hepatic enzyme activities compared to AP treated cells ($p<0.01$); ALT decrease: 30 $\pm$ 5, 34 $\pm$ 1 and 62 $\pm$ 3, respectively; AST decrease: 26 $\pm$ 2, 19 $\pm$ 1 and 35 $\pm$ 1, respectively. The rest of HE with low AO, such as boldo (34 $\pm$ 2%), could not decrease ALT or AST activities. Conclusion: Only non-toxic HE with high AO could achieve hepatic protector effect. Just QE could decrease ALT and AST activities to similar values of those of NAC.

161. (68) **INTERFERON ALPHA 2B (IFN-α-2B) BUT NOT VITAMIN K2 SUPPLEMENTATION REDUCES LIVER CARCINOGENESIS IN MICE**

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In HCC, IFN-α monotherapy is not satisfactory and its effects remain controversial. However, the combination of IFN-α with other anti-cancer drugs, have had promising results. Vitamin K2 is present in dairy products and has been recommended as a micronutrient supplement in humans. Several studies have indicated that vitamin K2, may play a role in controlling the growth of HCC. Objective: to evaluate if the therapy of IFN-α-2b with vitamin K2 has a synergistic inhibitory action on DEN-induced carcinogenesis in mice. Methods and Results: 14-day-old mice were injected ip with 25 mg/kg DEN (control group with tumor, CT). After 10 months mice were divided into 4 groups: CT; IFN: CT mice that received IFN-α-2b 6.5x105U/kg ip 5 times/week/3 weeks; VK2: CT mice that received vitamin K2 5 mg/kg ip 5 times/week/3 weeks; and IFN+VK2: CT mice which received both drugs. Animals were euthanized after treatments and livers were obtained. There were no differences in body and liver/body weight ratio between groups. CT mice treated with IFN-α-2b had fewer tumors/liver and a trend to be smaller in size. VK2 and IFN+VK2 groups did not show differences respect to CT in number and size tumors. Immunoblot analysis showed increase proapoptotic Bax protein in IFN and decrease expression in IFN+VK2 groups (+51%* and -41%* respectively) respect to the CT group. PCNA protein expression showed a decrease (-60%*) in IFN group respect to CT. Finally, immunoblotting showed increase E-cadherin expression in IFN and VK2 groups (+290%* and +145%* respectively) and no differences in N-cadherin protein expression between all the studied groups. (*$p<0.05$vsCT). Conclusion: Our findings indicate that IFN-α-2b, but not vitamin K2, contributes to reducing liver cancer in mice treated with DEN. Moreover, VK2 blocks the positive effects of IFN-α-2b. This is in agreement with our *in vitro* studies in SK-HEP-1 cells as well as with our studies performed in in the early stages of liver cancer development in rats

162. (79) **ALPHA-TOCOPHEROL INTAKE: INFLUENCE OF PREVIOUS NUTRITIONAL STATUS ON LIVER VITAMIN E LEVELS AND OXIDATION PARAMETERS**

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Liver plays a preponderant role in the distribution and metabolization of vitamin E and this in the oxidative balance of macromolecules. Objective: to analyze the effect of different amounts of α-tocopherol (α-T) consumption on the α- and γ-tocopherol (γ-T) hepatic levels and the oxidative balance of lipids and proteins in the liver.
Experimental model: Six groups (n=6 per group) of well-nourished *Wistar* rats at weaning were used. Three of them went through severe protein malnutrition and renourished with an experimental diet containing 20% of casein according to AIN93 (Re-nourished group = RN: R, RE, REX). Well-nourished controls (WN: C, CE, CEX) received animal stock diet since weaning. α-T was administered for 42 days in the diet and as a supplement in drinking water. The RE and CE groups received in the drinking water, α-T equivalent to +4.5% and REX and CEX +45% of the intake from the diet. Liver α-T and γ-T levels were determined by HPLC; lipid oxidation by TBARS assay and protein oxidation by carbonyl groups quantification. Results were expressed as the Mean ± S.E. Statistics: One-way ANOVA.
RN presented higher α-T and lower γ-T liver levels than the WN groups (39±10 vs. 23±5 and 1,0±0,1 vs. 1,6±0,2 *μ*g/g tissue, respectively; p<0,05), even though the 20% casein diet ingested by RN animals contained twice as much γ-T as the stock diet received by WN. TBARS: RN= 0,18±0,01; WN= 0,24±0,02 (*μ*g MDA/g tissue; p<0,05). Carbonyl groups: RN= 1,71±0,11; WN= 0,76±0,06 (nmoles carbonyl/mg protein; p<0,05). The differences between the groups were given by the nutritional status and not by supplementation. RN had higher α-T levels and lower lipid oxidation and WN had higher γ-T levels and lower protein oxidation in the liver.
Regardless of variations in the intake of the tocopherol isomers, the liver accumulation capacity of α-T and its action on the oxidative balance on lipids and proteins, was determined by the previous nutritional status.

163. (106) **METFORMIN PREVENTS RENAL SODIUM RETENTION AND BLOOD PRESSURE INCREASE IN RATS EXPOSED TO CHRONIC HIGH FAT DIET**

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Kidneys are sensitive to high fat diet (HFD) showing intracellular lipid accumulation, tubular atrophy, interstitial fibrosis and glomerulosclerosis. Metformin, a biguanide drug, is mainly use for insulin resistance therapy. The aim of this study was to analyze the preventive effect of 8 weeks metformin therapy over sodium excretion, systolic blood pressure (SBP), and renal tissue integrity in male Sprague-Dawley rats under HFD. The animals (body weight of 180-200 gr) were randomized on 4 groups (n=4/each one): control (C, standard diet and tap water to drink), HFD (C + 50% w/w fat added to C diet), control+metformin (CM, C + 500mg/kg/day of metformin diluted in the water), HFD+metformin (HFDM, HFD+CM). Results: Metformin improved fractional sodium excretion (FENa%, HFDM: 0.34±0.09 vs HFD: 0.16±0.02; p<0.05), urinary sodium excretion (UNa.V, mEq/24hs, HFDM: 0.86±0.13 vs HFD: 0.45±0.06; p<0.02), and SBP (mmHg, HFDM: 128±1 vs HFD: 138±2; p<0.02). Daily urine excretion and GFR showed no variation during the studied period between HFDM vs HFD. Body weight, triglyceridemia and glycemia were also improved by metformin comparing HFDM vs HFD (p<0.01 for each), while hyperinsulinemia induced by HFD was not significantly modified under metformin treatment. Histological analysis with H&E stain showed that metformin treatment reduced intracytoplasmatic inclusions in the tubular cortex cells of HFD; while Picrus Sirius red showed that metfomin could not prevent the interstitial fibrosis observed in HFD; but transmission electron microscopy showed a minor foot processes effacement in HFDM vs HFD, improving glomerular structure. In conclusion: Metformin showed beneficial effects by improving fractional and urinary sodium excretion, SBP and renal tubular and glomerular structure and ultrastructure in this experimental model.

164. (126) **EFFECTS OF DIETARY SALVIA HISPANICA L. SEED UPON RENAL ALTERATIONS OF LIPIDIC AND GLYCOSIDIC METABOLISM IN A RAT MODEL OF METABOLIC SYNDROME**

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Objective: The main goal of this study was to assess the effects of dietary Salvia hispanica L. seed (chia) upon renal alterations of lipidic and glycosidic metabolism in a rat model of Metabolic Syndrome (MS), induced by a sucrose-rich diet (SRD).
Materials and Methods: Male Wistar rats received an SRD (% energy: 60 sucrose, 23 corn oil, 17 protein) for 3 months. After, half of the rats were fed with an SRD containing chia seed as a source of fat instead of corn oil for 3 months (SRD+chia group). The other half continued with the SRD until 6 months (SRD group). The reference group consumed a standard chow diet through the experimental period (RD group). In all dietary groups were assessed: 1-Glucose and triglyceride serum levels and the weight of perirenal adipose tissue. In the renal cortex: 2-Content of triglycerides (TG); 3-the activity of key enzymes of de novo lipogenesis: Acetyl-CoA carboxylase (ACC), fatty-acyl-CoA synthase (FAS) and glucose-6-phosphate dehydrogenase (G6PD); 4-the activity of key enzymes of gluconeogenesis: Glucose-6-phosphatase (G6Pase), fructose-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK). The statistical analysis was performed by ANOVA. P values lower than 0.05 were considered statistically different.
Results: Compared with the SRD group, the SRD+chia group displays a significant decrease of perirenal adipose tissue (p<0.05) and a lowering of TG levels in both serum and renal tissue (p<0.05). In the renal cortex of this dietary group a significative decrease (p<0.05) of ACC, FAS, and G6PD was observed. Besides, the normalization of glycemia in the SRD+chia group was related to a significant reduction of the activity of FBPase and PEPCK reaching values similar to those observed in the RD. No change in G6Pase activity (p>0.05) was observed between SRD and SRD+chia groups.
Conclusion: The results achieved suggest that chia seeds could be a beneficial nutrient to counteract the renal alterations of MS.

165. (144) **ACTION OF IODINE AS AN ANTAGONIST OF THE THYROID DISRUPTING ACTION EXERTED BY NITRATES PRESENT IN GROUNDWATER.**

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Recently we have demonstrated that nitrates present in groundwa-

ter exert a disruptive action. Nitrates could compete with Iodine for thyroid cotransporter NIS, being amphibian larvae highly sensitive to this effect. Objectives: evaluate the antagonistic action of iodine and groundwater contaminated with nitrates on metamorphosis, morphological and histological changes in amphibian. Materials and method: Xenopus Laevis larvae at: 22ºC ± 2ºC, pH: 7 to 7.8 and light-dark cycle: 12hs-12hs, immersed in: a) filtered drinking water: control (C) (n= 5), b) groundwater (G) (n= 8), c) groundwater with arrested larvae at stage 56NF, to which is added 2.5 µg/L potassium iodine (G+I) (n= 8), d) filtered drinking water plus 0.007 mg/L of potassium perchlorate: positive control (PC) (n= 8). Results: the analysis of the groundwater used shows a concentration of nitrates greater than 40 mg/L (INTI, National Food Code). The metamorphosis was completed for: 100% of the C, 37.5% of the G ($p<0.001$) (RR: infinity), 62.5% of the G+I ($p<0.01$), (RR: 0.67) and 0% of the PC ($p<0.0001$), (RR: infinity) (CI: 95%). The total metamorphosis time was: C: 52±1, G: 65±2, G+I: 54±1 days ($p<0.001$), CI: 95%. Weight and height showed significant decreased in the 58NF stage in group G and G+I vs C and PC ($p<0.01$) CI: 95%. In stage 66NF for weight there were no differences ($p<0.05$), the height was significantly decreased for the group G and G+I vs C ($p<0.02$), CI: 95%. Histologically (58NF) the colloidal area increase: G+I vs G, PC and C ($p<0.001$), CI: 95%; and increases epithelial height G and G+I vs PC and C ($p<0.001$), CI: 95%.The follicular hyperplasia grade was in G, G+I and PC: 2 and in C: 0 ($p<0.001$) CI: 95%. Conclusion: During metamorphosis, morphological and histological changes regulated by thyroid hormones are affected by action of nitrates present in groundwater; these effects could be reversed in the amphibian, by the action of iodine.

166. (158) **EFFECT OF THE PHARMACOLOGICAL COMBINATION OF ATORVASTATIN AND METFORMIN ON BIOMARKERS OF INFLAMMATION AND OXIDATIVE STRESS IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME**

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Introduction: Oxidative stress (OS) and inflammation would be the pathogenic promoters of the structural alterations observed in metabolic syndrome (MS). Atorvastatin and Metformin could improve cellular functionality by restoring the redox state and inflammatory process, preventing MS lesions. Objective: An experimental model of MS assessed the pharmacological response of atorvastatin and metformin over inflammation and OS biomarkers. Materials and methods: 40 male Wistar rats were used (8 per group): A)Control, B)SM, C)SM+Atorvastatin, D)SM+Metformin, and E)SM+Atorvastatin+Metformin. SM was induced by 10% fructose in drinking water during 6 weeks. Atorvastatin was administered in doses equivalent to 10 mg and metformin in doses equivalent to 500 mg, during 45 days. Fibrinogen (mg/dL), nitric oxide (NO)(μM) and superoxide dismutase (SOD)(U/mL) were quantified by spectrophotometry. The results were analyzed with ANOVA and hotelling as a post hoc test, p significance level<0.05. Results: Fibrinogen concentration increased in (B)(288.83±6.8) with respect to (A)(203.33±6.8) ($p<0.001$), and in groups (C)(A) 196±7.45), (D)(242±7.45) and (E)(104,33±6,8) a diminution of fibrinogenemia was evident($p<0.001$). Levels of NO significantly decreased in (B)(14.76±1.86) compared to (A)(27.09±1.95)($p<0.001$) and normalized their levels in the groups (C)(25.48±2.06), (D)(22.2±2,33) and (E)(31.25±2,18)($p<0,001$). SOD activity in (B)(178.64±10.23) increased significantly contrasted with (A)(134.5±10.73)($p<0.001$), similar response evidenced in (D)(195.71±12,82) and (E)(222,17±15.17)($p<0.001$), however in group (C)(145.71±12.82) there was a significant decreased from (B) ($p<0.001$). Conclusion: Atorvastatin exhibited its pleiotropic effects by normalizing biomarkers. Metformin improves the inflammatory and OS profile by enhancing the activity of the antioxidant system. Combined administration demonstrated pharmacological synergy by producing regression of OS and inflammatory state.

167. (160) **EFFECT OF THE INTAKE OF BREAD WITH LENTIL FLOUR ON CALCIUM ABSORPTION AND BIOMECHANICAL PARAMETERS IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RATS**

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Lentils are nutritive foods with a high amount of minerals but also anti-nutritional factors that can affect their absorption. However, the great amount of fiber provides functional properties and bread made with this flour, can be a product that could help to maintain a healthy diet.
Objective: the aim of this work was to study the effect of the intake of bread with lentil flour during 60 days, on calcium absorption and biomechanical parameters, using a Wistar rat model.
Methods: animals were divided in three groups (n=8/group) and fed after weaning with different diets: control (C) according to AIN 93, diet containing lentil flour (LF) and diet with wheat and lentil flour bread (80:20%) (LFB). During the last five days of the experience, food intake was recorded and feces were collected to calculate Apparent calcium absorption (APCa%). At the end of the study, rats were anesthetized and the cecum from each animal was excised, split open, and the pH of the cecal content was measured. Right femur was also excised and Limit elastic load (Wy), Diaphyseal stiffness (Wy Dy) and Maximum fracture load (Wf max) were measured. Results: the results showed that the cecal content of LF and LFB presented a lower pH than C (6.39±0.14 vs 6.52±0.19 vs 7.13±0.14; *p< 0.0001*). ApCa% were lower in LFB compared with LF and C (68,0±6,4 vs 69,1±7,4 vs 73,6±2,8; *p<0.05*). Values of Wy were significantly higher in groups LF and LFB than in C (147,2±13,5 vs 137,2±25,9 vs 102,8±15,4;*p<0.01*) as well as results of Wy Dy (268,2±29,1 vs 260,5±39 vs189,9±26,9 ; *p<0.001*). Wf max was also higher for LF and LFB than for C (182,8±13,7 vs 176,1±28,3 vs 124,3±12,7;*p<0.0001*).
Conclusions: the results indicate that factors present in lentil didn't affect calcium absorption. Intake of lentil flour on baked goods showed a prebiotic effect improving bone quality. Financed by UBACyT Nº 20020170100148BA, 20020130100126BA and PICT 2016-3047.

168. (162) **MICROSOMAL TRIACYLGLYCEROL TRANSFER PROTEIN (MTP) INHIBITION, BY HYPOCHOLESTEROLEMIC DRUG LOMITAPIDE, FAVORS TUMOR DEVELOPMENT**

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Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as very low density lipoproteins. There is evidence that MTP might be involved in other cellular processes, including the pathogenesis of different diseases; however, no studies were performed yet to evaluate whether MTP plays a role in cancer.

The MTP inhibitor lomitapide binds directly to MTP thereby inhibiting the synthesis of triglyceride-rich VLDL in the liver. Therefore, the objective of this work was to study the effect of MTP inhibition on tumor development. Adult male C57BL/6 mice were subjected to a model of chemical hepatocarcinogenesis. Animals were randomly divided into two groups (5 mice/group). One group (Control) received vehicle (methylcellulose, gastric probe) and another group received 5 mg/kg bw/day lomitapide (gastric probe) for 3 weeks. At the end of the treatment, mice were sacrificed, livers were excised and weighed and tumors on the liver surface were counted. After treatment, lomitapide-treated mice showed increased liver/body weights ratio (2-fold) and higher number of tumors (2-fold) than control mice. As expected, plasma levels of triacylglycerol and ApoB-100 were decreased (-40% and -60%, respectively) in lomitapide-treated mice compared to control mice. Liver histology analysis showed no differences between groups on tissue and tumor architecture; however, lomitapide-treated mice presented less remaining normal liver parenchyma. Conclusion: these studies represent the first steps in the evaluation of the role of MTP in cancer development, and demonstrate that MTP may be participating in tumor growth.

169. (208) D**ECREASED CHRONIC INFLAMMATION WITH RESVERATROL, ALPHA-TOCOPHEROL, AND PIPERINE DIET SUPPLEMENTATION IN NEUTROPHILS OF PATIENTS WITH METABOLIC SYNDROME**

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Chronic inflammation and oxidative stress are processes associated to metabolic syndrome (MS). Currently, despite advances in the knowledge and therapeutics of MS, the mentioned processes are poorly diagnosed and treated in this disease. With the aim of evaluating the effectiveness of resveratrol supplementation on chronic inflammation associated to MS, clinical-biochemical biomarkers were analyzed in the blood of voluntary individuals participating in the study with a diagnosis of MS (n=92). The inclusion criteria were met 22 patients with an average of 68 ± 5 years (13 men, 9 women), whose diagnosis of MS was made based on the diagnostic criteria of the National Cholesterol Education Program, Adult treatment Panel III, 2002. Patients received a dietary supplement along with their usual treatment, whose formula is composed of resveratrol (50 mg), alpha-tocopherol (25 mg) and piperine (5 mg) for a period of 3 months. Piperine increases resveratrol and alpha-tocopherol absorption. Control was the patient himself in baseline conditions, avoiding interindividual variables and bioavailability of active principles. Volunteers were bled before initiation and after 3 months of treatment. Biochemical markers assessed in plasma: glucose, HDL cholesterol, triglycerides, ferritin, ultrasensitive C reactive protein (CRP); and in neutrophils: oxygen consumption (ΔO_2, evaluated using a Clark type oxygen electrode) and chemiluminescence, determined with a photon counter. Results showed: triglycerides decreased by 25% ($p<0.05$), ferritin 10% ($p<0.05$), ultrasensitive CRP 33% ($p<0.0001$) in plasma; and in PMN, oxygen consumption decreased by 55% ($p<0.0001$) and spontaneous chemiluminescence 25% ($p<0.05$) after treatment. To our knowledge, this is the first study which has been carried out showing that MS patients were able to reduce chronic inflammation by the administration of a dietary supplement based on resveratrol, alpha-tocopherol and piperine, together with conventional therapy.

170. (221) **EVALUATION OF OXIDATIVE STRESS AND ANTIOXIDANT DEFENSES IN SKELETAL MUSCLE OF DYSLIPEMIC-INSULIN RESISTANT RATS CHRONICALLY FED A SUCROSE-RICH DIET**

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Nowadays oxidative stress (OS) has been recognized as a central contributor to metabolic disorders. In rats chronically fed a sucrose-rich diet (SRD), we previously showed that impaired adipose tissue and heart muscle function was associated with lipid accretion, increased OS and depletion of the antioxidant system. However, in skeletal muscle (SM), although lipid accumulation was related to insulin resistance (IR), the participation of OS remains unclear. This study aimed to explore the possible involvement of OS in the development of IR in SM (gastrocnemius). Male Wistar rats were fed a SRD for 6 months (percentage energy: 60 sucrose, 23-corn oil, 17 protein). A reference group consumed a control diet all the time. In SM were analyzed: (i) triglycerides (TG), long-chain acyl-CoA (LCA-CoA) and diacylglycerol (DAG) content; (ii) OS markers: reactive oxygen species (ROS) levels and lipoperoxidation as thiobarbituric acid reactive substances (TBARS) content; (iii) reduced glutathione (GSH) levels. Besides, plasma levels of glucose, TG, free fatty acids (FFA) and TBARS and whole-body peripheral insulin sensitivity (euglycemic-hyperinsulinemic clamp) were measured. Results were analyzed by Student´s t-test. Chronic administration of SRD significantly increased the accumulation of lipid species in gastrocnemius muscle. Also, dyslipidemia, moderate hyperglycemia, elevated levels of FFA and IR, characteristics of the experimental model, were observed. In addition, plasma levels of lipoperoxidation (TBARS) significantly increased in animals that received SRD. Surprisingly, the lipid accretion in SM of SRD-fed rats at this treatment duration was not accompanied by significant changes in the levels of ROS, TBARS or GSH. The results reached in the present study expand the current knowledge about the mechanisms underlying diet-induced lipotoxicity in SM. The link between OS and IR in SM is still under discussion and is an issue to be elucidated in future research.

171. (242) **OFFSPRING BEHAVIOR IS AFFECTED BY FRUCTOSE-INDUCED INSULIN RESISTANCE IN WISTAR RAT DAMS**

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Maternal nutrition before and during pregnancy plays a critical role in fetal brain development. Epidemiological studies have shown an association between maternal obesity and adverse neurodevelopmental outcomes for offspring. The effects of pregestational insulin resistance (PIR) on intrauterine programing of fetal brain is a lesser-explored area. The goal of this study was to investigate the impact that PIR has on neurodevelopmental reflexes and behavior during adulthood. Offspring behavior was evaluated using open field (OF), marble burying, tail flick and elevated plus maze (EPM) tests. Wistar adult rats received fructose enriched water (10 % or 20% w/v) before pregnancy to induce PIR, and during gestation.

No modifications on the developmental milestones nor neonates size were observed when comparing offspring of control and PIR dams. Smelling, rearing and climbing frequency as well as duration during exploratory behavior testing in the OF test decreased in PIR dams progeny. Analysis of minute-by-minute duration in the EPM assay evidenced that the PIR group spent more time in the central square and less in the closed arms compared to control group. Furthermore, offspring of PIR dams showed a more frequent stretch-attend posture in the central square. Offspring exposed to maternal fructose intake had a greater latency of tail withdraw in the tail flick test and buried more marbles in the marble test.

We conclude that maternal exposure before and during gestation to fructose does not affect primitive reflexes such as body righting mechanisms, coordination, strength, sensory system maturation,

and labyrinthine reflex. On the contrary, adult rat behavior was significantly altered. Particularly, our results evidence an increment on anti-anxiety like behavior; longer decision-making time and risk assessment. Obsessive-compulsive disorder behavior and altered nociception were also noted.

172. (257) **IRON INTAKE AND ANCESTRY IN ADULTS OF BUENOS AIRES**

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High iron (Fe) intake in carriers of *HFE* gene mutations (C282Y, H63D, S65C) can result in overload and eventually, in hereditary hemochromatosis (HH). HH is more common in men than in women. C282Y is characteristic of northern Europe, while H63D is in the Mediterranean countries. European ancestry is estimated to be 81% in Buenos Aires city. Therefore, given high meat intake in Argentina, ancestry and its relationship with possible alterations in Fe metabolism were studied in clinically healthy adult males. 54 male blood donors (20-58 years) attending Hospital de Clínicas (UBA) (2018) were enrolled. The daily Fe intake (FeI), heme Fe intake (hem FeI) and non-heme FeI intake (non-heme FeI) were evaluated using a food consumption frequency questionnaire (ARGENFOODS and USDA). A genealogical survey was carried out, and ancestry informative markers (AIMs) were studied in whole blood using the multiplex PCR-APLP technique (30 markers). C282Y, H63D and S65C were investigated in blood by DNA extraction (Accupred Genomics DNA Kits) and PCR-RFLP. The FeI, non-heme FeI and heme FeI were, respectively (mg/d): mean ± SD (range): 22.7 ± 8.6 (9.6-47.2); 20.7 ± 7.9 (8.3-42.2); 2.0 ± 1.1 (0.3-5.0). Fortified flours (30 mg Fe/kg) provided 35.7% of total FeI. The FeI was> 6 mg Fe/d (Estimated Mean Requirement) in all participants, and 3.0% exceeded the Maximum Tolerable Intake Level (45 mg Fe/d) (NAS, 2001). H63D was present in 13/54 (24%) male donors; only 7/13 (53.8%) answered to have a high European self-perceived ancestry. An average European contribution of 83% (range: 99% - 66%) was estimated by the AIMs. These results suggest that a significant number of clinically healthy individuals could believe that their European ancestry is fewer than that estimated by a genetic analysis. Therefore, they could be at risk of Fe accumulation given feeding habits in Argentina (55.5 Kg meat /per cápita/y, FAO 2013, and Ley 25630/2002). *Universidad de Buenos Aires, UBACyT 20720150100004BA.*

173. (335) **DIETARY *SALVIA HISPANICA* L. (CHIA) SEED INDUCES TISSUE REMODELING AND MODULATES HORMONE- SENSITIVE LIPASE PROTEIN LEVELS IN DIFFERENT FAT PAD DEPOTS OF SUCROSE-RICH DIET-FED RATS**

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Visceral fat accumulation (central obesity) is associated with both structural and functional changes in adipose tissue (AT) that contribute to the development of whole-body metabolic disorders. Since obesity and other associated metabolic disorders have reached epidemic proportions, potential therapeutic strategies targeting AT are of main research interest. We have previously shown that *Salvia hispanica* L. (chia) seed was able to reduce the increased visceral adiposity and improve the altered insulin sensitivity (IS) present in rats chronically fed a sucrose-rich diet (SRD). The aim of this work was to evaluate the effect of chia seed on the morphology and key molecules of insulin signaling pathway and lipolysis in different visceral AT depots of SRD-fed rats. Male Wistar rats were fed a SRD for 3 months. Half of the animals continued with the SRD until month 6, the other half was fed a SRD in which the fat source, corn oil, was replaced by chia seed from month 3 to 6 (SRD+chia). Another group consumed a reference diet all the time. We analyzed: body weight (BW), energy intake and visceral adiposity index (VAI). In epididymal (eAT) and retroperitoneal adipose tissue (rAT): fat cell size, fat cell number and lipid content, total hormone sensitive lipase –HSL- and AKT protein mass levels (Western Blot). IS (euglycemic–hyperinsulinemic clamp) and serum glucose, insulin, triglycerides and FFA levels were also determined. The addition of chia seed to SRD-fed rats: a-significantly decreased ($p<0.05$) the increased VAI, although BW and energy intake was similar to those of the SRD-fed rats, b- In both eAT and rAT: reduced ($p<0.05$) the increased fat cell size and lipid content and decreased ($p<0.05$) the HSL protein levels. No changes were observed in eAT and rAT AKT protein mass levels. Besides, chia seed corrected the moderate hyperglycemia, dyslipidemia and IS. This work provides novel information on the beneficial effects of chia seed in AT remodeling and dysfunction.

174. (349) **UNDESIRABLE LITTER'S METABOLIC CONSEQUENCES DUE TO MATERNAL MALNUTRITION THROUGHOUT PREGNANCY.**

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It is widely accepted that diet quality supply during gestation programs pup´s development. Our aim was to evaluate the impact of fructose rich diet (FRD) intake in adult females whose mothers consumed FRD during pregnancy. For this purpose on pregnancy day 1, dams were provided either with tap water alone or containing fructose (10% w/v; FRD) and fed *ad libitum* with chow up to delivery. Weaned female pups were maintained until 60-days old with normal diet *ad libitum*. Randomly, one group of pups was sacrificed (C and F considering maternal diet) and the remaining were split into two subgroups: one received normal diet (CC and FC) and the other FRD (CF and FF) for three weeks. Body weight (BW) and caloric intake (CI) were recorded from day 21 until 81 of age, data were analyzed by split plot ANOVA. At both sacrifice days (60 and 81) blood was collected and retroperitoneal adipose tissue (RPAT) was excised and weighed. T-tests were performed for 60 day´s results and 2-way ANOVA for 81 ones (programming and diet as variables). At age 60 days, F females only showed differences in CI in an age x programming dependent manner, with lower CI than C rats as age increases, but no metabolic changes were seen. At 81 days, all groups increased their BW in an age dependent manner, but programming and adult diet increased it even more. CI increased by FRD, showing FF rats the highest CI among groups. While plasma glucose levels did not vary, insulin increased by adult FRD. Triglycerides levels in FF animals were the highest, over all other groups. Moreover, programming by FRD induced higher levels of leptin in line with higher RPAT mass. In conclusion, FRD intake during pregnancy induces a programming effect on female litters, installed at age 81 days. Indeed, because maternal FRD intake effect appeared, the unbalanced diet consumption seems to trigger a metabolic misprogramming in adulthood. This effect could probably be due to the *in utero* high FRD pups' exposure. PICT 2017-2334

175. (353) **HEALTH, FOOD AND NUTRITIONAL SITUATION DURING MANDATORY ISOLATION DUE TO COVID-19 IN UNSL PERSONNEL**

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Food and supplement consumption, nutritional status, presence of diseases, physical activity practice and psychological factors were evaluated during compulsory isolation due to COVID-19 in teachers and non-teachers (in activity) 40 years and older, at UNSL, during the March-July period of the year 2020. The mentioned variables were evaluated in 62 individuals through a virtual survey (previously validated). For data analysis, descriptive statistics were performed. Most of the respondents (>60%) maintained the same consumption of the protective food groups with respect to their usual diet ("cereals, legumes and derivatives"; "vegetables and fruits"; "dairy", "meats and eggs" and "oils, nuts and seeds"). There was a noticeable decrease in the consumption of canned products (52%), ultra-processed foods (45%) and alcohol (40%) and an increase in the consumption of infusions (65%). 50% of those surveyed presented normal nutritional status, 32.3% overweight and 17.7% obese. 24.2% of those surveyed reported consuming nutritional supplements (vitamins and minerals, collagen and / or sports supplements). The most prevalent diseases were thyroid disorders (22.6%), arterial hypertension (19.4%), hypercholesterolemia (14.5%), and hypertriglyceridemia (9.7%). 72.6% of those surveyed maintained their practice of physical activity. 8% of those surveyed expressed feelings of stress, anxiety or depression due to the current pandemic situation. On the positive side, the consumption of healthy foods was maintained, the consumption of processed foods and alcohol decreased, and the consumption of infusions increased. In addition, the practice of physical activity was maintained and stress was manifested in a small group. As negative aspects, excess weight in 50% of the cases and the presence of chronic diseases stand out.

176. (366) **COPPER TOXICITY AND PROTEIN OXIDATION IN RAT HEART DUE TO CHRONIC OVERLOAD AND THE PROTECTIVE EFFECT OF VITAMIN E**

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Previous results indicated that chronic copper [Cu(II)] overload is toxic for liver and brain. Toxicity is associated with oxidative damage (OD) on biomolecules mediated by the generation of reactive oxygen species, mainly hydroxyl radical and organic hydroperoxides. The objective of this work is to assess whether OD due to chronic overload of Cu(II) in rats involves changes in redox homeostasis in heart (H) and the effect of vitamin E (Vit.E) on it prevention. Male Sprague Dawley rats (80-90 g) received standard diet and drinking water with 0.5 g/L Cu(II) (0.05%w/v, n=36) as $CuSO_4$ for 21 days, and Vit.E (100 mg/day, 5 g/kg food) over 5 days before Cu(II) overload. The following parameters were assessed in tissue homogenates: protein oxidation, measured as carbonyl groups (CO), phospholipid oxidation, measured as the content of thiobarbituric acid reactive substances (TBARS) and NADPH oxidase activity. In mitochondria, the oxygen consumption (ΔO_2) was measured with a Clark-type oxygen electrode. A 7-fold increase of CO was observed with Cu(II) overload (day 14, $p<0.001$), whereas Vit.E prevented protein oxidation (decreased 91% CO before Cu(II) treatment). Rats which received Vit.E or combined Vit.E and Cu(II) treatments for 21 days and showed lower levels of TBARS in the organ (30%, $p<0.01$ and 20%, $p<0.05$, respectively). NADPH oxidase activity decreased 100% with Cu(II) (day 7, $p<0.001$), 56% (day 14, $p<0.001$) and 45% (day 21, $p<0.05$). NADPH oxidase activity decreased with Vit.E (76%, day 7, $p<0.01$; and 36%, day 14, $p<0.05$) with Vit.E ($p<0.001$), and the combined treatment, decreased this complex enzyme 60% at days 7 and 14 ($p<0.001$). Cu(II) treatment decreased ΔO_2 (27%, day 7, $p<0.05$) and combined treatment 33% ($p<0.01$), 69% ($p<0.01$) and 18% ($p<0.5$) from day 7 to 21. Results indicate that Cu(II) chronic overload induces cytosolic, membrane and mitochondrial protein oxidation in heart which impairs their biological function whereas Vit.E protects cytosolic proteins.

177. (393) **ASSESSMENT OF SODIUM INTAKE OF YOUNG ADULTS IN A 3-YEAR STUDY**

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Elevated sodium intake is related to NCDs such as hypertension and cardiovascular diseases, being a huge public health problem. Aim of the study: to assess the sodium intake of young adults in a 3-year study. 754 university students (571 women, 183 men), aged 26±5 years, were studied from 2017 to 2019. A spot urine was obtained to determine Na, K by direct ISE and creatinine by Jaffé method. INTERSALT and Tanaka prediction equations were used to estimate 24-h urinary sodium excretion (24-hUNa,mg/d). Spot urine Na/K ratio was calculated. Throughout the 3-year study, 24-hUNa estimated by both equations pointed out an increased sodium dietary intake in more than 70% of women and 80% of men, exceeding WHO recommendation of 2 g/day. Na/K ratio was >1 in 90% of students. 24-hUNa estimated by INTERSALT was significantly higher in men than in women in 2018 and 2019 (2916.8±760.2 vs 2202.5±542.3; 2929.9±811.3 vs 2260.7±570.8; $p<0.001$). However, it was only significantly higher in men compared to women in 2018 when Tanaka was used (2888.3±896.5 vs 2608.4±761.3; $p<0.03$). 24-hUNa of women estimated by Tanaka was significantly higher compared to INTERSALT (2017: 2754.3±829.8 vs 2265.5±589.4; 2018: 2608.4±761.3 vs 2202.5±542.3; 2019: 2835.0±901.8 vs 2260.7±570.8; $p<0.001$). In men, a significant difference was only observed in 2017 (2867.0±793.2 vs 2091.0±941.1; $p<0.001$). There was no difference in 24-hUNa of women estimated by INTERSALT along the 3 years. However, 24-hUNa of men was lower in 2017 compared to 2018 and 2019 (2091.0±941.1 vs 2916.8±760.2 and 2929.9±811.3; $p<0.001$). When Tanaka was used, there was no difference in 24-hUNa of men along the study, but in women it was lower in 2018 than 2019 ($p<0.05$). Regardless of the used prediction equation, an excess in sodium intake was observed in young adults over time, being a risk factor for NCDs. Usually men's sodium intake was higher than women's. Tanaka seems to predict higher 24-hUNa values than INTERSALT, mainly in women.

178. (424) **INSULIN-LIKE GROWTH FACTOR TYPE 1 IN PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA AND ITS RELATIONSHIP WITH HEME BIOSYNTHESIS INTERMEDIATES**

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Acute Intermittent Porphyria (AIP) is caused by an hereditary disorder of heme biosynthesis, produced by a decrease in the activity of porphobilinogen deaminase, associated with an increase in the expression of the regulatory enzyme of this pathway, δ-aminolevulic synthetase 1 (ALAS1), accumulating the neurotoxic precursor δ-minolevulic acid (ALA) generating recurrent and intense seizures with neurological involvement.

Carbohydrate treatment rapidly reduces ALA production; the therapeutic effect is related to the ability of both glucose and insulin to modulate ALAS1 activity. Decreased levels of insulin-like growth factor 1 (IGF1) may alter the metabolism of specific carbohydrates and lipids, altering the flow of nutrients to the liver.

As IGF1 acts complementary to insulin, we decided to evaluate IGF1 levels and its relationship with heme metabolism in an AIP patients. In a population of 82 genetically diagnosed individuals, the biochemical parameters (BP) of AIP: ALA, porphobilinogen (PBG) and total porphyrins (TP) and the IGF1 levels were measured. Three groups were classified according to symptoms: Latent (L): no symptoms; Manifested (M): presented attack and BP values returned to normal; Subclinical Manifested (SM): presented attack and their BP values remained elevated.
To compare the BP and IGF1 levels between L, M and SM groups, the variables were categorized as: IGF1-n (normal) and IGF1-l (low) and for the BP: ALA, PBG and TP as normal and elevated.
There is only a significant association in the SM group ($p = 0.0029$): 84% of the patients have elevated ALA / IGF1-l and when we verify the relation between IGF1 with the three high BP simultaneously, the SM group shows a significant association ($p = 0.008$): 80% of patients with IGF1-l have the three BP high. [Irwin-Fischer bilateral and Chi square Pearson, $p < 0.05$].
We can conclude that there is a significant relationship between IGF1 levels and heme biosynthesis in AIP-SM patients.
Key words: Acute intermittent porphyria, acute attack, Insulin-like growth factor type 1

179. (439) **CHARACTERIZATION OF THE CAIMAN OIL OBTAINED FROM FATTY DEPOSITS AND ITS POTENTIAL USES IN HUMANS. A HEALTHY AND SUSTAINABLE ALTERNATIVE**

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The high economic value that crocodilian leather and meat has today is recognized. However, there are other derivatives such as fats from these animals, which are discarded and could be used as sources of natural oils with potential applications in food. In recent years, the demand for natural oils by the industry has increased, seeking natural alternatives, especially when we mention fatty acids (FA) of ω-3 and ω-6 families whose relationship can have different effects on health. The objective was to obtain caiman oil from fatty deposits of *Caiman latirostris* for its potential use as a dietary supplement. Oil was extracted from fat by melting at 60 °C. The FA profile was analyzed by gas chromatography. Oxidative stability was evaluated through peroxide index (PI), anisidine index (AI) and Kreiss index (KI). A microbiological study was carried out on the oil, which showed absence of total aerobic mesophilic bacteria, total coliform bacteria, Escherichia coli and Salmonella. The FA profile showed high content of oleic acid (33.4%) and linoleic acid (28.8%). Likewise, the presence of 2% α-linolenic acid was evidenced. The PI values were kept below 6 meq O2 / Kg oil, while KI was negative and AI was not detectable. Since the consumption of a product derived from caiman fat is not part of the usual diet of the population, it was essential to choose a reliable analytical methodology with high performance, high oxidative and hydrolytic stability, without microbial load and with nutritional quality and low cost. Nutritionally, obtained caiman oil results in an excellent source of essential FA, being a healthy and sustainable alternative that could be incorporated as part of the human diet.
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180. (503) **ANTHOCYANINS AS INHIBITORS OF α-GLUCOSIDASE AND PANCREATIC LIPASE ACTIVITY**

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Food components providing glucose and fat-lowering effects in the body can be useful to attenuate the negative health effects associated to high-carbohydrates and/or high-fat diets. Here, anthocyanins were studied as potential inhibitors of α-glucosidase (α-G) and pancreatic lipase (PL) activities, two key enzymes responsible for carbohydrates and fats digestion. The anthocyanin profile of extracts obtained from bilberry (BB), blackcurrant (BC) and black rice (BR) was characterized. The capacity of extracts, pure anthocyanins, and mixtures (representative of anthocyanin extracts composition) to inhibit the activity of α-G and PL was evaluated using enzymatic assays, including acarbose and orlistat as positive controls for α-G and PL, respectively. In a concentration range of 20-320 µg/mL, extracts showed a dose-dependent inhibitory effects on α-G activity. The calculated IC50 were 87 ± 7, 76 ± 7 and 236 ± 25 µg/mL for BB, BC and BR, respectively. Some pure anthocyanins showed inhibitory actions (cyanidin-3-*O*-glucoside>delphinidin-3-*O*-glucoside>petunidin-3-*O*-glucoside) whereas the mixtures were ineffective at the assayed range concentration. In a broader concentration range (0-2000 µg/mL), extracts did not show significant inhibition on porcine PL activity, but most of the pure anthocyanins were effective (petunidin-3-*O*-glucoside>cyanidin-3-*O*-glucoside>delphinidin-3-*O*-glucoside>malvidin-3-*O*-glucoside). No inhibition was observed for peonidin-3-*O*-glucoside and mixtures of compounds. In summary, anthocyanins would contribute only marginally to the in vitro α-G inhibition. Regarding PL activity, their inhibitory capacity was reduced when present in extracts or mixtures, suggesting complex interactions with other components under the in vitro used conditions. In vivo studies are needed to confirm the effects of extracts/pure anthocyanins on enzymatic activities observed here. UBACyT 20020170100586BA, PIP-CONICET 11220170100585CO, PICT 2018-03052.

181. (536) **OxLDL-INDUCED INFLAMMATION IN ISOLATED AORTA: EFFECTS OF SHORT CHAIN FATTY ACIDS.**

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Oxidized low density lipoproteins (OxLDL) has been implicated in NLRP3 inflammasome activation in the vascular wall. Short-chain fatty acids (SCFA) produced by gut microbiota, could provide beneficial effects in attenuating endothelial dysfunction and inflammatory response associated with metabolic diseases.The aim of this work was to evaluate select short chain fatty acids (SCFA) on the OxLDL-induced NLRP3 activation in aorta by using an ex-vivo model. Thoracic aortas from male Sprague Dawley rats were cut in rings, and incubated with OxLDL (0-200 mg/ml) or vehicle for 0-120 min. At the end of the incubation periods, expression of proteins (NLRP3, procaspase-1, caspase-1, pro-IL1beta, TNFalpha) and detection of 4-hidroxinonenal adducts (4HNE) were measured by western blot in the aorta rings, and interleukin levels (IL1beta and IL18) by ELISA in the incubation media. OxLDL produced modifications in all the measured parameters, with different time-course and degree of response. The earliest responses (10 min) were observed for NLRP3 expression in aorta (increments of 25, 125, and 100% for 50, 100 and 200 mg/ml OxLDL, respectively $p<0.05$, compared to vehicle), and for IL18 levels in the incubation media (increments of 128, 200, and 300% for 50, 100 and 200 mg/ml OxLDL, respectively $p<0.05$, compared to vehicle). To evaluate SCFA effect on this model, before treatment with OxLDL, aortic rings were pre-incubated with 10 µM butyrate, propionate, or acetate. Preincubation with each SCFA prevented OxLDL-induced modifications in different parameters: acetate prevented IL18 increased (-32%, $p<0.05$), butyrate prevented TNFalpha increased (-21%, $p<0.05$), and propionate prevented only 4HNE detection. In conclusion, OxLDL activates NLRP3 inflammasome in isolated aorta, and the assayed SCFA inhibited that activation at different levels of the inflammasome cascade. UBACyT 20020170100586BA, PIP-CONICET 11220170100585CO, PICT

2018-03052.

NEFROLOGÍA

182. (103) **ELIGLUSTAT PROTECTS FROM DAMAGE CAUSED BY SHIGA TOXIN TYPE 2 IN HUMAN RENAL TUBULAR EPITHELIAL CELLS**

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Shiga toxin-producing *Escherichia coli* is responsible for Hemolytic Uremic Syndrome (HUS), a cause of renal failure in children. We have previously shown that C-9 and Eliglustat (EG), inhibitors of glucosylceramide synthase and globotriaosylceramide (Gb3), prevent the cytotoxic effects of Shiga toxin type 2 (Stx2), in human cortical renal tubular epithelial cells (HRTEC) primary cultures and HK2 cell line. The aim of this work was to evaluate the efficacy of EG, elucidating EG treatments necessary to achieve total protection against Stx2 in HRTEC and HK2. Cells were incubated with Stx2 (1 ng/ml, 24 and 72 h) and pre-incubated with or without EG (1-500 nM, 6 and 24 h), followed by co-incubation with same dilutions of EG and Stx2 (24 and 72 h). Total number of cells stained with Hoechst was counted in microphotographs and compared with cell viability measured by neutral red uptake. Early and late apoptosis and necrosis was evaluated by annexin V/propidium iodide staining. Tubulogenesis was evaluated in HRTEC grown on matrigel. Treatment of cells with Stx2 significantly decreased cell confluence and viability and the number of cells attached ($p<0.001$). In HRTEC, Stx2 increased early and late apoptosis, and necrosis compared to non-treated cells ($p<0.01$). Furthermore, Stx2 inhibited cell aggregation and tubulogenesis on matrigel. HRTEC preincubated with EG (50 nM, 24 h or 500 nM, 6 h) totally prevented Stx2 effects on HRTEC measured as cell count, viability, apoptosis, necrosis and tubulogenesis ($p<0.05$). Preincubation of HK2 cells with EG (1 nM, 24 h or 10 nM, 6 h) totally prevented Stx2 effects on cell viability and confluence. EG alone did not produce cytotoxic effects *per se*. In conclusion, EG protects human renal tubular epithelium against Stx2 cytotoxicity being HRTEC more sensitive than HK2. Treatment with EG could be a novel substrate inhibition therapy to neutralize Stx2 action and prevent renal damage in patients with HUS. Study supported by PUE0041, CONICET.

183. (284) **EFFECTS OF SHIGA TOXIN TYPE 2 IN PREGNANT AND NON-PREGNANT FEMALE RATS**

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Shiga toxin-producing *Escherichia coli* causes acute renal failure and Hemolytic Uremic Syndrome. It was reported that inhibition of nitric oxide (NO) by Shiga toxin type 2 (Stx2) enhanced renal damage in mice and baboon models of Stx-mediated HUS. The aim of the work was to study the evolution of the damages caused by Stx2 in P compare with NP rats. Pregnant Sprague-Dawley rats, at day 8 of gestation, and NP rats were ip inoculated with 0.5 ng Stx2/g body weight (PS, NPS) or diluent (PC, NPC). Some PS and PC rats were treated with 1mg/ml L-NAME, NO inhibitor, in drinking water (PLS, PLC) from 24h before ip injection to 4 days post-injection (dpi). Rats were individually housed, checked for water and food intake, and weighted every 24h until 30 dpi. At 4 dpi, blood and 24h-urine samples were collected to determine urinary flow and free water clearance (C_{H20}). Then, rats were euthanized and kidneys were removed for histopathological observations. NPS and PS rats showed a decrease in food intake and weight with respect to controls ($p<0.05$). PS rats increased food intake and recovered weight at 5 dpi, while NPS rats showed an improvement at 14 dpi. The water intake increased in NPS and PS rats compared to controls until 7 dpi ($p<0.05$). In NPS at 4 dpi, the rise in water intake coincided with an increase in urinary flow and C_{H20} respect to NPC ($p<0.05$), different from what was observed in PS. The renal cortex of NPS presented significantly more necrosis and atrophied tubules than PS ($p<0.05$). Preliminary results in PLS rats showed that L-NAME significantly increased renal necrosis compared with PLS and PLC rats ($p<0.05$). In conclusion, PS rats suffered less renal damage and recovered from the Stx2 effect faster than NPS rats. L-NAME increased Stx2 effect in PLS suggesting that physiology changes caused by pregnancy, like increasing in NO production, may contribute to protect maternal kidney from Stx2 effects.

184. (359) **MOLECULAR MECHANISMS INVOLVED IN RENAL ALTERATIONS TRIGGERED BY ENDOTHELIN INHIBITION IN THE RAT DURING THE POSTNATAL PERIOD**

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We had previously shown that Endothelin (ET) inhibition during the early postnatal period (PNP) with bosentan (20 mg/kg/day), a dual ET receptor antagonist (ERA), leads to alterations in both ET-1 and ET receptors expressions in adult rats during a high sodium intake. We had also shown that ET inhibition during PNP increases apoptosis in the kidney and this could be a consequence of an imbalance between nitric oxide (NO) and superoxide (O_2^-). It is known that the delicate balance between NO and O_2^-, besides contributing to development, is important for renal sodium handling.
The aim of this work was to evaluate in 7 day old rats (male and female controls and ERA-treated rats): renal mitochondrial NO/O_2^- ratio, nitric oxide synthase (NOS) activity in different renal structures estimated by NADPH-diaphorase technique, renal NOX4, ET_A and ET_B expressions by Westernblot and renal pre-pro ET-1 by real time PCR. Four experimental groups were studied: control males (Cm), males treated with bosentan (ERAm), control females (Cf) and females treated with bosentan (ERAf). Two-way ANOVA was used for statistics.
We found an effect of sex for pre-pro ET-1 expression ($p<0.05$), being higher in m than in f and for ETB expression ($p<0.02$), being higher in f than in m. However ET_A/ET_B ratio was not significantly different between groups.
On the other hand, we found a decrease in NADPHd activity in immature cortical renal structures only in ERAm ($p<0.05$) and in macula densa in both ERAm and ERAf vs their controls ($p<0.05$) and a clear tendency to decrease NO/O_2^- ratio in ERA-treated animals. NOX4 expression also had a tendency to increase in both ERAm (0.70 ± 0.18 vs 0.87 ± 0.17) and ERAf (0.64 ± 0.18 vs 0.86 ± 0.06) vs their respective controls. This tendency could explain the tendency to decrease NO/O_2^- ratio in ERA-treated animals. The expression of renal pre-pro ET-1 and ETB receptor has sex differences in early life but it is not affected by ET inhibition at this stage.

185. (378) **EFFECTS OF ESTROGENS ON RENAL PROXIMAL TUBULE EPITHELIAL CELLS**

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We have previously demonstrated that 17β-Estradiol (17βE) stimulates cell proliferation through classic estrogen receptors (ER) and the G protein-coupled estrogen receptor 1 (GPER-1), in primary cultures of human renal cortical tubular epithelial cells (HRTEC). We also observed that 17βE decreases the expression of Na+ K+ ATPase (NKA) in primary cultures. The aim of the present work is to study the effects of 17βE on cell proliferation and the expression of NKA in a human renal proximal tubular epithelial *cell line* (*HK-2*) and its comparison with previous results in HRTEC and in studies *in vivo*

with ovariectomized (oVx) Wistar rats. HK-2 were treated with 17βE (10 nM, 24 h) with or without an agonist (G-1) or an antagonist (G-15) of GPER-1. Cell proliferation was measured by bromodeoxyuridine (BrdU) uptake. The expression of NKA was assayed by western blot. In HK-2, 17βE stimulated the BrdU uptake (26%) compared with control cells ($p<0.05$). The treatment of HK-2 with G-15 (100 nM) inhibited 17βE effect on cell proliferation. The treatment with G-1 (1000 nM) inhibited the BrdU uptake as observed in HRTEC ($p<0.05$). The treatment of HK-2 with 17βE and with G-1 (10 nM, 24 hs) decreased NKA expression compared with control cells ($p<0.05$), demonstrating that estradiol exerts these effects through GPER-1. These results agree with previous studies in HRTEC, where an increase of D1DR (dopamine receptor) expression was associated with the decrease of NKA. These results also match with previous studies in female adult Wistar rats, in which the oVx produced an increase of NKA expression in renal medulla while there was a decrease of D1DR, both in cortex and medulla. Likewise, hormonal replacement on oVx animals with 17βE diminished the expression of NKA in renal medulla. In conclusion, our present results show that HK-2 cell line can be a valid *in vitro* model for better understanding of molecular and cellular renal mechanisms regulated by female sex hormones like estrogen.

186. (444) **A MULTIVARIATE RELATIONSHIP BETWEEN LABORATORY DATA DURING THE EVOLUTION OF TYPICAL HEMOLYTIC UREMIC SYNDROME CHILDREN POPULATION**

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Hemolytic uremic syndrome (HUS) is a systemic disease characterized by variable degrees of acute nephropathy, thrombocytopenia and microangiopathic hemolytic anemia. Laboratory and clinical parameters contribute very closely to progression of HUS. To better understand HUS evolution, the association between a set of laboratory data and a set of clinical parameters of a HUS population is investigated in this study.

We conducted a retrospective study of patients (n = 20) attended with diagnosis of typical HUS in the Pediatric Service of the Hospital Posadas from January 2012 to July 2020. 70% were women, with a mean age of 2.19 year. All laboratory data including those from the emergency department (admission), hospitalization, up to the first post-discharge check-up by external clinics were standardized in innovative report formats.

We perform the graphical representation of the evolution over time of several of the important clinical parameters (creatinine, hematocrit, hemoglobin, among others). We find the creatinine curve relevant with well-defined moments in its evolution: rise, plateau and decline. We emphasize that 50% of the patients present a similar descent slope (- 0.353 +/- 0.022 mg/dL/day) regardless of the maximum value reached by creatinine. Also, analytic platform KNIME was used to evaluate the multivariate relationship between laboratory data and the evolution plasma creatinine values. We observed a strong correlation between the plasma values of creatinine-urea (positive, r = 0,818), platelets-uric acid (negative, r = 0,610) and direct bilirubin-uric acid (positive r = 0,735).

The study should be complemented with the comparison of qualitative variables, as well as with new parameters such as albuminuria, podocyturia, etc.), in order to generate a model of prediction of patient evolution during the acute period of HUS the disease and after it.

187. (468) **NATRIURETIC PEPTIDES AND RENAL INFLAMMATION IN TWO MODELS OF HYPERTENSION: DEOXYCORTICOSTERONE ACETATE-SALT AND RENOVASCULAR 1 KIDNEY-1 CLIP**

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Objectives: The aim was to demonstrate the hypothesis that in DOCA-salt (DS) and renovascular 1K-1C (RV) hypertensive models, the development of hypertension and the changes in natriuretic peptides (NP) secretion could be related to simultaneous over expression of NP receptors and inflammatory and fibrotic biomarkers in kidney.

Materials: Male Sprague-Dawley rats were randomly divided in three groups: Control (C), DS (30 mg/kg-Salt 1% W/W and RV. After 6 and 12 weeks, systolic blood pressure (SBP) was measured by the tail cuff method; plasma ANP and BNP levels were determined by commercial radioimmunoassay; mRNA expression of receptors NPR-A and NPR-C were measured in kidney by RT-PCR; and immuno-expression of inflammatory biomarkers (IL-6, TNF-α and NF-κB) and fibrotic biomarker TGF-β, by immunohistochemistry. Statistical analysis was performed by two-way ANOVA followed by a Tukey-Kramer test.

Results: SBP increased in DS and RV at 6 and 12 weeks (mmHg: C6:118+2; DS6:194+2*; RV6:184±2*; C12:121±1; DS12:193±4*; RV12:203±4*). Plasma ANP increased in DS and RV (pg/mL: C6:170+68; DS6:689+143*; RV6:387±90*; C12:87±39; DS12:609±38*; RV12:294±57*), while plasma BNP rose only in RV (pg/mL: C6:39±6; RV6:99±8*; C12:42±7; RV12:94±10*). mRNA levels of NPR-A (AU, C6:0.096±0.011; DS6:0.232±0.015*; C12:0.092±0.020; DS12:0.328±0.039*) and NPR-C (AU, C6:0.184±0.020; DS6:0.721±0.131*; C12:0.131±0.026; DS12:0.302±0.039*) increased only in DS. All biomarkers increased in DS at 6 and 12 weeks (*), while TGF-β and TNF-α enhanced in RV only at 12 weeks (*$p<0.05$ vs C).

Conclusion: Results suggest that ANP is a better marker for volume overload, while BNP level was increased only in the RV model. mRNA expression of both NP receptors increased only in DS and correlated with plasma ANP levels. Furthermore, inflammatory biomarkers expression increased faster in kidney DS than in RV, suggesting that volume overload induces earlier inflammation than pressure overload.

188. (490) **ERYTHROPOIETIN IN URINE AS A NOVEL EARLY BIOMARKER OF CISPLATIN-INDUCED NEPHROTOXICITY IN RATS.**

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Erythropoietin (EPO) is a glycoprotein hormone produced primarily in the kidney in response to hypoxic conditions. There is little information about the role of endogenous EPO in urine. In this regard, we were pioneering in detecting EPO in rat urine. Cisplatin is a chemotherapeutic agent broadly used. Its primary dose-limiting side effect is nephrotoxicity. The aim of this work was to analyse EPO urinary excretion (EPOu) in rats treated with Cisplatin and compare it with traditional and novel markers of renal injury. Male Wistar rats were treated with different single doses of Cisplatin (2, 5 and 10 mg/kg b.w., i.p.; Cis2, Cis5 and Cis10, n=4, respectively). A Control group of rats (C, n=4) was processed. After 48 h of Cisplatin administration plasma and urine samples were collected. Urea and creatinine plasma levels (Ur_p and Cr_p) and total proteins levels in urine (Pr_u) were determined spectrophotometrically. EPOu and Neutrophil gelatinase-associated lipocalin (NGALu) were evaluated by immunoblotting. ANOVA/Newman-Keuls test, $P<0.05$: *a* vs C; *b* vs Cis2; *c* vs Cis5; *d* vs Cis10. Results: $\mathbf{Ur_p}$(g/L): C=0.32±0.01,

Cis2=0.28±0.01[d], Cis5=0.49±0.08[d], Cis10=1.23±0.09[a,b,c]; **Cr_p**(mg/dL): C=5.90±0,70, Cis2=5,45±0,78[d], Cis5=7.66±0.71[d], Cis-10=11,70±0.79[a,b,c]; **Pr_u**(g/gCr): 0.99±0.12, Cis2=1.06±0.09[d], Cis-5=1.38±0.28[d], Cis10=2.53±0.25[a,b,c]; **EPOu**(%): C=100±5, Cis-2=88±6[c,d], Cis5=141±11[a,b], Cis10=163±10[a,b]; **NGALu**(%): C=100±3, Cis2=128±9[d], Cis5=115±9[d], Cis10=179±26[a,b,c]. Ur_p, Cr_p and Pr_u, as well as NGALu were significantly modified only in Cis10 group. In contrast, EPOu was significantly modified at a lower dose of Cisplatin (5 mg/kg), allowing predicting renal perturbation, when no modifications of other markers of renal injury were still observed. These results could postulate EPOu as a novel early biomarker of cisplatin-induced nephrotoxicity in rats. Besides, EPOu could contribute to create a well-designed panel of biomakers that allows predicting renal injury in Cisplatin treatment.

189. (494) **EFFECT OF TESTOSTERONE ON RENAL MANEUVER OF HIGH SODIUM INTAKE IN NORMOTENSIVE RATS**

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Introduction. Sexual hormones have an important role in the regulation of renal function and blood pressure in humans and also in several experimental animal models. We studied the renal response to high sodium intake in male Wistar rats either orchidectomized (ORX) or ORX supplemented with testosterone (ORX-To).
Methods. Male Wistar rats were ORX at 60 days of life. At 138 days of life, half of them were supplemented with 5 mg testosterone propionate (s.c. pellets) and the other half remained ORX with cholesterol (ORX-cho) as vehicle. During the last 5 days of the study, half of rats in each group received normal sodium (NS, NaCl 0.24%) or high sodium intake (HS, NaCl 1% in drinking water). Glomerular filtration rate (GFR), renal plasma flow (RPF), sodium and water excretion and mean arterial pressure (MAP) were measured. To analyze the response of renal function upon HS, total alpha 1 subunit Na+,K+-ATPase mRNA (Atpa1a) expression by RT-PCR and the ratio between dephosphorylated and total Na+,K+-ATPase (NKA) as an activity index (dNKA/tNKA) were determined in renal cortex and medulla by Western blot.
Results. MAP under NS was similar between ORX-cho and ORX-To and did not change upon HS intake. ORX-To rats showed an increased dNKA/tNKA ratio in renal medulla under NS and HS diet and a lower cortical Atpa1a than ORX-cho with HS. Sodium and water excretion in ORX-To HS were lower than in ORX-cho HS (both $p<0.05$). GFR and RPF increased with HS in ORX-To and ORX-cho (both $p<0.05$), but the increment with To was higher than in ORX-cho ($p<0.05$).
Conclusion. The retention of sodium and water in To could be the consequence of a more active NKA. Despite sodium and water retention, MAP remains unchanged probably by a decrease in renal vascular resistance. Under HS intake the volume of the extracellular fluid and MAP are regulated through renal hemodynamic mechanisms. These findings provide a better understanding of the effect of male sexual hormones on renal function.

190. (483) **IDENTIFICATION OF GLOMERULAR BIOMARKERS IN URINE CELLS FROM PATIENTS WITH TYPE 2 DIABETES**

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Type 2 Diabetes Mellitus (T2DM) is characterized by the presence of insulin resistance and a prolonged asymptomatic period that makes early diagnosis difficult, so the identification of early damage markers such as those associated with diabetic nephropathy (DN) is extremely valuable. In this context, the involvement of CD39 and CD73 ectoenzymes in DN is not entirely clear, but many studies suggest that alterations in the extracellular ATP metabolic machinery trigger the pathophysiology of the disease. Our objective was to identify early markers of glomerular kidney damage and the expression of CD39 and CD73 in urine cells of T2DM patients. To this aim, the expressions of nephrin (NEF), podocalyxin (PDX), CD39 and CD73 were evaluated by immunofluorescence (IF) in renal explants and in the first morning urine of T2DM patients to determine the detachment of podocytes. In addition, the plasma profile of cytokines was analyzed by LengendPlex and flow cytometry. The IF revealed that PDX is an exclusive marker for podocytes, while NEF was widely expressed in tubules and Bowman's capsule cells. CD39 was detected in vasculature cells and CD73 was located ubiquitously in the kidney. Preliminary results showed that T2DM patients presented about 2 fold and 3 fold higher frequency of urine NEF+ and PDX+ cells, respectively, than control donors. Furthermore, the percentage of NEF+ and PDX+ cells expressing CD39 in the urine of T2DM patients were about 5 times higher than the corresponding cells from control donors. Finally, the median fluorescence intensity (MFI) of IL-6 (101,92±44,13), IL-4 (130,65±50,18) and TNF-a (130,65±30,14) in plasma from T2DM patients (n=4) tend to be higher that the control counterparts (n=5) (IL-6 69,26±9,26 ($p= 0.1435$); IL-4 93,91±7,43 ($p= 0.3819$); and TNF-a 46,49±0,69 ($p= 0.0824$)). In conclusion, the results suggest that urine from T2DM patients present increased detached podocytes, which express higher levels of purinergic pathway ectoenzymes.

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191. (74) **EVIDENCE FOR RESVERATROL MODULATING RAC1 ACTIVATION IN MICROGLIAL CELLS**

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Maternal immune activation (MIA) produces structural, metabolic and epigenetic changes in the fetus resulting in long-term neurological and neuropsychiatric consequences. 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. It has been determined that Rac1 plays a central role in the inflammatory response and microglial neurotoxicity in the CNS.
It has been demonstrated that resveratrol has anti-inflammatory, antioxidant properties that translate into neuroprotective effects in adults.
Objectives: To study the possible neuroprotective effects of resveratrol in the fetal CNS in a model of MIA induced by bacterial lipopolysaccharide (LPS) and to characterize Rac1 signaling pathways involved in the modulation of the microglial response.
Resveratrol was administered to Balb/c females on gestational day 15, which were then exposed or not to LPS. After LPS administration, maternal sera and amniotic liquid were collected to evaluate the expression of the proinflammatory cytokine IL-6. Additionally, cell cultures of the murine microglial cell line BV2 were exposed to different concentrations of LPS and resveratrol and variations in Rac1 activation were analyzed at different time-points.
Results: Preliminary results show that the LPS-triggered MIA induces IL-6 in the mother sera and in the amniotic liquid ($p <0.05$ respectively) while resveratrol prevents this effect.
The optimal concentration and time exposure of BV2 cells to LPS that induces maximal Rac1 activation are 100 ng and 15 min ($p < 0,05$). We observed that in vitro treatment with resveratrol interferes with the activation of Rac1 signaling pathways.
Conclusion: Maternal immune activation increases IL-6 production in peripheral blood and amniotic fluid. Rac1 activation decreases in the presence of resveratrol.

192. (83) **ANTIINFLAMMATORY ACTIONS OF TIBOLONE IN THE SPINAL CORD OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MICE**

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Tibolone (TIB) is a synthetic estrogenic compound with tissue-specific actions commonly used to prevent symptoms in postmenopausal women. The tissue selectivity of TIB depends on its specific local transformation to active metabolites that can exert estrogenic, progestinic and androgenic activities. Recently, antioxidant and anti-inflammatory actions of TIB have been described in the CNS. In this study we assessed the effect of TIB on the clinical outcome, inflammation and myelination in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice model of multiple Sclerosis (MS). To this purpose, female C57BL/6 mice were divided into control (CTRL), EAE receiving TIB (0.08mg/kg s.c every other day from the day of induction (EAE-TIB) or EAE receiving vehicle (EAE-V) groups. Spinal cord tissues were collected at day 17 post induction and subjected to qPCR and Western Blot analysis. EAE-TIB group showed decreased microglial reaction revealed by lower CD11B and toll like receptor 4 (TLR4) mRNAs expression respect of EAE-V ($p<0.01$ and $p<0.05$). NLRP3 inflammasome signalling pathway was assessed by analyzing NLRP3, IL-1β and IL-18 mRNAs and cleaved caspase 1 protein expression. EAE-V showed an aberrant expression of NLRP3 and activated caspase 1 vs CTRL mice, while TIB administration significantly decreased both parameters (EAE-V vs EAE-TIB; $p<0.05$ and $p<0.01$ respectively). IL-1β was hyperexpressed in EAE-V vs CTL and significantly decreased with TIB treatment ($p<0.05$ and $p<0.001$ respectively), while IL-18 showed no intergroup differences. In addition, the EAE-TIB group showed reduced myelin basic protein mRNA loss ($p<0.05$), and better clinical outcome compared to TIB untreated EAE mice. Interestingly, hypertrophic effects on the uterus was not evidenced by TIB administration. As a conclusion, TIB exerts anti-inflammatory and myelin protective actions in the spinal cord of EAE mice without uterine compromise, suggesting its potential value for MS treatment.

193. (87) **ALZHEIMER´S DISEASE AS A METABOLIC PATHOLOGY: INFLAMMATION AND INSULIN RESISTANCE IN A MOUSE MODEL OF AD. IN VIVO AND IN VITRO APPROACHES.**

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Insulin resistance and chronic inflammation are frequently associated with the development of cognitive disorders and neurodegenerative diseases such as Alzheimer's disease (AD). However, it is not yet clear whether there is a causal link between these two factors, and which one appears earlier in the pathology. Our objective was to study metabolic parameters and inflammation in a possible brain insulin resistance context in a transgenic model of AD, the PDAPP-J20 mouse at the age of 8 months. We hypothesized that an inflammatory environment could trigger insulin resistance in the brain, neurodegeneration and cognitive impairment. We found lower glycemic levels and altered phosphorylation levels of AKT measured by Western Blot -an indicator of the insulin signaling status- in the hippocampus of transgenic mice compared with control mice ($p<0.05$). However, we did not find significant differences in pAKT/AKT in the liver and hypothalamus. In addition, insulinemia was not affected. We also evaluated inflammation markers as GFAP and S100b immunoreactive areas by immunohistochemistry in the hippocampus, which were both increased in transgenic mice ($p<0.05$). Microglial soma size was also increased, suggesting a glial proinflammatory state. Finally, to understand the role of Aβ on astrocytes, we evaluated the effect of fibrillar Aβ on C6 cells in vitro, through the NFkB and the AKT pathways. Astrocytes exposed to fAβ showed increased nuclear translocation of NFkB and decreased AKT phosphorylation ($p<0.05$), suggesting astrocyte inflammatory activation and impaired insulin signaling, respectively. Our results show that inflammation and insulin signaling impairment in the hippocampus are found in an early stage of the pathology when there is yet little Aβ deposition, suggesting an early role of these pathways on the pathophysiology of experimental AD.

194. (93) **THE ADMINISTRATION OF TELLURIUM TO RAT MOTHERS IN THE LACTATION PERIOD AFFECTS THE NATURAL LATERALIZED EXPLORATION AND SOCIAL ACTIVITY OF THEIR OFFSPRING MATURING RATS.**

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Previous evidence from our laboratory showed that intact rats subjected to a chronic administration (51 days) of non-toxic K2TeO3 (Te, 0.39µg/L) the normal response of lateralized exploration and social activity of the offspring maturing rats was affected. In order to investigate if these modified behaviours were due to Te prolonged exposition, in the present work a short treatment (21 days) of Te administration to mother rats in the lactation period was applied. At 30 day-old, all offspring were tested in the Double Lateral Hole Board Labyrinth (DLHB), and 24 hours later in the Intruder to Resident Test (IRT), as previously described. The following groups were formed: Control rats (n=20) and Te-treated rats (n=18). Results found in the DLHB showed that Control rats had a left biased exploration (70±5.3 Vs. 45.5±4.1 Counts/3 min; left Vs right, $p < 0.01$), while Te-treated rats showed no side preference exploration (49±3.9 Vs 47.5±6.3 C/3min; Left Vs right, n.s.). In the IRT, control rats showed a latency to confront the intruder rat of 9±2.6 C/2min; while in the Te-treated rats latency was 32±8.3 C/2min, statistically different from Control group ($p < 0.02$). Duration of social interaction in the Control group was 93±5.5 C/3min; while in the Te-treated rats was 73±3.6 C/3min, statistically lower from Control group ($p < 0.01$). In conclusion, results suggest that it is not necessary a prolonged administration of Te in order to affect the exploratory and social activity in the offspring rats.

195. (114) **AMYLOID-β INDUCES ENDOTHELIAL ENDOPLASMIC RETICULUM STRESS IN ASSOCIATION WITH BLOOD-BRAIN BARRIER DISRUPTION IN EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE.**

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Alzheimer's disease (AD) is the leading cause of dementia. Among other histopathological hallmarks, it is characterized by the abnormal accumulation of Amyloid-β (Aβ) peptides. Vascular alterations and blood-brain barrier disruption are also evidenced in AD patients, in close association with perivascular amyloid deposits, which are composed mainly of Aβ 1-40. In this study we characterized the progression of vascular alterations in the hippocampus of PDAPP-J20 mice, a validated transgenic model for AD. We found a significant increment in morphological alterations in vessels from AD mice compared to age-matched controls ($p<0.05$), mainly in vessels surrounded by Aβ deposits. At ages in which parenchymal amyloid deposits were evident, we found an increased permeability of the blood-brain barrier, evidenced by the extravasation of systemically injected Evans blue and sodium fluorescein ($p<0.05$), in association with a decreased immunoreactivity for the endothelial tight junction protein occludin ($p<0.05$). Then, we obtained cerebral vascular fractions from AD and control mice, and measured the levels of vascular proteins through mass spectrometry and proteomics analysis. We identified 82 proteins whose levels were decreased in AD mice. The enrichment analysis showed that the most represented cellular processes in this sample were translation and protein synthesis. Aβ

was linked to endoplasmic reticulum stress (ERS) induction in the CNS. Human brain microvascular endothelial cells exposed to Aβ1-40 showed not only a decrease in the occludin detection ($p<0.05$) with concomitant decreased trasendothelial electrical resistance ($p<0.05$) but an increment in BIP and IRE-1α expression ($p<0.05$), confirming that Aβ caused ERS with disruption of the blood-brain barrier in this in vitro model. Our results suggest that ERS, down-regulation of translation and loss of proteostasis in vascular cells emerge as mediators for Aβ-induced endothelial alterations during the progression of AD.

196. (117) **METABOLIC LIPOTOXICITY-INDUCED NEUROINFLAMMATION: POTENTIAL ROLE OF GLIAL CELLS INTERACTION VIA EXTRACELLULAR VESICLES**

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Obesity and related metabolic disorders are important risk factors for brain aging, promoting alterations in the plasticity of limbic structures such as the hippocampus. Among the underlying mechanisms, chronic inflammation and insulin resistance are crucial factors, also associated with alterations of sphingolipid metabolism. Taking this into account, we intend to study mechanisms associated with the impact of metabolic disturbances on the brain. We aim to assess the inflammatory response induced by a lipotoxic context and the communication between glial cells mediated by the release of extracellular vesicles (EVs) as vehicles of damage propagation.
We previously found that C57BL/6 mice exposed to a high fat diet (HFD) presented neuroinflammation, decreased neurogenesis and structural synaptic alterations, together with spatial memory impairment. To assess potential mechanisms involved, we used an *in vitro* approach emulating the lipotoxic context with the saturated fatty acid palmitate (PA). Microglial cultures exposed to PA showed a pro-inflammatory profile and, after purification of EVs from the conditioned media (CM), we found that exosomes altered dendritic spine morphology of hippocampal neurons. Here, we show that in the presence of ceramide synthesis inhibitor Cambinol, the induced expression of IL1β in PA-exposed BV2 microglial cells was diminished ($p<0.05$). In the same line, Cambinol seemed to prevent the decreased phagocytic capacity of BV2 cells exposed to PA. Finally, regarding the interaction between glial cells, preliminary results showed that CM from PA-stimulated BV2 cells was able to induce the expression of IL1β in C6 astrocytic cell line. Interestingly, after EVs isolation, exosomes derived from PA-microglia exerted the same effect.
Our results suggest a role of ceramide pathway in the inflammatory context induced by PA and the potential involvement of exosome-like EVs in the propagation of the damage response between glial cells.

197. (120) **SUSTAINED INCREASE IN DNMT1 AND DNMT3A EXPRESSION IN RESPONSE TO THE INITIAL PRECIPITATING INJURY IN LITHIUM-PILOCARPINE EPILEPTIC MODEL.**

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Retrospective studies have shown that temporal lobe epilepsy (TLE) patients refer an initial precipitating event (IPE) during early childhood and a subsequent latency period in which seizures are absent. Latency period is a poorly studied subject in epilepsy, but it is proposed that essential steps in epileptogenesis occur during this period. The lithium-pilocapine model presents most of the characteristics of human TLE in experimental animals, including an IPE followed by a latency period. Epigenetics changes in the latency period have been described. On the other hand, important astroglial genes, such as Kir4.1 which is involved in K+ homeostasis, are heavily epigenetically-regulated genes. In the present work we induced an IPE (status epilepticus, SE) in male Wistar rats by administering 3 mEq/kg LiCl and 30 mg/kg pilocarpine. SE lasted for 20 min and then seizures were stopped with 20 mg/kg diazepam. Animals were sacrificed at 7- or 21- days post-SE (DPSE) and brains processed for biochemistry or fixed for immunofluorescence. We observed that Kir4.1 expression was reduced in hippocampal and cortical astrocytes concomitantly with increased GFAP expression and reactive gliosis in these areas. Kir4.1 gene (KCNJ10) decreased mRNA was also shown by RT-PCR and we consistently noticed an increase in the expression of DNMT1 and DNMT3a at 7- and 21-DPSE. By performing in silico analysis, we have also observed that proximal DNMT1 and DNMT3a promoters have consensus sites for NF-kB and Stat3 transcription factors that are known to be activated in reactive astrocytes. Considering published evidence regarding the participation of DNMT1 in the regulation of KCNJ10 in astrocytes, we here propose that the observed astroglial Kir4.1 downregulation induced by the SE-induced IPE is probably lying downstream of reactive astrogliosis, NF-kB/Stat3 activation and increased DNMT activity.
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198. (134) **SPLEEN-BRAIN INFLAMMATORY COUPLING IN A MODEL OF TEMPORAL LOBE EPILEPSY**

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A high percentage of patients with temporal lobe epilepsy (TLE), one of the most frequent neurological diseases, refer an initial precipitating event, such as complex febrile seizures during childhood, followed by a silent latency period (LP), until the onset of the chronic seizures phase. Using the lithium-pilocarpine rat model of TLE we have previously shown that neurodegeneration, reactive gliosis and macrophages brain infiltration occur during the LP and that early interventions limiting immune activation during the LP increase epileptic threshold during the chronic phase (Rossi et al., 2013; 2017). We here studied the peripheral immune cells participation in the LP that follows pilocarpine-induced SE. Male Wistar rats were treated with lithium-pilocarpine (127 mg/kg /30 mg/kg) developing SE, that were limited to 20 min by 20 mg/kg i.p. diazepam. Histological analysis of spleen sections 8 h or 1, 2, 3 days post SE (DPSE) showed a peak in the disorganization of the spleen white pulp at 1 DPSE. This disorganization seemed to be spleen-specific since we did not find significant changes in the gut-associated lymphoid tissue (GALT). On the other hand, we found increased abundance of CD4+ lymphocytes in the choroid plexus at 3DPSE, suggesting a brain-spleen inflammatory coupling. Accordingly, our loss of function studies performed on splenectomized and sham-operated rats (1w before lithium-pilocarpine-induced SE) showed that splenectomy decreased astrogliosis and neuroinflammation at 7DPSE. Our results suggest that peripheral immune system is probably responding to specific brain-derived clues triggered by the SE and the spleen is directly involved in the modulation of neuroinflammation that follows SE. Supported by PICT 2017-2203; UBACYT; and FONCYT fellowship (PS).

199. (164) **ANALYSIS OF THE IMPACT OF THE BODY MASS INDEX ON COGNITTIVE VARIABLES IN PATIENTS WITH SUBJECTIVE MEMORY COMPLAINTS**

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Cognitive problems are frequent in the entire population, but especially in older than 60 years. Many risk factors have been evaluated

as determinants of neurocognitive disorders. Relationship between nutritional status and cognitive problems has been established.
The aim of the present work is to evaluate the influence of the body mass index (BMI) in general cognitive state, functional level and the alterations in MRI.
The study was analytical prospective observational, with patients who attended the consultation due to spontaneous demand for cognitive symptoms. The Montreal Cognitive Assessment (MoCA) and Functional Assessment (Kats-Lawton Index) were performed. The Body Mass Index was measured. The following characteristics were evaluated in MRI: atrophy, white matter hyperintensities (WMH), hemorrhages and infarcts. Frequency distribution was established and expressed as percentages or absolute frequencies, mean and standard deviation were determined in continuos variables. In non-parametric bivariate analyzes, the Chi-square test was used, in parametric ANAVA. Linear and logistic regression models were performed for the others analyzes. A level of significance was established for all cases of $p < 0.05$.
Seventy-six patients were studied with a mean age of 68.39 ± 11.86 years, predominantly female, the years of schooling were 08.13 ± 04.40. The MoCA test score had a mean below the cut-off point of 22.36 ± 06.30. For BMI, the score was 26.47 ± 05.34. Linear regression analysis demonstrated a positive association between BMI and MoCA score (CI: LI-16.49, LS-24.41) para ($p < 0.0001$). An increase in BMI was associated with WMH ($p = 0.0113$) and bleeding ($p = 0.0073$) in a logistic regression model. Functional level did not show statistically significant association with BMI.
The increase in BMI has a negative impact on cognition, and the alterations found in brain MRI. These results support the evidence for the relationship between nutrition and cognition.

200. (165) **UNRAVELING THE MITOCHONDRIAL ROLE IN CEREBRAL CORTEX OXIDATIVE DAMAGE DURING ENDOTOXEMIA**

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Mitochondria play an essential role in inflammatory processes such as sepsis or endotoxemia, contributing to organ-cellular redox metabolism, being the energy hub of the cell, and emerging as an important center of action of second messengers. In this work, we aimed to elucidate the energy state, redox balance and mitochondrial remodeling status in cerebral cortex in an experimental model of endotoxemia. Female Sprague-Dawley rats were subjected to a single dose of LPS (ip 8 mg kg^{-1} body weight) for 6 h. State 3 O_2 consumption and inner mitochondrial membrane potential were found increased, whereas ATP production was observed decreased, possibly indicating a low efficient oxidative phosphorylation process as this scenario was accompanied with a decreased P/O ratio. O_2^- production and both systemic and tissue NO markers were observed increased in treated animals. The existence of nitrated proteins suggests an alteration in the local redox balance and possible harmful effects over the energetic processes. Mitochondrial architecture analysis showed an increase in de novo mitochondrial synthesis (increased expression of PGC-1α and TFAM) and fusion process (increased expression of OPA-1). The observed elongation of mitochondria correlates with the occurrence of mild mitochondrial dysfunction and increased levels of systemic NO. Our work presents novel results that contribute to unravel the mechanism by which the triad endotoxemia-redox homeostasis-energy management interact in the brain cortex, leading to propose an interesting target to base future developing therapeutics for preserving this organ from inflammatory and oxidative damage.

201. (175) **REGULATION OF $GABA_A$ RECEPTOR EXPRESSION INDUCED BY PROLONGED BENZODIAZEPINE STIMULATION DEPENDS ON THE ACTIVATION OF L-TYPE VOLTAGE-GATED CALCIUM CHANNELS**

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Persistent stimulation of $GABA_A$ receptors by positive allosteric modulators, such as benzodiazepines, barbiturates, neurosteroids and ethanol, induces adaptive changes that result in tolerance. In particular, the clinical use of benzodiazepines has been limited by the development of tolerance to most of their pharmacological actions. Although different alterations in the structure and function of the $GABA_A$ receptors have been described, the signaling pathways activated by sustained exposure to benzodiazepines remain unknown. The aim of this work was to elucidate the signaling cascade triggered by prolonged benzodiazepine treatments that lead to downregulation of $GABA_A$ receptor alpha 1 subunit. To this end, primary neuronal cultures from rat cerebral cortex were treated with diazepam (25 μM), a classical benzodiazepine, for 48 h. At the end of this incubation, cells were collected for biochemical and molecular biological experiments. Flunitrazepam binding experiments showed that diazepam induced uncoupling between GABA and benzodiazepine sites (40 %, $p<0.05$), which was blocked by flumazenil, picrotoxin, or nifedipine. Quantitative real-time PCR and nuclear run-on assays demonstrated that diazepam produced a transcriptional repression of $GABA_A$ receptor α1 subunit gene (45 % decrease, $p<0.05$) that was inhibited in the presence of nifedipine and a protein kinase A (PKA) inhibitor. These findings suggest that benzodiazepine treatment stimulates the calcium influx through L-type voltage-gated calcium channels which in turn activates a PKA-dependent cascade finally leading to changes in $GABA_A$ receptor expression. These changes may be responsible for the uncoupling between GABA and benzodiazepine site interactions. Elucidation of the signaling pathway activated by benzodiazepines provides a new spectrum of possible molecular interventions to extend the initial clinical benefits of benzodiazepines and thus, prevent the development of tolerance.

202. (203) **INCREASED SEIZURES SUSCEPTIBILITY AND REACTIVE GLIOSIS IN MALES SUBJECTED TO EXPERIMENTAL FEBRILE SEIZURES**

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Febrile seizures occur in 3–5% of children between 6 months and 5 years of age. Retrospective studies in adult epilepsy patients show an initial precipitating injury, usually febrile seizures, during childhood. Using an animal model of hyperthermic seizures (HS), we have previously shown that male HS-exposed animals exhibit a significative reduction in the convulsive threshold and moderate reactive gliosis with an atypical astrocytes distribution in the pyriform cortex and other brain structures while female did not develop SE and exhibited lower reactive gliosis compared to males. Here we extended the analysis of gender differences by studying the distribution of S100B+ glial cells and microglial response in HS-exposed animals. Rat pups (10-11 postnatal days old, PND) were placed in a glass chamber, and their core temperature was raised by a regulated stream of moderately heated air (39-42°C). Body temperature was measure at baseline, seizure onset and every 2 min during the seizures. Hyperthermic temperatures (39.5–42.5 °C) were maintained for 30 min. The seizures onset was monitored behaviourally, and consisted of an acute sudden arrest of hyperthermia-induced tonic freeze postures and occasional oral automatism (biting and chewing) and often body flexion. Rats were then placed on a cool surface, monitored for 5 min before being returned to their mothers. At PND35 rats were deeply anesthetized, fixed and brains processed for immunohistochemistry and morphometrical studies. We observed an increase in the area occupied by S100B + cells in fe-

males, although there was an increase in the number of S100B cells in both sexes. On the other hand, reactive microgliosis was more prominent in males compared with females. Our results strongly suggest that males are more susceptible to HS exposure and this could be related to their future susceptibility to develop epilepsy.
Supported by grants: UBACYT, PICT 2017-2203, PIP CONICET 479

203. (205) **ASTROGLIAL PHENOTYPES IN TRAUMATIC BRAIN INJURY AND THEIR RELATIONSHIP WITH NEURONAL DEGENERATION**

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Astrocytes are key players in the Central Nervous System injury. By not completely defined pathways, reactive astrocytes may suffer a pathological remodeling engaging a pro-inflammatory phenotype that is very stable and promote further neuroinflammation and neurodegeneration. We here aimed to define the spatio-temporal distribution of astroglial phenotypes after traumatic brain injury and the consequences for neuronal survival and behavioral parameters. Following a stereotaxic stab wound injury (0.8 mm needle, coordinates 2 mm posterior and lateral to Bregma; 1 mm depth) performed in C57BL/6 mice and immunohistochemistry on brain sections, we classified GFAP reactive astrocytes in five different phenotypes defined using Sholl analysis (Auzmendi et al., Molec. Neurobiol. 2019). While at 1 day post-injury (DPI) GFAP+ astrocytes were not different from contralateral non-injured hemisphere, at 3DPI and 7DPI highly reactive phenotypes colocalized with altered neurons in lesion penumbra. At 14DPI highly reactive astrocytes and altered neurons were abundant only in the lesion core. Pro-inflammatory gain of function paradigm was achieved by administering LPS (5 mg/Kg i.p) in lesioned animals, and that resulted in a greater number of complex reactive astrocytes at 7DPI ($p<0.05$) and a population of C3+ astrocytes. On the other hand, loss of function paradigm with chemical NFkB blocker sulfasalazine (150 mg/kg i.p) significantly reduced highly reactive astrocytes ($p<0.05$) and showed reduced neuronal death. Animal motor deficits were analyzed by computer-assisted open field, but at 7DPI we were unable to detect significative differences among groups probably due to the small lesion size. We conclude that increased GFAP+ higher complexity astrocytes are associated with increased neuronal death and that NFkB pathway is likely to be involved in the pathological conversion to the pro-inflammatory-neurodegenerative phenotype.
Grants: UBACYT, PICT 2015-1451 and 2017-2203, PIP 479.

204. (214) **MICROGLIA AS THE TRIGGERING SPARKLE FOR CHROMATIN REMODELING IN PRO-INFLAMMATORY ASTROCYTES**

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Conversion of astrocytes to a pro-inflammatory phenotype leads to exacerbated neuroinflammation and neurotoxicity, therefore understanding this mechanism has become a main interest as a novel pharmacological target. We here aim to understand an epigenetic mechanism which may lead to a sustained astrocyte response expanding inflammation and neuronal death.
We exposed primary cultures of cortical astrocytes containing different amounts of microglia (below 1% and up to 20%) to 25 ng/ml Lipopolissacharide (LPS) for different periods of time to promote pro-inflammatory conversion. Astroglial and microglial morphology was analyzed using immunofluorescence. Nuclear localization of p65 subunit was assessed as parameter of NFkB activation using immunofluorescence for p65/GFAP/DAPI or p65/IBA/DAPI. As an indicator of chromatin remodeling, we studied the levels of acetylated histone 3 at lysins 9 and 14 (H3K9K14ac) using immunofluorescence for H3K9K14ac/GFAP/DAPI. This epigenetic mark is known to be promoted by NFkB activation. Pro-inflammatory conversion of astrocytes was confirmed by analyzing expression of pro-inflammatory cytokines.
Results show that LPS-induced astroglial conversion towards a pro-inflammatory phenotype evidenced by changes in morphology, activation of NFkB and cytokine expression is microglia-dependent. This astroglial pro-inflammatory phenotype correlates with global changes in nuclear H3K9K14ac only when they are co-cultured with microglia.
Our work evidences a mechanism of gene regulation by chromatin remodeling which may underlie long term cellular changes in astrocyte phenotype conversion. Our results suggest a global reconfiguration of the chromatin which could be pharmacologically targeted to reduce neuroinflammation. **PICT-2018-00920 (joven), ISN-CAEN_2020 (category B),** ***PICT 2017-2203, PICT 2015-145.***

205. (220) **PRELIMINARY EFFECT OF THE ADMINISTRATION OF *E. COLI* LIPOPOLYSACCHARIDE (LPS) ON THE ASCORBYL RADICAL ($A^{\cdot}$) CONTENT IN BRAIN FROM RATS OVERLOADED WITH FE**

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The aim of this study was to evaluate the effect of LPS in brains from rats treated with acute Fe-dextran. Sprague Dawley rats were intraperitoneally injected with a single dose of Fe-dextran (500 mg/kg) and LPS (4 mg/kg). The nitric oxide (NO) content was determined in blood by Electronic Paramagnetic Resonance Spectroscopy (EPR), with hemoglobin at 77K. The administration of LPS significantly increased ($p\leq0.01$, ANOVA) the NO content in the blood of the control animals (1.9 ± 0.1 AU), in the presence of either Fe-dextran, or LPS or LPS+Fe-dextran (1.5-, 10.5- and 14.2-folds, respectively). After 6 h of treatment, the administration of Fe-dextran or Fe-dextran+LPS, showed a significant increase of the Fe content in total brain, determined by acid mineralization, in the absence (20-fold), and in presence of LPS (17-fold). The labile Fe pool (LIP) content, determined using calcein, was increased 6 h after Fe administration, and returned to control values after 8 h. The content of $A^{\cdot}$, determined by EPR, significantly increased after 6 h, in animals overloaded with Fe-dextran in the absence of LPS (41%, $p<0.05$), without changes in the brains of animals treated with LPS or with LPS+Fe-dextran, as compared to the control values. These results suggested a protective effect against the production of $A^{\cdot}$ of the simultaneous acute administration of Fe-dextran and LPS. This effect could be due to (i) the ability of NO generated by the LPS to chelate Fe with the formation of complexes which would favor the gradually incorporation of the metal, and decreasing its catalytic capacity, or (ii) since Fe-dextran administration leads to an increase in the nuclear levels of Nrf2/keap1, the activation of the antioxidant response mediated by changes in the glutathione metabolism related enzymes would occur, which could be a critical factor in the observed response. Further studies are necessary to identify the mechanisms underlying this treatment.

206. (226) **REVERSIBLE ALTERATIONS IN GLIAR FIBRILAR ACIDIC PROTEIN (GFAP) AND TYROSINE HYDROXYLASE (TH) EXPRESION AFTER ACUTE ACETAMINOPHEN (APAP) INTOXICATION IN BRAIN AREAS INVOLVED IN LOCOMOTION AND MEMORY REGULATION.**

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We have previously demonstrated that liver toxicity by APAP not resulting in acute organ failure produces hypolocomotion associated with decrease in dopamine levels and reactive astrogliosis in selective brain areas. The aim of the present work was to evaluate if these effects are still present a week after APAP intoxication. For this purpose, male Wistar rats were divided in 4 groups, dosed with APAP (1g/kg; i.p.) or vehicle. After one or seven days of treatment, rats were anesthetized and intracardiacally perfused with PAF. Immunohistochemistry for glial fibrillary acidic protein (Gfap) and tyrosine hydroxylase (TH) expression was performed on free-floating coronal sections. The percentage of reactive area in pre-frontal cortex (Pfc), Nucleus accumbens (NAc), dorsal striatum (DStr) and non-motor cortex (Cx) was quantified using imageJ (NIH). Since control one and seven day treated rats did not show any differences, samples were pooled as a single control group. One day after APAP intoxication, TH expression was decreased in DStr and NACs by 21% [t (2,11)=18.34, p=0.0003] and 20%; [t (2,11)=6.179, p=0.00159], respectively and restored to normal values by 7 days after treatment. However, no significant changes in TH expression were detected in Pfc and Cx at either time point. Moderate reactive astrogliosis was also detected at day one in all dopamine-rich areas of the brain examined, with significant increments in GFAP immunoreactivity by 26% for Pfc [t (2,10)=9.702, p=0.0045]; 88% for DStr [t (2,10)=9.702, p=0.0045] and 25% [t (2,12)=8.156, p=0.0058] for NAc, returning to basal value by day 7. However, no induction of GFAP was detected in Cx and no alterations in astrocyte numbers were observed in any of the areas analyzed at either time point. In conclusion, our results demonstrate that the astrogliosis and altered dopaminergic level are transitory alterations during APAP intoxication in rats and therefore, full recovery in locomotive function is expected.

207. (227) **GLAUCOMA INDUCES REDOX IMBALANCE IN THE PRIMARY VISUAL CORTEX IN A RAT EXPERIMENTAL MODEL.**

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Glaucoma is the first irreversible cause of blindness worldwide and damages structures in the brain, such as the primary visual cortex. The aims were to elucidate the antioxidant enzymes activities, the intracellular sources of oxidant species (OS) and the signaling pathways involved in the redox metabolism in the primary visual cortex in an experimental glaucoma model.

3-month female Wistar rats were operated by cauterizing two of the episcleral veins: glaucoma group (GG n=12); the control group (CG n=12) received a sham procedure. Seven days after surgery rats were euthanized and the primary visual cortex was dissected (CICUAL FFyB n° 3314). Antioxidant enzymes activities, GSH metabolism, NOX activity, iNOS expression, Nrf2 and NF-κB activation were evaluated.

When compared to CG, GG showed 147% increase in NOX activity (*p<0.05*), increasing the steady state concentration of OS. NOX4 expression was 90% higher in GG (*p<0.05*). iNOS expression was increased in GG (47%, *p<0.05*), induced by NF-κB activation (48%, *p<0.01*). As an adaptive response to OS, there was a 40% and 55% increase in SOD (*p<0.01*) and GPx (*p<0.05*), respectively. However, there was an alteration in glutathione metabolism in GG shown as a 40% and 53% decrease in GR expression (*p<0.05*) and GCL activity (*p<0.05*), respectively, which leads to a decrease in GSH/GSSG ratio (55%, *p<0.001*). In addition, a decrease in Nfr2 expression was shown in GG (40%, *p<0.05*). This scenario leads to proteins (140%, *p<0.001*) and lipids (70%, *p<0.001*) damage.

These results suggest that glaucoma induces damage to the primary visual cortex, such as oxidative modifications to macromolecules, due to an enhancement in OS (H_2O_2 and NO) production from NOX family and iNOS. In this context the Nrf2 impairment could lead to a deficient antioxidant response.

208. (289) **SHIGA TOXIN 2 (STX2) AND LPS FROM ENTEROHEMORRHAGIC *ESCHERICHIA COLI* (EHEC) PRODUCE A TLR4-INDEPENDENT MICROGLIAL REACTIVITY AND PRO-INFLAMMATORY CYTOKINES**

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Stx2 from EHEC is the main cause of hemolytic uremic syndrome that is defined clinically by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Stx2 can also lead to encephalopathy with motor, cognitive and emotional impairments in 30% of cases. We have studied that Stx2 can bind to neurons through its receptor Gb3. Other authors have reported that Stx2 may bind to TLR4 in leukocytes, which leads to its activation and cytokine release. Given that microglial cells (MC) are CNS resident macrophages, we hypothesize that MC would respond to Stx2 through a TLR4 receptor. Therefore, our aim was to determine by *in vivo* and *in vitro* studies whether Stx2 produces MC activation through TLR4 and cytokine release. Mice were subjected to the following sublethal treatments: vehicle (control) or Stx2 (3ng)+LPS (800ng), to determine MC reactivity by immunofluorescence and cytokine release by flow cytometry. *In vitro* assays of MC were obtained from TLR4 KO rat brains which were treated with either control, LPS (50ng/ml), Stx2 (50 or 200ng/ml) or Stx2+LPS. One way ANOVA analysis, and Bonferroni post-hoc analysis were performed for i*n vivo* and *in vitro* studies. After 24h of treatment, Stx2+LPS treated mice showed an increase in the expression of IBA1 as well as in the number of MC ($p<0.001$). Further, after 6 hours of toxin treatments a significant increase of IL6, TNF alfa and INF gamma was observed ($p<0.001$). *In vitro* studies showed that 200ng/ml of Stx2 and Stx2 (200 ng/ml)+LPS produced an increase in the area occupied by MC respect other treatments ($p<0.001$). Further, the expression levels of IBA1 and the number of MC were increased in Stx2 (50 and 200ng/ml) and Stx2 (50 and 200ng/ml)+LPS with respect to LPS alone and the control ($p<0.05$). We concluded that co-treatment of Stx2+LPS produced early anti-inflammatory cytokines in the brain which could be related with MC reactivity through a TLR4 independent pathway.

209. (297) **STRESSFUL LIFE EVENTS IN ALZHEIMER'S, PARKINSON'S AND ISCHEMIC STROKE DISEASES**

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Chronic and Posttraumatic Stress Disorder are related with greater risk of developing different neuropathologies. While neither is necessary or sufficient for dementia they have been acknowledged as one of many factors intervening in pathologies such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Isquemic Stroke (IS). Psychological stress can trigger physiological responses, suggesting that stress could be considered as a prodromal symptom in such conditions. To examine the development of a neurological disorder AD, PD and IS after a stressful life event (SLE) in a cohort of mod-

erate neurological patients which have experienced a psychosocial stressing event prior to initial symptoms. The interactions of Sex, Race, Age, and Economic and Cultural level with the main factor Condition or with respect to the SLE tested in a three-way ANOVA, yielded no significant values. The two-way analysis of the Condition and Stressful Event factors was conducted pooling all data. Interestingly, significant differences were found in Time-to-diagnosis and Evolution time of the disease after the different SLE in AD and PD for the comparison between Death of a familial against Work dismissal (P<0.00001 and P<0.05, respectively), while no differences were found in the IS group (P=0.43 y P=0.14). These results invalidate global comparisons of the main factors levels; further analyses were restricted to the factor Death or serious illness of a close relative. In the restricted dataset no significant interaction effects were found in time-to diagnosis between condition and demographic variables. These observations suggest that psychosocial stress might be considered as a risk factor for the development of neurological conditions in aged subjects. The existence of significant differences in Time-to-diagnosis and Evolution Time between the diseases demonstrates that death or serious illness of a close relative has different impacts on the development of neurological conditions.

210. (298) **INCREASED EGR1 EXPRESSION IN THE MEDIAL PREFRONTAL CORTEX AND ALTERED MOTOR AND BEHAVIOURAL PARAMETERS IN THE OFFSPRING FOLLOWING MODERATE PATERNAL ALCOHOL INTAKE**

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Previously, we observed that male alcohol intake affects sperm biochemical parameters and DNA integrity. The resulting progeny was underweight during their first weeks of life, and overweight in their adulthood, and showed altered spleen cell populations. Aim: To assess the effects of moderate paternal alcohol consumption on different physical and behavioral parameters of its offspring (F1) development. Additionally, we screened for potential modifications in the expression of candidate genes in the medial prefrontal cortex (mPFC) of F1 mice. CF1 male mice were exposed (A) or not (C) to 15% (v/v) ethanol in drinking water ad libitum for 15 days, following which they were mated (1:1) with non-treated CF1 females. Two to 17 days old F1 mice were subjected to developmental tests (surface righting, grasping reflex, negative geotaxis, limbs suspension, grip strength, cliff aversion, ambulation tests), and two to four months old F1 mice to behavioral tests (open field or OF, elevated plus maze, object context recognition test and social dominance tube test). Brains from adult F1 mice were dissected, and those from A weighed less than those from C ($p<0.05$). Since mPFC is implicated in reward circuits and social cognition, expression levels of genes expressed in this region were evaluated through RT-qPCR. Pups from A presented a delay in surface righting at day 7 after birth ($p<0.0001$). Hind grasping reflex was also delayed in A male ($p<0.01$) and female ($p<0.001$) pups. The OF test showed that adult F1 A males spent longer in the center of the maze than adult F1 C males did ($p<0.05$). F1 A adults were less socially dominant than F1 C adults for both male ($p<0.0001$) and female ($p<0.05$). *Egr1* expression was higher ($p<0.05$) in F1 A adult males than in F1 C adult males. Altogether, these results suggest that moderate paternal alcohol intake has a detrimental effect on its progeny´s social skills, associated with altered expression of mPFC genes involved in neural plasticity.

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211. (307) **MITOCHONDRIAL FUNCTION IN BRAIN AND HEART OF SENILE RATS EXPOSED TO HYPOBARIC HYPOXIA. ROLE OF NITRIC OXIDE.**

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In previous studies, we observed that exposure to hypobaric hypoxia (HH) can induce a cardioprotective effect that involves mitochondrial nitric oxide (NO) and differently affects cortical and hippocampal mitochondrial function. In the present work, we evaluated the impact of senility on mitochondrial function in left ventricle heart, cerebral cortex and hippocampus after an acute exposure to HH.

Male Wistar rats of 22 months of age were subjected to a simulated 4,400 m altitude (58.7 kPa=440 mmHg) in a hypopressure chamber during 48h. A group of the same number of sibling rats remained as controls at sea level atmospheric pressure (101.3 kPa=760 mmHg). Oxygen consumption, mitochondrial membrane potential, hydrogen peroxide (H_2O_2) production and nitric oxide (NO) levels were measured in cortical and hippocampal mitochondria. In addition, cytochrome oxidase activity and NO production were evaluated in mitochondrial fractions isolated from left ventricle heart. Differences were considered significant when $p<0.05$.

Hypobaric hypoxia induced mitochondrial depolarization (18%) and decreases in both state 4 (43%) and state 3 (37%) respiratory rates associated with an increment in NO (48%) and H_2O_2 (23%) production in cerebral cortex. In the hippocampus, we observed a decrease in mitochondrial membrane potential (18%) and an increase in NO production (46%), without changes in oxygen consumption and H_2O_2 production after HH exposure. Regarding heart left ventricle mitochondria, HH was able to decrease NO production by 47% and to induce cytochrome oxidase activity by 29%, as compared with controls.

Results indicate that the mitochondrial response to HH would be dependent on the changes in NO production and in the degree of mitochondrial membrane polarization. According to oxygen demand and sensitivity of each tissue, the modulation of both NO levels and mitochondrial membrane potential changes would integrate in a response mechanism and/or endogenous protection against hypoxia

212. (315) **PIAS4 IMPAIRS TAU HOMEOSTASIS**

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Tauopathies are neurodegenerative diseases characterized by the formation of intracellular hyperphosphorylated tau deposits. Notably, aberrant tau accumulation in brain often co-occurs with other protein aggregates, suggesting that there might be common factors supporting their deregulation. Accumulating evidence points to the PIAS SUMO E3-ligases as SUMOylation inductors of several key proteins involved in neurodegeneration, modifying their solubility, activity, or stability. Taking this into account, we hypothesize that these enzymes could be common regulatory factors implicated in neurodegenerative processes. The aim of this work is to determine the role of PIAS family in the regulation of tau protein homeostasis. By means of a western blot (WB)-based screening in HT22 cells overexpressing human 2N4R WT tau (hTau) together with PIAS family members, we observed that only PIAS4 increased total tau levels (+1.75, $p<0.001$). This result was corroborated in N2a cells lines stably expressing endogenous levels of hTau (N2a_hTauWT). PIAS4 promoted not only total tau but also phospho(P)-tau accumulation (total tau: +0.63, $p<0.0001$; PS396 -tau/total tau: +0.48, $p<0.05$; PS214-tau/total tau: +1.8, $p<0.001$; PS202-tau/total tau: +1.6, $p<0.001$, PS[396/404]-tau/total tau: +0.89, $p<0.01$). In addition, the SUMOylation mutant cells, N2a_hTauK340R, exhibited a similar response upon PIAS4 expression. Accordingly, our niquel affinity purification experiments showed that PIAS4 was unable to induce

SUMO conjugation to tau, indicating that it is not a tau SUMO-E3 ligase. Finally, our preliminary autophagic flux assays revealed that PIAS4 might be blocking selective autophagy degradation of tau.
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213. (317) **CHRONIC EXPOSURE TO FLUOXETINE DURING PRE-PUBERTY IMPAIRS RAT SOCIAL INTERACTION IN A SEX-DEPENDENT MANNER**

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Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), has been approved to treat major depressive and obsessive-compulsive disorders in pediatric patients along with some off-label uses. Several concerns were raised when it was determined that Fluoxetine could lead children to suicidal thoughts and behaviors. Although well characterized for adults, little is known about the effect of Fluoxetine on pediatric patients. The aim of this work was to evaluate the effect of early exposure to Fluoxetine on social interaction, stereotypical and exploratory activities, and anxiety. Male and female Wistar rats were daily administrated (sc.) with Fluoxetine (10 mg/kg) or saline between postnatal days (PND) 16-35 and behaviorally evaluated at PND 30-35. Concerning social behavior, Fluoxetine treatment in males dramatically reduced social play behavior measured as the number of pinnings and social preference evaluated in a three-chamber task. On the contrary, Fluoxetine did not modify these behaviors in females. Also, only in male, Fluoxetine treatment increased stereotypical behaviors measured as the number of self-grooming events and enhanced anxiety-like behavior indicated by a reduction in the time spent in the open arms of an elevated plus-maze. Notably, while in males Fluoxetine treatment did not affect exploratory activity, in females it decreased the number of hole-pokings. Regardless of sex, Fluoxetine treatment did not modify locomotor activity and increased serotonin immunoreactivity in the hippocampus These results strongly indicate that pre-puberal rat exposure to Fluoxetine targets social interaction, exploration, stereotypical and anxiety-like behaviors in a sex-dependent manner. Our results also highlight male vulnerability to modulation of serotonin levels during infancy and pre-puberty.

214. (320) **MILD VPA BEHAVIORAL PHENOTYPE IN FEMALE RATS: EVIDENCE OF STRUCTURAL SYNAPSE REMODELING IN THE MEDIAL PREFRONTAL CORTEX AND THE HIPPOCAMPUS**

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Autism spectrum disorders (ASD) are a group of severe neurodevelopmental disabilities of unknown etiology, characterized by social interaction deficits and increased stereotyped behaviors. Although ASD incidence is four times higher in boys than in girls, sex differences have not been clarified. The rat model of autism induced by prenatal exposure to valproic acid (VPA) has been characterized in males (MVPA), while female rat VPA (FVPA) phenotype is still controversial. The aim of this work was to further characterize the behavioral profile of FVPA and explore structural synapse markers, cell adhesion molecules and microglia morphology in the medial prefrontal cortex (mPFC) and the hippocampus. At early postnatal days (PND)7-15, and similar to MVPA, FVPA showed growth and maturation deficits: delayed eye opening, lower body weight, altered negative geotaxis, higher latencies to nest seeking response and a deficit in swimming performance. At PND30-35, like MVPA, FVPA showed a reduced number of interactions in the social play behavior test, but they exhibited distinctive pinning features. Contrary to MVPA that showed an exploratory deficit and increased stereotypical activities, FVPA matched control female rat behavior. Notably, at PND35, mPFC of FVPA and MVPA showed an increase in synaptophysin (SYN) and neural cell adhesion molecule (NCAM) and a similar ramified/unramified microglia (Iba+) ratio. However, the polysialylated form of NCAM (PSA-NCAM) was increased in FVPA but decreased in MVPA. In the hippocampus, both FVPA and MVPA showed reduced SYN labeling and increased NCAM but only FVPA displayed a higher proportion of unramified microglia. Also, PSA-NCAM levels were preserved in FVPA but reduced in MVPA. To sum up, FVPA exhibit a mild behavioral phenotype accompanied with a distinctive microglia profile and NCAM/PSA-NCAM ratio that may facilitate structural synapse remodeling and plasticity.

215. (411) **$GABA_A$ RECEPTOR MODULATION BY KETONE BODIES**

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Ketone bodies are produced from the β-oxidation of fatty acids during ketogenesis. In humans, acetyl-CoA is the end product of fatty acid catabolism. Three types of ketone bodies can be synthesized from acetyl-CoA: acetone, β-hydroxybutyrate and acetoacetate. Ketogenesis is increased under conditions of low glucose (eg, fasting), low insulin or excessive alcohol consumption. This increase also occurs during certain diets, with low carbohydrate and high fat consumption, indicated to reduce the probability of seizures in epileptic patients or to alleviate the alcohol withdrawal syndrome. Additional beneficial effects of ketogenic diets have been described in models of Alzheimer's disease and amyotrophic lateral sclerosis. The actions of ketone bodies on neurotransmission have been poorly explored and the mechanisms responsible for the therapeutic benefits of ketogenic diets remain under study.
Recently Pflanz et al. (2019) studied for the first time the effects of ketone bodies on ligand-activated ion channels and described acetone as a positive modulator and β-hydroxybutyrate as a negative modulator of GABAAα1β2γ2 receptors. In this context, we studied the modulatory effects of ketone bodies on GABAergic neurotransmission, evaluating the sensitivity of different subtypes of GABAA phasic and tonic receptors. Human $GABA_A$ρ1, $GABA_A$α1β2, $GABA_A$α5β3 y $GABA_A$α4β3δ were expressed in *Xenopus laevis* oocytes and chloride currents were recorded by two-electrode voltage-clamp. Results with acetone (100 to 300 mM) showed inhibitory effects on GABAAρ1 and potentiating effects on GABAAα1β2, GABAAα5β3 and GABAAα4β3δ responses (~EC10). Acetone effects on oocytes baseline were controlled in every experiment. Further experiments will be carried out to characterize acetone modulation and evaluate β-hydroxybutyrate effects on these receptors.

216. (413) **METABOLISM AND EFFECTS OF TESTOSTERONE IN THE SPINAL CORD FROM WOBBLER-ALS MICE AFTER TREATMENT WITH EXOGENOUS TESTOSTERONE**

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Amyotrophic lateral sclerosis (ALS) patients present motoneuron degeneration leading to muscle atrophy, dysphagia and dysarthria. The Wobbler (WR)-ALS mice, a recognized model of this disease, shows a selective loss of motoneurons, astrocytosis and

microgliosis in cervical spinal cord (CSC). The incidence of ALS is greater in men; however it increases in women after menopause, suggesting a role of sex steroids in ALS. Testosterone is a complex steroid that exerts its effects directly via androgen (AR) and indirectly via estrogen receptors (ER) after aromatization into estradiol. Its reduced-metabolite 5a-dihydrotestosterone acts via AR. This study analyzed the metabolism of testosterone in the spinal cord and studied testosterone effects on myelin basic protein (MBP) and rotarod performance in male symptomatic WRs. Controls or WRs received empty or testosterone-filled silastic tubes for 2 months. The CSC from testosterone-treated WRs showed: 1) similar androgen levels to untreated control, and 2) increased levels of testosterone ($p<0.05$), and its 5a-reduced metabolites, 5a-dihydrotestosterone ($p<0.01$) and 3b-androstanediol ($p<0.001$), but 3) undetectable levels of estradiol compared to untreated WRs. Testosterone-treated controls showed comparable steroid concentrations to untreated controls. CSC from WRs showed low number of oligodendrocyte CC1+cells ($p<0.05$) and high immunoreactivity for MBP ($p<0.05$) vs. controls. In testosterone-treated WRs, we showed increased number of CC1+cells ($p<0.01$) associated to high % of immunoreactive area for MBP ($p<0.05$) vs. WRs. Clinically, testosterone treatment in WRs improved rotarod performance ($p<0.05$). Collectively, our findings indicate a promyelinating effect of testosterone in the CSC of Wobbler-ALS mice. These results coincided with a high concentration of androgen-reduced derivatives after testosterone treatment suggesting that the profile of steroid metabolites may have a beneficial role on disease progression.

217. (420) **GENERATION AND CHARACTERIZARION OF A HUMAN INDUCED PLURIPOTENT STEM CELL LINE FROM A FAMILIAL ALZHEIMER'S DISEASE PSEN1 T119I PATIENT**

Isaja L[1], Rodriguez Varela MS[1], Marazita M[1], Mucci S[1], Itzcovich T[1], Chrem-Méndez P[1], Niikado M[1], Allegri R[1], Martinetto H[1], Sevlever GE [1], Scassa ME [1], Surace E[1], Romorini L[1].

1. Fundación para la lucha contra las enfermedades neurológicas de la infancia (FLENI-CONICET)

Alzheimer's disease (AD) is a neurodegenerative proteinopathy which is the main cause of dementia in adults. It is estimated that 5% of cases are caused by inherited mutations in genes such as Presenilin-1 (*PSEN1*). Previously, our group reported a novel heterozygous variant in *PSEN1* (c.356C>T; p.T119I) in an Argentine family with early- and late-onset AD. In order to functionally validate this variant, we aimed to generate a human induced pluripotent stem cell (hiPSC) line from dermic fibroblasts (DF) of a male mutation carrier. To this purpose, we first infected patient DF with a STEMCCA lentiviral vector encoding the Yamanaka reprogramming factors (*OCT-4*, *KLF4*, *SOX2* y *c-MYC*) and obtained two clones, termed FFAD1.2c4 and FFAD1.2c8. Both clones exhibited typical hiPSCs morphological characteristics (formation of compact multicellular colonies with a high nucleus/cytoplasm ratio and distinct colony borders) and high Alkaline Phosphatase (AP) activity. Moreover, we also observed robust expression of different stemness-associated markers (OCT-4, NANOG, SSEA4, TRA1-80, and TRA1-60),) analyzed by immunofluorescence and RT-qPCR. Also, using a non-directed method of differentiation (embryoid bodies assay) we demonstrated that at least FFAD1.2c4 had the pluripotent potential to be differentiated into cells from the three germinal layers (mesoderm, endoderm and ectoderm) as judged by RT-qPCR and immunofluorescence analysis of Smooth muscle actin (SMA), Alpha-fetoprotein (AFP) and Nestin differentiation markers expression, respectively. Finally, both clones exhibited the same normal karyotype (46, XY) and *PSEN1* T119I genotype as the parental DF cells and silenced ectopic expression of Yamanaka gene. Overall, we successfully obtained an hiPSCs line (FFAD1.2) carrying the AD *PSEN1* T119I genotype.

218. (428) **NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AS A MODEL FOR STUDYING NEURAL STRESS AND CDK5 SIGNALING**

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Neurodegeneration is a complex multifactorial process that causes progressive loss of structure or function of neurons. CDK5/p35 complex is involved in neuronal homeostasis. However, stressful stimuli induce calpain-mediated cleavage of p35 to p25. p25 in turns forms a more stable CDK5/p25 hyperactive complex that participate in neurodegenerative mechanisms. Although CDK5 signaling has been intensively study in animal models, there are currently no good *in vitro* models for studying its participation in human neuronal homeostasis and neurodegenerative processes. In this work we aimed to generate an *in vitro* human model for studying CDK5 signaling and neural stress based on the neuronal differentiation of human embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively). First, hESCs (H9 line) and hiPSCs (FN2.1 line) were derived to neural stem cells (NSC), which were then differentiated into generic neurons using 2D protocol. Phenotypes were validated by immunofluorescence of lineage specific markers (Sox-1 and Sox-2 for NSC, MAP2 and Tuj-1 for neurons). Next, neurons were subjected to stressful stimuli (rotenone 1μM for 24h and calcium ionophore A23187 2μM for 2h) and an increase in mitochondrial membrane potential was observed (measured using Mitoprobe JC-1 Assay). Moreover, morphologic changes (axonal spheroids appearance) were found in derived-neurons upon stressful stimuli. Finally, we found that p35 is highly expressed (quantified by RT-qPCR and western blot) in derived-neurons and that rotenone and A23187 treatments induced its cleavage to p25 (analyzed by western blot). Further, p35 cleavage was mediated by calpains as proteolysis was inhibited with a calpain inhibitor (ALLN, 50μM). In conclusion, neurons-derived from hESCs and hiPSCs are potentially a good *in vitro* human model for studying the relevance of CDK5 signaling in neural stress as they responded to stressful stimuli inducing calpain-mediated cleavage of p35 to p25.

219. (435) **THE INFLAMMATORY PROFILE OF CIRCULATING MONOCYTES IS ALTERED IN PATIENTS WITH MOOD DISORDER**

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Mood Disorders (MD) affects over 350 million people around the world. Despite the public impact of MD, its etiology remains unknown. In recent years, evidence reveals a key role of the immune system in the development and maintenance of MD. We have recently shown that plasma proinflammatory IL-12 and IL-6 were increased in patients with depression and suicidal behavior. Interestingly, these cytokines positively correlate with altered monocytes activation and proportions.

We aim to determine the differences in the profile and activation status of circulating monocytes in patients with MD vs healthy controls (HC) at baseline and after a 6-month follow-up. Psychiatrists used the International Psychiatry Interview (MINI) to diagnose MD, and the Hamilton Depression Rating Scale (HADRS) to define active disease (AD) or non-active disease (NAD). Blood samples were obtained, and the classical, intermediate, and non-classical monocyte subsets were analyzed by FACS. After 6-month, patients were re-evaluated for depressive and inflammatory status. The study was approved by IRB, each participant gives written consent.

MD sample was 23% male, 77% female with a 25-62 year age range. The percentage of classical monocytes was reduced in MD (72.0 ± 2.6, N=23) vs HC (87.0 ± 1.7, N=5) together with an increase of the intermediate fraction, MD (11.8 ± 1.6) vs HC (4.4 ± 0.5). After segregation, the AD patients showed the most significant change in terms of percentage of classical ($p<0.001$) and intermediate monocytes

(p<0.05) compared with HC; meanwhile, no significant differences were observed yet between AD (N=12) vs NAD (N=11) patients. Interestingly, after follow-up, five patients with improved HDRS scores showed significant changes in the percentage of classical (p<0.01) and intermediate (p<0.05) monocytes compared to the baseline. These data suggest that the monocyte profile is altered in patients with MD, and these changes could be useful to monitor the cellular inflammatory status at baseline and follow-up.

220. (441) **THE COLD MIMETIC SYNTHETIC MOLECULE ZR17-2 PREVENT RETINAL LESIONS IN A MODEL OF EXPERIMENTAL COMPRESSION OF THE OPTIC NERVE**

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Injuries to the optic nerve (NO) are a common damage that produces antegrade and retrograde axonal degeneration, with death of retinal ganglion cells and vision loss. Previously, we showed that ocular temperature reduction after experimental compression of NO (intraorbital optic nerve crush, IONC) significantly diminishes vision loss, and effect probably mediated by cold inducible RNA-binding proteins (CIRP). ZR17-2 is a cold mimetic synthetic molecule, able to prevent CIRP degradation in culture cells. Our aim was to evaluate the effect of ZR17-2 on retinal lesions due to NO trauma. Adult male rats (n=28) were equally distributed in groups: 1a) CTL (left eye, LE): simulation of surgery with intravitreal injection of vehicle; 1b) IONC (right eye, RE): IONC with intravitreal injection of vehicle; 2a) CTLZR (LE): simulation of surgery with intravitreal injection of ZR17-2; 2b) IONCZR (RE): IONC with intravitreal injection of ZR17-2. Each animal was its own control. Animals were euthanized five days after surgery and eyes processed for TUNEL, or placed in a dark room fifteen days after surgery for 12 hs and inspected for scotopic electroretinography (ERG) and oscillatory potentials to evaluate the integrity of the visual pathway. IONC and IONCZR depicted significant lower a- and b-waves amplitude than their respective controls; whereas, CTL and CTLZR did not show significant changes between them. In addition, IONC and IONCZR showed significant b-wave decrease related to CTL and CTLZR respectively. IONCZR showed also a significant increase in the b-wave versus IONC. Oscillatory potentials showed similar variations than ERG. On the other hand, retinas of CTL and CTLZR showed a few TUNEL+ cells, whereas IONC showed a significant increase related to CTL and IONCZR. In conclusion, the present work corroborates the effectiveness of ZR17-2 *in vivo* to prevent retinal lesions constituting an innovative treatment for NO trauma, and enabling it use in future preclinical trials.

221. (477) **CHARACTERIZATION OF REACTIVE OXYGEN SPECIES EFFECTS ON $GABA_A$ RECEPTORS**

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Endogenous reactive oxygen species (ROS) were involved in neuronal signalling and plasticity, in normal physiology, aging and neurodegenerative disorders. Besides, GABAergic neurotransmission was shown to be sensitive to redox agents. We previously demonstrated that tonic responses mediated by homomeric $GABA_{A\rho1}$ receptors can be modulated by hydrogen peroxide (H_2O_2), ascorbic acid, glutathione and nitric oxide, through thiol modification of cysteines. We also identified endogenous redox agents that modulate $GABA_A$ receptors involved in fast inhibitory neurotransmission in the retina and hippocampus, but the molecular mechanisms of action remain elusive. We analysed the effect of H_2O_2 on different heteropentameric $GABA_A$ receptors by using heterologous expression of these GABA-gated Cl^- channels in *Xenopus laevis* oocytes, followed by two-electrode voltage-clamp recordings of the GABA-evoked ionic currents. H_2O_2 induced dose-dependent, reversible and voltage-insensitive potentiating effects on $GABA_{A\alpha1\beta2}$ and $GABA_{A\alpha5\beta3}$ receptors. In contrast, $GABA_{A\alpha1\beta2\gamma2}$ and $GABA_{A\alpha5\beta3\gamma2}$ receptors activity were not altered during exposure to H_2O_2, suggesting that the γ2 subunit conferred a relative insensitivity to these endogenous redox agents. H_2O_2 effect on $GABA_{A\alpha1\beta2}$ was partially prevented by irreversible alkylation of sulphydryl groups with NEM. Concentration-response curves in the presence of H_2O_2, compared to control values, showed a leftward shift and an increase in the maximal response ($EC_{50\ GABA}$= 2.7(2.4 to 3.0) μM, nH=1.46±0.12; $EC_{50\ GABA+H2O2}$=1.9(1.5 to 2.4)μM, nH=1.85±0.38). As observed for many redox agents acting on $GABA_{A\rho1}$ receptors, the degree of potentiation exerted by H_2O_2 on $GABA_{A\alpha1\beta2}$ responses decreased as GABA concentration increased. Additional experiments are being performed to elucidate the mechanisms of action underlying the effects of H_2O_2 on $GABA_A$ receptors.

222. (574) **FK506-BINDING PROTEIN 51 (FKBP51) EXPRESSION IS MODULATED UPON SERUM DEPRIVATION AND MAY PLAY A ROLE IN THE REGULATION OF THE AUTOPHAGY PATHWAY IN THE HYPOTHALAMIC CONTEXT.**

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FK506-binding protein 51 (FKBP51) is an Hsp90 co-chaperone that has been described to regulate the activity of the glucocorticoid receptor (GR) and is therefore critical for the regulation of the stress response. In order to act as a GR regulator, our group has demonstrated that FKBP51 has to be SUMOylated, a process enhanced by cellular stress. FKBP51 also acts as a metabolic sensor playing an important role in energy and metabolic homeostasis. At the brain level, the hypothalamus has a central role in the control of body homeostasis, neuroendocrine outputs, and feeding behaviour. It does so by sensing and integrating signals from the periphery and effecting appropriate physiological changes. Hypothalamic FKBP51 is induced by fasting, and elevated hypothalamic expression promotes obese phenotypes. Interestingly, several studies have described a role for hypothalamic autophagy in the control of food intake and energy balance. At the molecular level, FKBP51 has been shown to bind Beclin1 and alter its phosphorylation status, promoting the induction of the autophagy pathway. It does so by interacting with PHLPP and AKT1 and thereby favouring AKT's dephosphorylation, which leads to Beclin1 recruitment and dephosphorylation. Furthermore, synthetic glucocorticoids and antidepressants act synergistically with FKBP51 in the induction of autophagy. Our preliminary western blot results show that FKBP51 expression is up-regulated upon starvation in the GT1-7 hypothalamic cell line (35.5±3.3%, p<0.05). This correlates with decreased phosphorylation of AKT1 and Beclin1 (p-AKT1: 61.2± 8.1%, p<0.05; p-beclin1: 77.3±4.5% p<0.05) and with the induction of autophagy markers measured by western blot assays (42.6±4.2%, p<0.05). Also, we demonstrate by co-immunoprecipitation assays that FKBP51 interaction with AKT1 and Beclin1 is dependent on FKBP51 SUMOylation, suggesting that SUMO conjugation to FKBP51 may have an impact in the regulation of the autophagic pathway in this context.

ONCOLOGÍA

223. (419) **PIN1 VALIDATION AS A MOLECULAR TARGET FOR GLIOBLASTOMA TREATMENT**

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The Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1(PIN1) is currently the only enzyme described with the ability to recognize and isomerize the phosphorylated Serine/Threonine-Proline (pSer/Thr-Pro) motif. Through this mechanism, PIN1 has the ability to regulate the structure of a subset of proteins, and consequently controls diverse cellular functions. Thus, both PIN1 overexpression as its involvement in various oncogenic pathways has been reported in several cancers types, including glioblastoma. Glioblastoma is characterized as a complex and lethal disease with limited therapeutic resources so far. Due to the need to develop new therapies, there is an interest to explore the role of PIN1 in glioblastoma progression. However, studies on the contribution of this protein in glioblastoma are limited. Thus, the aim of this work was to study the contribution of PIN1 in a glioblastoma progression using LN229 cells, seeking to validate this protein as a molecular target. With this objective, we developed a Pin1 K.O. (knockOut) LN229 cell variant using CRISPR/Cas9 technology. Both, Pin1 K.O. and wildtype models were compared in different cellular processes related to tumor progression such as: cell migration by transwell assays, cell cycle progression by propidium iodide staining and cytometry, doubling time by colorimetric quantification and finally, in vivo tumor progression xenografts. Results revealed that lack of PIN1 expression in glioblastoma cells suppressed their malignant phenotype by reducing migration, cell cycle progression, and increasing doubling time. Furthermore, Pin1 deletion also resulted in the loss of LN229 ability to form tumors in nude mice. These results highlight PIN1 contribution in glioblastoma progression, in turn, validating this protein as an attractive molecular target for development of novel glioblastoma treatments.

224. (544) **HV1 INCREASED ACTIVITY IS ASSSOCIATED TO A HIGH GLYCOLITIC FLUX**

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It is well-known that most of cancer cells present an increased glycolytic flow related to their metabolic reprogramming (Tanner et al., 2018). This metabolic change, considered an emerging hallmark of cancer, conduces to an exacerbated lactate secretion and produces large amounts of protons. We have previously demonstrated that the inhibition of Hv1, an ion channel capable to extrude H^+ of cells, induces intracellular acidification and apoptosis in leukemic Jurkat T cells (Asuaje et al., 2018). In this work we present evidences showing that Jurkat T cells cultivated in presence of high (25 mM) glucose concentration increases (15%, $p<0.01$; N=9) lactate secretion per cell (commercial kit) compared with the cells cultivated in physiologic (5.5 mM) glucose concentration, suggesting that, the high glucose concentration in the culture medium increases the glycolytic flow. Then, we studied in deep the role of Hv1 channel in this metabolic context observing that the cells cultivated in presence of glucose 25 mM increases Hv1 channel activity (3 fold of ion current increment, $p<0.001$, N=10) measured by patch clamp technique; intracellular pH (0.12 units of pH increment, $p<0.01$, N=2) determined by using BCECF-AM prove; and cell proliferation (20% of increment, $p<0.01$, N=3. Doubling time). Moreover, when the same parameters were evaluated in presence of a specific Hv1 channel blocker (ClGBI, 200 μM), we found that ClGBI produced a drop of intracellular pH ($p<0.01$, N=2), a slight decrease of extracellular lactate concentration (25%, $p<0.05$ vs. DMSO 0.2%, N=4) and a significantly increase of cell death ($p<0.05$, anexineV-IP). These results suggest that high concentration of glucose induces an increment of glycolytic rate and cell proliferation in Jurkat T cells, and both of them were diminished by Hv1 inhibition, showing a relevant function of Hv1 channel in the metabolic change of these cells.

225. (9) **CONTRIBUTION OF MAPKS PATHWAYS, PI3K/AKT AND ER STRESS TO MURINE MELANOMA CELL DEATH INDUCED BY A TRIAZOLYL PEPTIDYL PENICILLIN**

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The triazolyl peptidyl penicillins (TAPs) are novel hybrid compounds having in their structure a penicillanic core linked to a peptide portion via a triazole group. In a previous study, we showed that the derivative containing the dipeptide Leu-Phe (TAP7f) triggers an endoplasmic reticulum (ER) stress response, induces apoptosis and activates p38, JNK and PI3K/AKT pathways in murine B16-F0 melanoma cells. In order to investigate the role of these signaling cascades in the mechanism of action of TAP7f, B16-F0 cells were transfected with specific mutant dominant negative constructs or control vectors and treated with TAP7f. Results showed an increase in cell proliferation from 26±3% (control vector) to 42±4, 36±3, 38±4% after transfecting cells with p38, JNK or PI3K-I dominant negative mutants, respectively (*$p<0.05$). We further examined whether the activation of p38 and JNK pathways was related to the ER stress response. To this end, MAPKs phosphorylation levels were determined by Western blot after pre-incubation with AICAR, an ER stress inhibitor. Our results revealed that p38 and JNK activation was markedly reduced after treating AICAR pre-incubated cells with the penicillin derivative. On the other hand, we evaluated whether PI3K-I/AKT pathway was involved in the apoptotic response induced by TAP7f. After cell transfection with the PI3K-I dominant negative vector, a significant recovery of full-length PARP-1 and an almost complete decrease of Bax levels were observed compared to cells transfected with the control vector. In summary, our results suggested that PI3K/AKT activation together with JNK and p38 MAPKs contribute to the apoptotic cell death induced by TAP7f. We also demonstrated that JNK and p38 MAPK are activated downstream the ER stress response. The knowledge of the role played by the intracellular signals that regulate cell death could certainly contribute to find new intracellular targets for melanoma treatment.

226. (10) **AHCYL1 AS A POTENTIAL PLAYER IN LUNG CANCER**

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AHCYL1 is a protein involved in cellular homeostasis. However, its potential contribution to pathogenesis, in particular cancer, remains unclear. We aim to explore its role focusing on lung cancer. AHCYL1 KD by shRNA caused an upregulation of pluripotency markers (such as POU5F1, CD133, and CD44), in addition to higher sphere-formation capacity in vitro and tumorigenesis in vivo. A meta-analysis of transcriptomic data from public databases (TCGA) revealed AHCYL1 is expressed in all grade II-III lung cancer samples, but is inversely correlated to stemness-related genes and directly correlated to differentiation genes expressions.
Moreover, AHCYL1 is upregulated primary biopsies of lung cancer compared to relapsed tumor samples characterized by higher tumorigenic potential. Preliminary data of immunohistochemical analysis in lung cancer patient samples revealed less AHCYL1 positive staining in samples corresponding tohigher stages. Statistical significance was evaluated considering a cut-off p-value ≤ 0.05. Understanding AHCYL1's role in lung cancer would contribute to better strategies for diagnosis and therapy.

227. (11) **GLIOBLASTOMA STEM CELL NUMBER REDUCTION**

BY SPECIFIC iNOS INHIBITOR IN HUMAN CELL LINES

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Introduction: Glioblastoma (GBM) is the most common and aggressive brain tumor. Despite the introduction of chemotherapy with temozolomide (TMZ), the survival does not exceed 2 years. Cancer stem cells (CSC) has been associated to tumor recurrence. On the other hand, it has been suggested that iNOS (inducible nitric oxide synthase isoform) enzyme can sustain the CSC niche.
Objective: Evaluate the expression of iNOS and the effect of its inhibitor, S-methylisothiourea (SMT), alone or in combination with TMZ, on the development of CSC on human GBM cell lines.
Methodology: Human GBM lines LN229, U251 and U87 were seeded in monolayer (2D) and under spheral (3D) conditions (low adhesion and high dilution). Viability in 2D was determined by MTS. The number of CSC was established by sphere forming efficiency (SFE) in relation to the seeded cells, and the diameter of the spheres was measured. iNOS expression was determined by immunofluorescence.
Results: The three lines expressed iNOS. In 2D, SMT (50 μM) only reduced LN229 cell line growth (26% inhibition); however, in 3D, it decreased SFE in the three lines (inhibition, LN229 42%; U251 61%; U87 48%) and their diameters (inhibition, 33%, 17% and 28%, respectively). The combination of SMT with TMZ 250 μM, further inhibited SFE (LN229 57%, U251 70%, U87 50%) compared to SMT or TMZ alone.
Conclusion: iNOS inhibition in combination of actual TMZ therapy, could be a useful reducing chemotherapy-resistant CSC. Further studies on the mechanisms of action will allow establish the differences observed between GBM cell lines.

228. (17) **MODULATION OF SPHERE-FORMING STEM-LIKE CELL POPULATIONS WITH CHEMOTHERAPEUTIC DRUGS USING A MURINE MIXED CELLULAR BREAST CANCER MODEL**

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Introduction: Breast cancer (BC) is the tumor with the highest incidence in women and a significant cause of cancer-related morbidity and mortality worldwide. The standard treatment depends on several factors including stage, histology and expression of molecular markers. Adjuvant or neoadjuvant therapy are mechanisms that facilitate tumor resection and chemotherapy (CT) is a widely approach used many times as concomitant neoadjuvant therapy with radio, endocrine or immunotherapy. Cancer Stem Cells (CSC) are a minority tumor cell population, associated to the lack of treatment response. The development of an *in vitro* assay that can predict treatment response and that could be useful for patients with this pathology is an important issue. Objective: Evaluate CSC response post-treatment CT drugs like doxorubicin (Doxo) and paclitaxel (Ptx) using a murine BC mixed cell line (LM38-LP). Results: Through a cell viability assay in monolayer, measured by MTS, IC50 values were obtained, being 1,5 μM for Doxo and 0,5 μM for Ptx. Cancer sphere forming assay in low-attachment conditions, an accepted method for CSC quantification, was carried out with or without Doxo or Ptx. Both CT compounds decreased the number of CSCs, measured as sphere formation efficiency ($p<0,0005$), spheres size by an average of 30% ($p<0,005$) and decreased the remained survived cells by 65% with Doxo and 35% with Ptx ($p <0,005$). Histological study of spheres revealed that both, CT drugs generated a more disintegrated and eosinophilic structure regarding control. Also, doxo or Ptx induced an increase of pluripotent markers Oct4, Sox2 and Nanog by qPCR ($p<0,0001$ vs control). Conclusion: Both drugs have inhibited the number of CSC, their survival rate, but have increased their pluripotent capacity. An in vivo assay analysing tumoral spheres growth rate under these treatments, will give us relevant information about their tumorigenic potential.

229. (19) **ANTIANGIOGENIC EFFECT OF A PENICILLIN DERIVATIVE IN MELANOMA**

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In a previous study, we demonstrated that TAP7f, a compound formed by penicillin linked to the dipeptide Leu-Phe through a triazole group, inhibited melanoma metastasis by the downregulation of β-catenin and integrin αVβ3. Since these molecules may be involved in the promotion of melanoma angiogenesis, we further explored the antiangiogenic effect of TAP7f. In this sense, we found that in HMEC-1 human endothelial cells, TAP7f inhibited cell proliferation (IC50 23±4 μM, 72h) and also cell migration by 36±8% (5 μM, $p<0.001$) and 52±5% (10 μM, $p<0.0001$) after 24 h of incubation. The penicillin derivative also blocked endothelial cell tube formation (75±3%, 18 h, $p<0.0001$) and altered actin cytoskeleton organization. It must be noted that all assays employed TAP7f in non-cytotoxic conditions. When gel plug assays were performed, TAP7f inhibited the neovascularization induced by 250 ng/ml of fibroblast growth factor 8 days after plug implantation in C57/BL6J mice. Furthermore, when B16-F10 metastatic melanoma cells were injected intradermally in these mice, we observed a decrease in tumor vascularization after a daily treatment with 10 mg/kg of TAP7f for 5 days. Moreover, we found that TAP7f (10 μM) reduced VEGF (vascular endothelial growth factor) expression levels (63.7±4%, 24 h, $p<0.0001$) in B16-F10 cells. When we evaluated TAP7f in vivo antitumor effect in a mouse melanoma model employing B16-F10 cells, we observed a reduction of ~50% ($p<0.001$) in tumor volume (5 doses of 20 mg/kg for 2 weeks). Additionally, tumor tissues from TAP7f-treated mice stained with anti-CD31 antibody exhibited a decreased number of endothelial cells and Western blot analysis of tumor lysates showed that TAP7f downregulated VEGF expression (68±6%, $p<0.001$).
In conclusion, our results suggest that TAP7f not only exerts antitumor and antimetastatic effects, but also behaves as an antiangiogenic agent. These features position this penicillin derivative as a promising agent for melanoma treatment.

230. (25) ***IN VIVO* PHOTOTOXIC ACTION OF A CATIONIC Zn(II) PHTHALOCYANINE IN A MURINE MELANOMA MODEL**

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Melanoma is the most aggressive form of skin carcinoma, highly resistant to traditional therapies. Photodynamic therapy (PDT) is an alternative modality, which combines a photosensitizer, visible light and molecular oxygen to produce reactive oxygen species that selectively destroy target tissues. We have previously demonstrated that the cationic Zn(II) phthalocyanine Pc13 is a potent photosensitizer that promotes cell death after irradiation in a panel of melanoma cell lines.
In order to evaluate the *in vivo* efficacy of PDT with Pc13, we em-

ployed a syngeneic model of C57BL/6 mice bearing subcutaneous B16F0 melanoma tumors. First, Pc13 biodistribution was examined after intratumoral administration using an *in vivo* imaging system. We found that maximum retention of the photosensitizer in the tumor was reached at 3 h post administration. Moreover, the presence of Pc13 in liver, intestines and kidneys suggested possible elimination pathways of this phthalocyanine. Laser irradiation (250 J/cm^2) after 3 h of intratumoral injection of 2 mg/kg Pc13 significantly reduced tumor volume at the end of the experiment (65%, $p<0.01$), compared to control groups. Body weight and histological characteristics of different tissues stained with hematoxylin-eosin were not altered in treated-mice, indicating non-toxicity of Pc13-PDT. Histological analyses of tumor sections showed a marked increase of necrotic areas (57% vs. 5%) and a reduction of PCNA staining after treatment. Furthermore, increased levels of Bax, active caspase 3 and diminished expression of Bcl-2 were observed in treated-tumors by Western Blot and immunofluorescence assays, indicating apoptotic cell death. In addition, higher levels of LC3-II and Beclin-1 demonstrated the participation of an autophagic response after Pc13-PDT. In conclusion, our results showed that PDT with Pc13 is a promising and non-toxic antitumor modality for melanoma treatment that efficiently induces cell death and reduces tumor growth *in vivo*.

231. (26) **CDU/5-FC SUICIDE GENE BYSTANDER EFFECT IS MEDIATED BY 5-FU AND ENZYME CODING INFORMATION IN HUMAN MELANOMA CELL LINES**

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Background: The yeast cytosine deaminase::uracil phosphoribosyl transferase (CDU) fusion protein [associated to its prodrug 5-fluorocytosine (5-FC)], was proposed as a suicide gene (SG) therapy approach for human melanoma. Previously, we reported the cytotoxic effects of this system on 4 melanoma cell lines (A375, hM1, hM4, hM9). Here, we extended the study to 4 additional cell lines (hM2, hM10, SB2 and M8).
Objective: To explore the mechanisms enhancing the cytotoxic effects of CDU/5-FC SG system on human melanoma cells.
Methods: Dose-response experiments for 5-FC and 5-fluorouracyl (5-FU) were performed on CDU lipofected and unlipofected cells, respectively. Conditioned medium (CM) from CDU lipofected cells was obtained after 48 h incubation with or without 5-FC. Cell survival was determined by the acid phosphatase assay and proliferative capacity of SG surviving cells by colony formation assay.
Results: A 5-FC concentration dependent decrease in CDU-lipofected cells viability was observed ($p<0.05$). The SG system mimicked 5-FU effects on cell viability. The two SG-resistant cell lines (hM2 and hM10) were also less susceptible to 5-FU. The clonogenic capacity of CDU-lipofected surviving cells was strongly diminished when they were pretreated with 10 μM 5-FC ($p<0.05$) and completely abolished with 100 μM 5-FC or 5-FU ($p<0.01$). Analyzing the contribution of the CDU/5FC-treated cells released factors to the bystander effect, we found that 5-FU accounted for most of the CM citotoxicity. Interestingly, the CDU and/or CDU-coding information was also delivered to the CM. Thus, when CM recipient cells were exposed to 5-FC, it was activated by CDU, resulting in cell death ($p<0.05$).
Conclusion: The CDU/5-FC SG therapy system appears as a promising adjuvant treatment for advanced melanoma. Its bystander effect is attributable to 5-FU and enzyme-coding information released by lipofected cells. Further studies are needed to assess the nature of all the molecule(s) involved.

232. (32) ***IN VITRO* COMPARATIVE ANALYSIS OF HSVTK/GCV AND CD::UPRT/5FC SUICIDE GENE SYSTEMS IN CELL DYNAMICS OF CANINE MELANOMA CELL LINES.**

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Our group demonstrated the efficacy of herpes simplex virus thymidine kinase/ ganciclovir (HSVtk/GCV) suicide gene (SG) for local control of canine melanoma in a clinical setting.
Aim: As a preclinical study to improve the antitumor efficacy of gene therapy, we explore the *in vitro* effectiveness of the SG system cytosine deaminase/uracil phosphoribosyl transferase fusion enzyme (CDU::UPRT, CDU) and its prodrug 5-FC (5-fluorocytosine; CDU/5FC).
Methods: Prodrug concentration-response experiments were performed on lipofected cells (monolayers and spheroids) in 3 canine melanoma cell lines derived from veterinary patient tumors. Cell viability was measured 5 days post-lipofection by the acid phosphate assay (APH) and clonogenic survival 7 days after reseeding cells by Chrystal Violet staining. Cell death mechanism was determined with acridine orange/ ethidium bromide (AO/EB) and extracellular vesicles (EVs) in the conditioned media (CM) were isolated by 500 xg; 2k xg and 12k xg centrifugation.
Results: The canine melanoma cells were very sensitive to both SG systems in both spatial configurations ($p<0.05$) and surviving cells to both SG treatments showed a significant decrease in their clonogenic capacity ($p<0.001$). AO/EB cell staining showed that during the process of apoptosis, there was an accumulation of vesicles in the perinuclear region and cell periphery. This happened at 24 h in cells exposed to CDU/5-FC, and at 48 h in *HSV*tk/GCV exposed cells. When isolated by differential centrifugation of their respective CM, the 500-2200 nm vesicles were the most toxic fractions from *HSV*tk/GCV cells ($p<0.0001$), while the highest cytotoxicity from CDU/5-FC lipofected cells´ CM was in the fraction containing 30-150 nm vesicles ($p<0.001$).
Conclusions: The present work shows that CD/5FC has a differential effect on cell dynamics and its bystander cell death is mediated by the release of small vesicles and 5-FU, supporting CDU/5-FC as a candidate for a novel *in vivo* protocol.

233. (33) **BRAF INHIBITION DIMINISHES CELL VIABILITY VIA PKC ALPHA (PKCA) IN THYROID CANCER CELLS**

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Thyroid carcinoma (TC) is the most common endocrine neoplasia. Its incidence has increased in the last 40 years worldwide. It comprises a group of tumors of different lineage and biological behavior. About half of TC are driven by an acquired activating mutation in the BRAF oncogene. While targeted therapies have improved outcomes in melanoma patients, most TC patients become resistant or recur suggesting that new or additive non-cross-reactive therapies are needed. We have previously shown that PKCa mediates TSH and thyroid hormones proliferative effects in TC. Recent evidence indicates that together PKCa overexpression and BRAF mutation should contribute to tumorigenesis and resistance to anticancer therapies. We found that by inhibiting BRAF expression with RNAi in anaplastic TC cells with BRAF mutation, PKCa expression decreases as well, suggesting that the latter is found downstream of BRAF. Furthermore, a decrease in the expression of the cell proliferation marker PCNA was observed in BRAF-depleted cells by western blot analysis. Also, TC cells were sensitive to increasing doses of the BRAF inhibitor widely used in the clinic vemurafenib/PLX4032 in a dose-dependent manner ($p<0.0001$) by Cell Titer Blue (CTB) assay. To begin to study the combined inhibition of PKC and BRAF, CTB assays were performed with increasing doses of vemurafenib in presence or absence of the PKC inhibitor GF109203X at selective concentrations in follicular TC cells carrying BRAF mutation. We confirmed the dose-dependency of vemurafenib and found that the combination leads to a significant decrease in cell viability ($p<0.5$). Our results establish that the effective dual PKCa and BRAF blockade can significantly drive tumor proliferation inhibition. The results

obtained could provide new therapeutic targets and alternatives to the treatments currently used for this disease. Despite its increasing incidence and mortality in many cases, TC constitutes a very poorly studied area in our country.

234. (37) **GLYPICAN-3 (GPC3) MODULATES THE ADHESION PROPERTIES OF BREAST CANCER CELLS**

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Glypican-3 (GPC3) is a proteoglycan downregulated in breast tumors. Previously, we showed that GPC3 prevents metastatic spread and regulates the epithelial-to-mesenchymal transition (EMT), suggesting its role as metastasis suppressor. However, events underlying this modulation have not completely described yet.
The aim of this study was to examine the effects of GPC3 on cell morphology and adhesion patterns, as well as on the expression of molecules associated with these properties. We employed human cell lines genetically modified. We silenced GPC3 expression in MCF-7 cells, while it was over expressed in MDA-MB231.
Our results showed that GPC3 expressing cells exhibit an epithelial phenotype and reorganize their actin cytoskeleton. By phalloidin-FITC staining, we observed that GPC3 expressing cells lose their stress fibers and place the actin in a cortical ring. We also checked the expression of linage markers by WB. We found higher levels of the epithelial marker E-cadherin in GPC3 expressing cells, while the expression of the mesenchymal marker vimentin was reduced.
We evaluated whether GPC3 modulates the cell adhesion to extracellular matrix components, showing that it impairs the ability of MDA-MB231 cells to adhere to FN ($p<0.001$, ANOVA Bonferroni´s tests) and LN ($p<0.0001$), as well as to plastic ($p<0.0001$). On the other hand, the GPC3 silencing did not change the adhesion of MCF-7 cells either to FN or plastic, but reduced their adherence to LN ($p<0.001$). We also analyzed the expression of adhesion proteins by WB. Supporting our results, we found that MDA-MB231-GPC3 cells have lower β1 and β4-integrin levels, while no significant changes were found in MCF-7 sublines.
In sum, here we demonstrated that GPC3 modifies several tumor cell properties, like morphology, cytoskeleton organization and adhesion, and modulates proteins related to these processes. Altogether, our results support the key role of GPC3 in the EMT regulation, and then breast tumor progression.

235. (39) **A NOVEL SOLUBLE ISOFORM OF THE HUMAN TGF-β TYPE 2 RECEPTOR EXERTS STRONG ANTITUMOR ACTIVITY IN COLORRECTAL CANCER-DERIVED CELL LINES**

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TGF-β signaling pathway is a key regulator of cancer progression, particularly in colorectal cancer, where 90% of microsatellite instable (MSI) tumors exhibit mutations in the TGF-β receptor type 2 (TGFBR2) gene. Here, we show that lentiviral-mediated overexpression of TGFBR2-SE, a recently discovered soluble isoform of the human TGF-β type 2 receptor, fused to the human IgG1 Fc fragment (TGFBR2-SE/Fc) reduces *in vitro* cell proliferation and migration while induces cell cycle arrest and apoptosis in the primary human colorectal cancer-derived cell line HCT116. Moreover, TGFBR2-SE/Fc impairs tumorigenicity of BALB/c nude athymic mice xenografts, increasing the survival rate of the animals. Tumors overexpressing TGFBR2-SE/Fc were considerable smaller or even unable to be established as only 3 out of 6 mice developed tumors in the TGFBR2-SE/Fc group. Mechanistically, TGFBR2-SE/Fc downregulates TGF-β canonical pathway and leads to the activation of tumor suppressor genes such as p21, p57 and p53, as well as to the inactivation of cell cycle progression elements such as cyclin B1 and Id1. These findings suggest a strong antitumor activity of TGFBR2-SE/Fc based on blocking TGF-β signaling pathway and Smad2/3- independent changes in gene expression supporting the further exploration and development of TGFBR2-SE/Fc as a new biopharmaceutical for the treatment of solid tumors.

236. (42) **VASCULAR NORMALIZATION OF TRIPLE NEGATIVE MAMMARY ADENOCARCINOMAS TREATED METRONOMICALLY WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS)**

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CY is an alkylating drug with toxic action on proliferating cells. LOS is an antagonist of angiotensin II receptor, used to treat hypertension. It was postulated that the antiangiogenic effect of metronomic chemotherapy (MCT) could be obtained through a normalization of the abnormal tumor vasculature. Previously, we demonstrated that MCT with CY+LOS, in M-234p and M-406 tumor models, caused inhibition of tumor growth, increase of survival rate and was devoid of toxicity. We aimed to analyze the structural and morphologic changes in M-234p and M-406 vasculature after MCT with CY+LOS. Mice were challenged with each tumor (Day 0). On days 31 (M-234p) and 22 (M-406) tumor samples were taken from: 1) CONTROL: with tumor and no treatment, 2) TREATED: with tumor and treated in the drinking water, from days 5 and 8, respectively, with 2a) CY (25mg/kg/day), 2b) LOS (200mg/kg/day) and 2c) CY+LOS as 2a+2b. Samples were fixed, paraffin embedded, cut in 5µm slices and stained with H&E. The capillaries in CONTROL group showed a circumferentially incomplete inner lining layer of small cells, flattened nuclei, marked intercellular gaps and an underlying sheet of very thin and interrupted connective tissue. No pericytes were observed around the capillaries. Samples from CY+LOS group showed intra and peritumoral capillaries with structure and morphology similar to normal patterns in tissues without tumor. Endothelial cells provided a continuous and uninterrupted linning, with a well-defined basal membrane covered by pericytes. Samples from 2a and 2b tumors were similar to CONTROL group. Results were similar for M-234p and M-406 tumors. The CY+LOS treatment produced modifications of tumor vasculature consisting of normalization of tumor vessels that showed a morphology similar to normal mammary tissue. This changes may reduce hypoxia, increase tumor oxygenation, leading to a better delivery of drugs and a better therapeutic outcome for triple negative mammary tumors.

237. (44) **CHEMOTHERAPEUTIC DRUGS INDUCE THE ACTIVATION OF PROTEINS ASSOCIATED WITH TUMORIGENESIS AND DRUG RESISTANCE IN LOWER-GRADE TUMOR CELLS**

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Acyl CoA synthetase 4 (ACSL4) is an enzyme participating in the metabolism of arachidonic acid. ATP-binding cassette (ABC) transporters are transmembrane proteins that translocate low molecular weight molecules through ATP hydrolysis.We have previously shown that ACSL4 is involved in resistance to chemotherapeutic agents by regulating the expression of transporters; thus, the objective of this work was to study the effect of chemotherapeutic agents on ACSL4 and resistance mechanisms. The experimental mod-

el consisted in the chemotherapeutic challenge of adrenal cancer NCI-H295R and breast cancer MCF-7 cells, two lines characterized by low aggressive phenotypes and low expression of the ACSL4, ABCG2 and ABCC4 proteins. We evaluated cell functionality using proliferation (BrdU) and viability (MTT) assays, and compound exclusion (efflux) using fluorescent Hoechst 33342. ACSL4 and ABC transporters were evaluated by western blot (WB). NCI-H295R cell treatment with doxorubicin (20 nM) and cisplatin (200 nM) increased the expression of ACSL4 (WB-p <0.001), ABCG2 (WB-p <0.001) and ABCC4 (WB-p <0.05). The treatments also improved fluorescent compound exclusion (efflux-p <0.01), an effect reversed by the action of ABGC2 transporter inhibitor KO143. Combined treatments (chemotherapeutic agents and ACSL4 inhibitor) reduced the proliferation of NCI-H295R cells (BrdU-p <0.05). MCF-7 cell treatment with doxorubicin and cisplatin increased the expression of ACSL4 (WB-p <0.001) and ABCG2 (WB-p <0.05) and the phosphorylation of pAKT (WB-p <0.05) and pS6 (WB-p <0.01), components of the AKT/mTOR pathway. These results are in line with our previous observation that ACSL4 regulates ABGC2 expression through the regulation of the AKT/mTOR pathway. Therefore, ACSL4 may constitute a therapeutic target at the initial stages of chemotherapeutic treatment to prevent the activation of pathways associated with increased tumor aggressiveness.

238. (46) **HEMEOXYGENASE-1 IN THYROID CANCER PROGRESSION**

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Previous work from our group shows that Hemeoxygenase-1 (HO-1) is overexpressed in several types of tumor and the enzyme can be located in cell cytoplasm and/or nucleus. This subcellular distribution is caused by the cleavage of the C-terminus of HO-1 by calpain 1 (CAPN1), calpain 2 (CAPN2), cathepsin B (CTSB) and signal peptide peptidase (SPP). In thyroid cancer (TC), HO-1 potential utility as biomarker remains underexplored. The aim of this work was to study HO-1 expression in TC and its correlation with clinical-pathological data. Tumor biopsies (N=64) and fine needle aspiration biopsies (FNAB) (N=22) were used to asses HO-1 expression by inmunohistochemistry (IHC) and inmunocytochemistry (ICC), respectively. In addition, mRNA expression of HO-1, CAPN1, CAPN2, CTSB and SPP were analyzed by using GEPIA2 and Kaplan-Meier Plotter databases in *in silico* assays. In TC biopsies, overexpression (OE) of HO-1 by IHC was found in the tumor (T) respect to non-malignant areas to the tumor (NMT) (Mann Whitney test, p<0.0001). In T, HO-1 was expressed in the cytoplasm while in NMT, nuclear expression was found. HO-1 expression correlated with histological subtype by IHC (Chi^2, p=0.0006) and Bethesda classification by ICC (Chi^2, p=0.0470). *In silico* studies (ISS) corroborated IHC results in papillary TC (ANOVA, p<0.001). Stage IV female patients with HO-1 OE were associated with lower overall survival (Log rank, p=0.032). ISS showed that stage III male patients with OE of CTSB and female patients with OE of CAPN1 correlated with greater survival (Log rank, p=0.017; Log rank, p=0.027 respectively). However, in female and male stage IV patients, OE of CAPN2 was associated with lower survival (Log rank, p=0.0015; Log rank, p=0.039 respectively). Furthermore, SPP OE correlated with lower survival in female patients (Log rank, p=0.041). So far our results show that HO-1, CAPN2 and SPP overexpression together could be used as unfavorable biomarkers in TC.

239. (64) δ-**TOCOTRIENOL POTENTIATES THE INHIBITORY EFFECTS OF INTERFERON ALFA 2-B (IFN A) ON PROLIFERATION, MIGRATION, INVASION AND INCREASES APOPTOSIS IN HUMAN HUH7 HEPATOCARCINOMA CELLS.**

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Our group has previously postulated that δ-tocotrienol supplementation to interferon alfa (IFN α) therapy can be used as a strategy against liver cancer cells because combined treatment produced growth inhibition and induced apoptosis in SK Hep-1 tumor cells. According to our preliminary results in SK-Hep1 cells, we decided to check if they were repeated in another liver tumor cell line (HuH7), doing additional migration and invasiveness studies.

Cells were treated with 20000 IU/L IFN α and 25 uM δ-tocotrienol, an isomer of vitamin E (combined IFN-E-group). Also, treatments with each single compound were made (IFN-group and E-group). MTT assay was performed to determine cell viability at 72 h of treatment; wound healing assay was done at 24 h to determine cell migration. Invasion studies at 24 h were made in transwell chambers, and annexin v/propidium iodide assay was performed to determine apoptosis at 72 h. As expected, IFN-E-group showed a higher decrease in cell viability (-70%*#) compared with monodrug therapy: IFN-group (-10%*), E-group (-15%*). IFN-E-group displayed a significant decrease (-44%*#) in migratory activity compared with each individual treatment: IFN-group (-21%*) and E-group (-22%*). Also, IFN-E-group showed a significant diminution (-75%*#) in cell invasiveness compared with monodrug therapy: IFN-group (-25%*) and E-group (-55%*). Finally, IFN-E-group showed a higher increase in total apoptosis (+160%*#) compared with individual therapy: IFN-group (-40%*) and E-group (-43%*),(*p≤0.05 vs. control untreated cells; #p≤0.05 vs IFN-group and E-group). In summary, we demonstrate that the addition of δ-tocotrienol to IFN α therapy enhances the reduction of cell proliferation and migration/invasiveness capacities of Huh7 cells, as well as potentiates the increase in apoptotic cell death. In this regard, combined treatment of immunochemicals together with natural products, might open a potential clinical aproach for HCC treatment in the future.

240. (81) **miR-34a AND miR-137 AND THEIR TARGET PROTEINS WERE FOUND TO BE DOWNREGULATED IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS**.

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Acute Lymphoblastic Leukemia (ALL) is the most frequent cancer in children, characterized by clonal proliferation of early B- and T-lymphocyte progenitors. Up to 25% of children and more than 50% of adults suffer a relapse of the disease which significantly reduces patient's survival. Therefore, it is important to identify new biomarkers, which can be used to improve the disease prognosis and/or to predict treatment efficacy. Non-coding RNAs have been shown to play a key role in the development and progression of tumors. Recent studies point out that aberrant miR-34a and miR-137 expression leads to an increase in cell proliferation, as well as an abnormal response to chemotherapy in various types of cancer. Thus, we aimed to elucidate the role of this two microRNAs in ALL, specifically to study their association to tumor development and disease

progression. To achieve this goal, we first analyzed the expression of these two microRNAs in B-ALL and T-ALL cell lines, using normal lymphoid cells as controls. We found lower expression of miR-34a and miR-137 by RT-qPCR in cancer cell lines compared to control cells. Furthermore, we studied the main proteins regulated by these non-coding RNAs: SIRT1 and LSD1. We demonstrated by Western Blot that SIRT1 and LSD1 levels were significantly higher in ALL cell lines. Finally, we overexpressed miR34a in the ALL cell lines by transfection with an expression vector in order to confirm that the lower expression of this microRNA is associated with the tumoral phenotype. The restored levels of miR-34a resulted in a downregulation of SIRT1 protein levels and cell proliferation. Thus, these novel results provide new insight into ALL cell biology. Furthermore, these results encourage us to continue studying the role of miR-34a and miR-137 as an early diagnostic molecule or a possible effective target for disease treatment.

241. (92) **ADIPOCYTES IN THE BREAST CANCER MICROENVIRONMENT: DIFFERENT TUMOR CELLS, DIFFERENT WAYS TO RESPOND**

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Adipocytes are considered to be critical in the tumoral microenvironment of breast cancer. However, most studies have focused on linking obesity and cancer, ignoring changes on normal adipocytes. Therefore, we retrieved microarray data from a GEO dataset (GSE95827) to analyse transcriptional changes in 3T3-L1 adipocytes after 3 days of co-culture with MCF7 (Ad+MCF) or MDA-MB-231 breast cancer cell (Ad+MDA).

By GEO2R tool, we determined 245 up-regulated genes in Ad+MCF7 and 215 in Ad+MDA compared to adipocytes alone ($p<0.01$), but only 68 of them overlapped between conditions. Gene Ontology Analysis was performed and showed that the biological process enrichment is completely different between MDA-MB-231 and MCF7-co-cultured adipocytes. Even more, co-culture with MDA-MB-231 cells enriched adipocytes with genes involved in *Cellular response to interleukin-6* (GO:0071354) and *Positive regulation of NIK/NF-κB signaling* (GO:1901224), both related to inflammation. Transcription factors (TF) analysis by ISMARA platform predicted that NF-κB family members had the highest activity in Ad+MDA. To verify this result, we performed immunofluorescence assays detecting the presence of phosphorylated NF-κB subunit p65. MDA-MB-231 cells significantly led to a higher fluorescence intensity in adipocyte nuclei than MCF7 did when compared to basal condition. Moreover, mRNA levels of NF-κB targets as *cxcl1, cxcl5, il6* obtained from the analysed dataset were found up-regulated in Ad+MDA respect to adipocytes alone ($p<0.05$). Interestingly, expression levels of Il6 family members (Il6 and Il11) were up-regulated in MDA-MB-231 cell line compared to MCF7 cells in three different public datasets, which could explain NF-κB activation only in Ad+MDA.

These results suggest that breast cancer cells stimulate adjacent adipocytes in different ways leading or not to inflammation in adipocytes.

242. (96) **TGF- B1 IMPLICATIONS ON LUMINAL-MYOEPITHELIAL DIALOGUE IN BREAST CANCER**

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The mammary gland duct is composed by an internal cell line formed by luminal cells (LEP) surrounded by an external one of myoepithelial cells (MEP). It is still unknown how these cells contribute to the progression of ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). Some works suggest that TGF-β1 pathway may be involved in epithelial-mesenchymal transition and confer stem cell properties to DCIS cells. This implies that this pathway might be a potential pathological mechanism which drives the progression of DCIS into IDC.

The cellular model LM38 consists of three cell lines: LM38-LP (MEP and LEP), LM38-HP (LEP) and LM38-D2 (MEP). Previously, we described that only the bi-cellular LM38-LP cell line was able to develop in situ tumors after intraductal injections, suggesting that cell interaction could confer an advantage for tumor formation and progression. Moreover, we showed that treatment with conditioned medium of LM38-D2 induced viability on LM38-LP cells.

The analysis of TGF-β1 expression in the LM38 model showed higher levels of TGF-β1 mRNA in LM38-D2 compared to LM38-LP (qPCR, $p<0.01$). LM38 cells were treated with a recombinant TGF-β1 (1ng/μl) and an inhibitor of the TGF-β1 receptor SB431542 (10 and 20μM). We could observe that TGF-β1 treatment increased 40 per cent the viability of LM38-LP compared to the control, which is reduced in presence of SB431542 (crystal violet assay, $p<0.05$, $p<0.001$).

When evaluating TGF-β1 expression in LM38 fat pad tumors we observed that tumors generated by LM38-D2 showed higher expression levels on TGF-β1 than LM38-LP (IHQ, p=0,01). Expression of TGF-β1 presented an heterogeneous pattern in LM38-LP tumors which requires further characterization.

In conclusion, TGF-β1 could be one of the factors implicated in the LEP-MEP dialogue. These results suggest that TGF-β1 could play an important role in cellular cooperation in early stages of breast cancer.

243. (98) **CHARACTERIZATION OF AN IMATINIB-RESISTANT CML K562 CELL LINE: KI562. EFFECT OF 4-METHYLUMBELLIFERONE ON ITS METABOLIC ACTIVITY AND CD44 EXPRESSION.**

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CML is a myeloproliferative neoplasia whose first-line therapy are BCR-ABL inhibitors such as Imatinib (IM). CD44 levels correlates with bad response to therapy. Previously, we demonstrated that hyaluronic acid (HA) abrogates IM-induced senescence, while the inhibition of its synthesis with 4-methylumbelliferone (4MU) has a synergistic effect with IM on CML cells growth. The aim of this work was to obtain an IM resistant K562 derivate cell line and to study the resistance mechanisms involved, as well as, the effect of 4MU treatment. The Ki562 cells were obtained after culturing K562 cells with increasing doses of IM from 0.1μM up to 1μM. Control cells derivate of K562, Ko562, were kept in culture presenting the same aging but, without selection pressure of IM. None of these cells showed efflux pump activity (determined by flow cytometry, FC). Both cell lines had a similar frequency of the F359I mutation (evaluated by DNA sequencing). However, Ki562 cells showed higher levels of *BCR-ABL* than Ko562 cells (evaluated by qRT-PCR and WB, $p<0,01$). Both of them, expressed similar levels of surface CD44 (evaluated by FC), which was downregulated by 4MU as well as by IM only in Ko562 cells ($p<0.05$). 4MU decreased the metabolic activity on both cell lines (determined by XTT, $p<0.01$) without modifying the percentage of PI+ cells respect to untreated control (evaluated by FC). Moreover, the co-treatment with 4MU+IM inhibited metabolic activity more than each drug alone in both cell lines ($p<0.01$), without modifying the percentage of PI+ cells. We conclude that Ki562 cells would be an interesting model to study IM resistance since the overexpression of BCR-ABL is one of the mechanisms related to therapeutic failure in CML patients. The loss of CD44 modulation in Ki562 cells could also be involved in their resistance. In addition, 4MU shows a cytostatic effect and enhances IM effect on metabolic

activity, supporting the hypothesis of 4MU as a promising therapeutic strategy in CML.

244. (100) **CFTR OVEREXPRESSION MAY CONTRIBUTE TO COLORECTAL CANCER**

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CFTR mutations cause not only cystic fibrosis disease, but also increase the risk of colorectal cancer. However, its probably role in colorectal cancer from patients without cystic fibrosis has not been previously investigated. RAC3 is a nuclear receptor coactivator usually overexpressed in several tumors, required to maintaining the cancer stemness. We investigated the functional relationship between CFTR and RAC3 for maintaining cancer stemness in human colorectal cancer.
Previously we investigated cancer stemness using a stable transfection of shCFTR or shRAC3 in HCT116 cells, and we found that CFTR downregulation inhibits the cancer stem phenotype. We also found that CD133+ side population expresses higher levels of RAC3 and CFTR than CD133- and RAC3 overexpression increases CFTR expression.
To further investigate this, we performed bioinformatics analysis in both human colorectal cancer samples and Caco-2 cells. In order to do these we used two datasets: 1) CD133+ or CD133- side populations and 2) CFTRwt or CFTRmut cells.
First we analyzed the expression levels of CFTR mRNA in colorectal cancer samples from patients without cystic fibrosis using the Xena platform (TCGA). The CFTR mRNA without mutations was higher than the CFTR mRNA with mutations and this correlates with an increased expression of RAC3. Then we compared the gene expression between CD133+ cells and CFTRwt cells using different platforms (ConsensusPathDB, STRING, Cytoscape, GeneMANIA). We found a common gene expression pattern between them involved in inflammatory and nuclear receptor pathways that contribute to colorectal cancer development.
From these and our previous results we conclude that although CFTR mutation may increase the risk of colorectal cancer, there are other pathways by which CFTR overexpression may also contribute to this disease, maintaining the cancer stemness and inducing tumor development.

245. (107) **NORCANTHARIDIN AND LEVOGLUCOSENONE: NEW NATURAL ALTERNATIVES FOR BREAST CANCER TREATMENT.**

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Breast cancer is a major public health problem, being the second leading cause of cancer death among women in developed countries. Given the need for new therapeutic alternatives, in this work we have studied the antitumor activity of two different natural compounds in order to evaluate their potential use in clinical settings.
Norcantharidin (NCTD) is a demethylated form of cantharidin, an active component present in Mylabris beetles and levoglucosenone (Levo) is obtained after the pyrolytic treatment of soybean hulls. NCTD showed an IC50 of 35 and 56 µM in 4T1 (murine) and Hs578T (human) mammary cell lines respectively. Levo and structurally related derivatives (compounds 2, 3 and 4) were analyzed in LM3 (murine) and MCF 7 (human) cell lines showing an IC50 of 12 and 50 µM respectively.
In both natural compounds, anti-proliferative effects were associated with apoptosis induction. Nevertheless, NCTD also induced a time-sustained reduction in ERK activated levels (p-ERK). Moreover, in vitro, both compounds significantly reduced the adhesive and migratory capacities as well as secreted MMP-9 activity in a dose-dependent manner ($p<0.05$ Anova).
Although these parameters could have a direct implication in malignant progression, in vivo assays pretreating 4T1 cells with NCTD, showed a significant increase in experimental metastatic spread. However, applying the same experimental approach, Levo and compound 2 significantly reduced the number of LM3 lung nodules. Furthermore, systemic treatment of BALB/c mice induced a significant inhibition of tumor growth upon using compound 2 and a partial effect was obtained after employing Levo.
In sum, all the compounds analyzed show promising effects against breast cancer and may become in the future an important therapeutic alternative, either as a single drug or enhancing the effect of pre-existing therapies.
*L, Ariza Bareño and D, Delbart, are both first authors

246. (108) **A NOVEL ROLE OF AN OLD FRIEND: GLUTATHIONE PROTECTS CELLS FROM FORMALDEHYDE TOXICITY**

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Formaldehyde (FA) is produced inside cells as a byproduct of essential biological processes such as epigenetic demethylations and the one carbon cycle. We have previously shown that human colorectal carcinoma cells lacking the FA-metabolizing enzyme alcohol dehydrogenase 5 (ADH5) cannot cope with blood concentrations of FA, which can be reverted by thiol-containing antioxidants like N-acetylcysteine (NAC) or glutathione monoethyl ester (GSH-MEE). The rescue of FA toxicity by thiol-rich antioxidants might indicate that glutathione (GSH) participates in limiting the toxicity of FA. To address this hypothesis, we used CRISPR/Cas9 to inactivate the gene coding for the regulatory unit (GCLM) of the enzyme γ-glutamate-cysteine ligase (GCL), which conforms the GSH synthetic pathway, in HCT116 human colorectal carcinoma cells. The normalized viabilities against 150uM FA were WT= 72.5±7.4 μM; ΔADH5= 26.0±5.1 μM and ΔGCLM= 46.9±7.1 μM ($p=0.002$, Tukey comparison vs WT; n=5, mean ± SEM). Moreover, the normalized plating efficiencies (PE) in presence of 25 μM FA were WT= 90.1±6.3 %; ΔADH5= 7.9±2.7 % ($P<0.0001$) and ΔGCLM= 72.4±4.8 % ($P=0.038$), (n=5, mean ± SEM, Tukey?s comparison vs WT). To obtain mechanistic insights, we investigated in cell extracts the formation of S-hydroxymethyl-GSH (HSMGSH), which is the product of the reaction between FA and GSH, by ultraperformance liquid chromatography coupled to high resolution mass spectrometry. ΔADH5 cells showed a 1.85-fold increase in the ratio HSMGSH/GSH compared to WT ($P<0.0001$, Mann-Whitney test), indicating GSH reacts with endogenous FA forming HSMGSH in cells, and that ADH5 particiates in the metabolization of HSMGSH. In conclusion, GSH might prevent FA toxicity by forming HSMGSH, and thus limiting the reactivity of the aldehyde. This role of GSH might have an impact for patients with the human disease Fanconi Anemia and for carriers of mutations in the tumor suppressor BRCA2, whose cells are more susceptible to FA damage.

247. (115) **ANTIMETASTATIC EFFECTS AND BIOAVAILABILITY STUDIES OF SILIBININ WITH OXIDOVANADIUM(IV) CATION (VOSIL)**

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Objective: The flavolignan silibinin is the main component of silymarin. It has powerful anticancer and antimetastatic effects against malignant cell lines. In recent years, a great effort has been dedicated to the development of more effective and less toxic chemotherapeutic agents. In this sense, we evaluated the anticancer and antimetastatic activity on A549 cell line of silibinin and the complex VOsil, previously synthesized and characterized. Likewise, the ability of bovine serum albumin (BSA) to bind the compound was evaluated.

Methods: The effect of silibinin and VOsil on the human lung cancer cell line (A549) viability was measured (MTT assay). In addition, the effect of the compounds at non-cytotoxic concentrations (5 μM) on adhesion, migration and invasion was investigated. On the other hand, the interaction between both compounds and BSA was investigated using tryptophan fluorescence quenching.
Results: VOsil behaved as a more cytotoxic agent than the ligand at concentration 100 μM inhibiting 40 % of cell viability. The adhesion to fibronectin ability of cells treated with silibinin and VOsil decreased 34 and 58 %, respectively in comparison with the control. The number of migrating cells decreased about 50 % after VOsil treatment. Silibinin attenuated cell migration to a lesser extent (25%). A 40% and 23% reduction on cell invasion was observed when cells were treated with VOsil and silibinin, respectively. Usually, the oxidovanadium(IV) cation was less effective in all assays. Binding constant values for the interaction of silibinin ($9.88 \pm 0.95 \times 10^6$ $L.mol^{-1}$) and VOsil ($12.58 \pm 0.76 \times 10^6$ $L.mol^{-1}$) with BSA were determined, suggesting high affinity of the compounds toward the protein. Also, n values of 1.07 ± 0.06 (silibinin) and 1.48 ± 0.07 (VOsil) were obtained indicating an interaction with one binding site of BSA.
Conclusion: This study shows that the complexation enhances the biological effects of the free flavolignan.

248. (122) **EPSTEIN BARR VIRUS RECRUITS PDL1 POSITIVE CELLS AT THE MICROENVIRONMENT IN PEDIATRIC HODGKIN LYMPHOMA.**

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Classic Hodgkin lymphoma (cHL) is a lymphoid neoplasm in which the immune microenvironment contributes to the lymphomagenesis process. Epstein Barr virus (EBV) presence also influences cHL microenvironment composition and contributes to pathogenesis. Our aim was to evaluate the PD1 / PDL1 pathway and EBV influence on this pathway in pediatric cHL. Methods: 80 pediatric patients were analyzed. EBV presence was assessed by in situ hybridization (HIS), the expression of PDL1 in the microenvironment (PDL1mic) and PDL1 in the HRS tumor cells (PDL1HRS) and PD1 in the microenvironment (PD1mic) by immunohistochemistry (IHC) expressing the results as +cells/ mm2. PDL1 genetic alterations were analyzed in a subgroup of 37 pediatric patients by FISH, following the criteria of: genetic gain (PDL1 / CEP9 <3: 1), amplification (PDL1 / CEP9 ≥3: 1) or diplody (PDL1 / CEP9=1:1). The survival was evaluated in relation to the expression of PDL1 mic, PDL1 HRS and PD1 mic. Results: No significant differences were observed in the PD1mic count or in the PDL1 HRS count between the EBV + and EBV- cases (P> 0.05; Mann Whitney test). Unexpectedly, only 38% of pediatric cHL showed PDL1 genetic alterations by FISH (8% amplification, 16% gain and 13% gain + amplification) and no differences were observed in EBV + vs EBV- cases (p> 0.05, exact test of Fisher). In the cHL EBV + cases, a significant increase in PDL1mic + cells was detected in the microenvironment (p> 0.05, Mann Whitney test). Neither PD1mic nor PDL1 expression in HRS cells or in the microenvironment was associated with survival in pediatric patients (p> 0.05, log-rank test) .Conclusions: Although our group previously described an environment of high cytotoxicity in pediatric EBV + cHL, it could be counteracted by a PDL1 + cell niche in the microenvironment, leading to unsuccessful elimination of EBV+ HRS tumor cells.

249. (123) **ANTIPROLIFERATIVE ACTIVITY OF EXTRACTS FROM LIQUID CULTURE OF EDIBLE AGARICOMYCETES (BASIDIOMYCOTA) FUNGAL STRAINS ON HUMAN PROSTATE TUMOR CELLS**

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Fungi are eukaryotic organisms with absorbotrophic heterotrophic nutrition. They present growth with four phases: lag, exponential, stationary and senescent.The exponential phase presents a primary metabolism in charge of obtaining energy through nutrients and getting biomass. In the stationary phase they synthesize secondary metabolites (SM) that allow it to degrade the substrate and thus expand. Medicinal properties have been attributed for some of these SM. Studies on tumoral cells shown apoptosis induction, arrest of the cell cycle and impaired proliferation by effect of edible strains of the Agaricomycetes class. However, most of the studies were performed with the basidium and little is known about the effect of the extracellular medium (EM), where the SM are concentrated. In this study we aimed to evaluate the effect of the EM of different fungal strains on the viability of human prostate tumor cell. PC3 cells were cultured with EM (20%; 24h) from six different strains of fungi collected at exponential or stationary phase (days 7 or 21, respectively). We found that the EM of C. comatus, M. titans and G. lucidum, from day 21 significantly decreased ($p < 0.05$) the viability of PC3 cells, evaluated by the MTS assay; whereas both EM (7 and 21) from C. cylindracea, impair viability of PC3 cells ($p < 0.05$), with a significant decrease in the number of cells ($p < 0.05$). These effects correlate with changes in cell-cycle where S phase grows at the expense of G1 phase. Nevertheless, no changes were observed in apoptosis levels, analyzed by flow cytometry with ANXAV-FITC / IP staining. These results indicate that EM of different strains of Agaricomycetes affect the viability of tumor cells, and this effect is not only dependent of the strain, but also on the growth phase.

250. (127) **THE ROLE OF AMPK-MEDIATED AUTOPHAGY IN COLON CANCER AND ITS THERAPEUTIC IMPLICATION IN PHOTODYNAMIC THERAPY WITH METHYL-AMINOLEVULINATE**

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Colorectal cancer (CRC) is the second leading cause of cancer death. The increased resistance of CRC to conventional therapies led to the need to seek new treatments such as photodynamic therapy (PDT). However, autophagy has been recently linked to the resistance of different antitumor treatments including PDT. Although autophagy is considered mainly as a cell survival mechanism, autophagy-mediated cell death has also been described evidencing the dual role of this process whose effects on therapeutic resistance are not yet fully elucidated. The increase in adenosine monophosphate (AMP) levels in cells will activate the AMP-dependent kinase (AMPK), who induce autophagy through the inhibition of the mTOR complex. Based on the above, we propose to characterize the mechanisms that govern autophagy in CRC through the AMPK-induced pathway and its implication in the therapeutic response to PDT. For this, we used the GEPIA and Human Protein Atlas database to study the relevance of AMPK and its correlation with the main modulators of autophagy in CRC. We also established two autophagy induction systems using SW480 CCR cells. For the AMPK-dependent pathway, cells were incubated in phosphate buffer saline (PBS 1X, 15min - 2 h) and for the AMPK-independent pathway, Compound C (10-20 µM, 18 and 8 h) in growth medium was implemented. It was shown that the induction of autophagy, by both conditions, produces a decrease in cell viability (MTT) in CRC cells (p value < 0.0001) over time where apoptosis is the main mechanism of cell death (determined by an Annexin V/Propidium iodide assay 24 hours post-autophagy induction measured by flow cytometry). Lastly, it was shown that cells with active autophagy (dependent and independent of AMPK) does not affect the cell viability (MTT) of CRC cells (p value = ns) treated with PDT (1.8 J/cm^2; Me-ALA 0.5 mM); which suggests that neoadjuvant treatments, which induce autophagy through these pathways, could be beneficial for the success of PDT.

251. (132) **ADIPOCYTE LIPID METABOLISM REGULATION BY DEXAMETHASONE**

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Tumor Microenvironment (TM) has altered its metabolism in a way that favors tumor progression. TM metabolic differences can be found even between different breast cancer (BC) subtypes. In the ST000054 public database (Metabolic Workbench) we observed that the mammary TM were enriched with cholesterol (1.9 times respected to reduction breast samples, p-value <0.01).
Our goal is to understand how oncology relevant factors could alter TM metabolism. In previous studies, we demonstrated that TNF increased the triglyceride secretion and altered the pattern of fatty acid secretion. Therefore, we were interested in studying the regulation of lipid metabolism by an anti-inflammatory stimulus.
We observed by gas chromatography that the pattern of secreted fatty acids was altered in murine mammary gland adipose tissue conditioned media (C57BL-6j) after 48 h with or without dexamethasone (Dx) 500 nM. Among them, the proportion of palmitic acid increased (32.54% Dx vs 3.02% control). We also observed an enrichment of saturated fatty acids under Dx stimulus similarly of what we have seen with TNF (66.1% Dx, 30.3% TNF, 5.3% control).
To further understand this result, we analysed with GEO2R the gene expression from GSE62635 database where adipocyte differentiated 3T3-L1 were treated with Dx 500 nM for 6 days and then we studied pathways enrichment with the String platform. In the set of genes with a fold change<-1.5 and an adj.p.value<0.01, we identified the pathways described in KEGG as *Unsaturated fatty acid biosynthesis* (mmu01040, FDR=0.0014). Based on these data, we studied in depth each gene involved in this pathway and observed a correlation between the downregulation of the genes involved in the pathway and the enrichment of saturated fatty acids found in the conditioned media.
These results encourage us to deepen our studies of the metabolic adaptation of cells in the tumor microenvironment to anti-inflammatory stimuli and how it can impact the metabolism of tumor cells.

252. (133) **ROLE OF RHOA-ACTIVATOR IN BREAST CANCER PROGRESSION**

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Cells express different classes of fibronectin (FN)-binding integrins, which induce signalling pathways that integrate Rho-GTPases to convert integrin signals into specific effector functions. The aim of this work is to study the relationship between differential expression of integrins and specific activation of Rho-GTPases in cancer. Using genetically engineered cells, we observed that α5β1-integrins promoted the formation of small adhesions and low RhoA activation, while αVβ3-expressing cells showed large adhesions, thick stress fibers and high RhoA activation. To further analyse these cellular phenotypes, we looked for specific RhoA activators (GEFs). For this purpose, we performed Mass Spectrometry analysis follow by biochemical assays and observed that GEF-H1 activation is αVβ3-integrin dependent.
By bioinformatic analysis using a mRNA dataset (Oncomine) we found high GEF-H1 expression in human breast cancer compared with non-tumoral breast tissue. In addition, high GEF-H1 expression correlated with a lower patient survival (n= 65, p=0.0071). We also observed by immunohistochemistry a significant GEF-H1 over-expression in human breast cancer biopsies compared with normal tissue (n=72, p=0.0201). Furthermore, GEF-H1 protein expression levels correlate with the invasive potential of human and murine breast cancer cell lines. Using CRISPR/Cas9 technology, we generated GEF-H1-knock out (KO) clones in a murine invasive breast cancer cell. We observed a significant decrease in the proliferation, migration and invasion rates in GEF-H1-KO cells (p<0.001) and a correlation with a decrease in focal adhesion formation and signaling.
These results showed that GEF-H1-RhoA activation may mediate the signaling involved in controlling cell structure, proliferation, migration and invasion of breast cancer cells. In addition, the studies in human tumor samples suggest that GEF-H1 might be a potential tumoral biomarker.

253. (135) **NOVEL THERAPEUTIC APPROACHES WITH REPOSITIONED DRUGS FOR TRIPLE NEGATIVE BREAST CANCER TREATMENT**

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Triple negative breast cancer (TNBC) is a particularly aggressive type of breast cancer with poor prognosis and limited therapeutic options. Drug repositioning refers to new uses outside the scope of the original medical indications for existing drugs or compounds. Metformin (M) is an antidiabetic, propranolol (P) is a β-blocker, chloroquine (CQ) is used to treat malaria. We found that M+P combination could be effective for triple negative breast cancer (TNBC) treatment. Our aim was to widen the knowledge of the mechanisms responsible for the effects of M+P treatment and to study the effect of other repositioned drugs combinations on TNBC. We performed an *in vitro* screening of drugs under reposition on TNBC cells and determined that M+P and CQ+P were particularly efficient in reducing proliferation. They showed synergistic effect, affecting significantly apoptosis. M+P reduced phospho Erk levels after serum induction but did not affect the expression levels of the epithelial markers β-catenin and E-cadherin. *In vivo* treatment with M(2g/l)+P(25mg/l) in 4T1 and M-406 models reduced tumor growth and metastasis development, while CQ+P did not show antitumor effect. Evaluation of the effect of M+P on immune cells revealed an increase in intratumor M-406 Tregs (P<0.05). 4T1 tumors showed an increase on $CD4^+$ (P<0.01), Treg (P<0.05) and a decrease in Th17 (P<0.01) cells. Moreover, we proved that M+P affects different steps associated to 4T1 tumor dissemination and decreases the % of $Ki67^+$ cells in lung metastases (P<0.05). We developed an adjuvant model (M+P treatment after tumor excision) and observed that M+P was effective preventing metastasis development (P<0.05). In brief: 1) M+P treatment decreased tumor and metastasis growth affecting several steps of tumor dissemination and it modified intratumor immune cells populations. 2) M+P treatment could be useful for adjuvant TNBC therapy avoiding the toxicity and high costs associated to conventional chemotherapies.

254. (137) **DEVELOPMENT OF LOW GRADE DYSPLASIA IN ANAL SQUAMOUS EPITHELIA UPON SPECIFIC ACTIVATION OF K-RAS IN TRANSGENIC MOUSE SYSTEM**

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Anal SCC is a rare malignancy, accounting for only 4% of all cancers affecting the gastrointestinal tract. However, is one of the most common reported malignancy among men with HIV infection. ASCC treatment is associated with severe disease and treatment-related morbidity, therefore there is an urgent need to develop novel therapeutic approaches. The exact pathogenesis of ASCC remains unknown, the understanding of the molecular mechanisms involved have been hampered by the lack of animal models. Here, we took advantage of the driver line K14-CreER^{tam} that we have previously characterized as a system that targets the basal layer of the squamous epithelia. Our aim was to characterize the expression of the

driver system as well as the consequences of targeting activated *ras* to the anal epithelium. We breed the driver line with Rosa26 reporter animal line and with K-*ras knock in* mice. Tissues from these mice were used to study the phenotype and characterized by immunohistochemistry (IHC). The administration of tamoxifen to reporter mice resulted in the expression of EGFP in epithelia cells of the anal mucosa. K14-CreER^{tam}/K-ras$^{G12D/+}$ mice exhibited an anal phenotype 3 weeks after induction in contrast with a control group of K14-CreER^{tam} (K14-CreER^{tam}/K-ras$^{G12D/+}$: 76 days (median survival). Kaplan–Meier survival curve, p<0,0001). Anal lesions arising in K14-CreER^{tam}/K-ras$^{G12D/+}$ were diagnosed as low grade dysplasia with increased cell proliferation restricted to the basal layer. IHC of the anal lesions showed an abnormal expression of cytokeratins 6 and 14 (differentiation markers) and an increase in pS6 (most downstream target of the PI3K/Akt/mTOR pathway) expression in the upper layers. We conclude that our driver system targets specifically the anal epithelium and the activation of K-ras is sufficient to produce dysplastic changes on it, showing an increase in pS6 expression. These results warrant further analysis of the mechanism underlying the development of ASCC.

255. (138) **DECREASED MIR-133A-3P AND MIR-133B EXPRESSION IN TUMORS FROM HIGH-FAT DIET FED MICE IMPACTS IN PROSTATE CANCER DEVELOPMENT**

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Recent evidence has revealed that high-fat diet (HFD) plays a role in prostate cancer (PCa) development and progression. Our hypothesis is that HFD alters miRNAs expression favoring prostate tumor growth. To assess this hypothesis, C57BL/6J male mice were chronically fed with HFD or control diet (CD). Murine TRAMP-C1 PCa cells were injected s.c on HFD and CD fed mice. After tumor growth, mice were sacrificed, tumors were collected and miRNAs isolated and analyzed by GeneChip® miRNA 4.0 Array (Affymetrix) microarrays. We identified 18 down- and 8 up-regulated miRNAs (FDR<0.05) in tumors from HFD fed mice compared to CD mice. We used DIANA-TarBase v7 of the miRPath tool to identify the miRNA validated target genes. Additionally, KEGG signaling pathways were identified. Target genes for down-regulated miRNAs were involved in cancer-related and lipid metabolism pathways, while those derived from up-regulated miRNAs were more associated with processes related to metabolism of xenobiotics and chemical carcinogenesis. From these data miR-133a-3p and miR-133b emerged as potential tumor suppressor miRNAs. We evaluated the expression of hsa-miR-133a-3p and 133b in PCa compared to normal adjacent tissue (NAT) using the bioinformatic UCSC Xena resource. We found that hsa-miR-133a-3p and 133b were significantly downregulated in PCa compared to NAT and negatively correlated with gleason score. Kaplan Meier curves indicated that lower levels of hsa-miR-133a-3p and 133b correlates with a worse progression free interval. Also, we analyzed the expression of hsa-miR-133a-3p and 133b target genes. We found that BUB1, CENPF, XPO1 and RAN expression were significantly increased in PCa compared to NAT and positively correlated with gleason score. Also, high expression of these genes is associated with a worse progression free interval. In summary, HFD represses tumor suppressor miRNAs expression leading to an increase in oncogenes expression and thereby impacting on PCa development.

256. (140) **ALL-TRANS RETINOIC ACID AND LAPATINIB COMBINED TREATMENT IMPAIR BREAST CANCER STEM CELLS GROWTH AND METASTATIC POTENTIAL**

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Cancer stem cells (CSC) are resistant to chemo and radiotherapies. To validate CSC as therapeutic targets in breast cancer, we analyzed the effect of Lapatinib (Lp, HER2 inhibitor), ATRA or the combined treatments, on growth, cell cycle distribution and metastatic capacity of primary mammospheres (CSC enriched cultures) from HER2 negative cell lines (4T1, MCF-7 and T47D).

We determined by WB that HER2 is overexpressed only in CSC subpopulation of all cell lines analyzed. Primary mammospheres were treated for 96 h with Lp1μM for 4T1; 5μM for MCF-7, 2μM for T47D cells combined or not with ATRA 1μM. ATRA treatment alone or combined with Lp only significantly reduced 4T1 mammospheres diameters ($p^{<}$0.05 Anova test) and signs of cell death were also observed.

The combined treatment induced cell cycle arrest at G0/G1 phase after 48h of treatment in 4T1 mammospheres, analyzed by flow cytometry. However, this combination not significantly induces cell cycle arrest in MCF- 7 mammospheres. Finally, we performed an experimental lung metastasis assay in mice pretreated with ATRA and Lp and observed that combination reduced metastatic potential of 4T1 cells derived from mammospheres ($p^{<}$0.05 Kruskal Wallis).

In the present work we have demonstrated that the CSC component of HER2 negative cell lines overexpress such receptor. Moreover, Lp and ATRA combined treatment can successfully reduce mammospheres growth and metastatic potential in 4T1 experimental model.

257. (141) **BRAFI RESISTANCE INCREASES THE SENSITIVITY TO OXPHOS INHIBITORS OF MELANOMA CELLS**

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Near 50% of melanoma cells harbor BRAFV600E as a driver mutation. BRAFV600E inhibitors (BRAFi) are approved therapy agents for unresectable or metastatic melanoma. Despite their initial efficacy, BRAFi resistance is acquired at 6-8 months in 50% of patients. Metabolic adaptations seem to be implicated in the emergence of BRAFi resistance. In this context, the aim of the present work was to establish a BRAFi resistant melanoma cell line to investigate the response to different metabolic modulators. A375 (a BRAFV600E mutant melanoma cell line) was significant affected by 1 μM GSK2118436 (Dabrafenib, BRAFi) showing a decrease in cell number. Despite this sensitivity, we were able to establish A375 resistant variant (A375-R) by increasing the concentration of GSK2118436 for four months. A375-R presented a significant increase in GSK2118436 and PLX4032 (other BRAFi) IC50s both as monolayers and spheroids culture. Then, we studied the effect of 9 different metabolic modulators: 2-deoxyglucose (2-DG); oxamate; 6-aminonicotinamide (6-AN); metformin (MET); antimycin A; dichloroacetic acid (DCA); methotrexate (MTX); BPTES and Everolimus (RAD001). A375 and A375-R presented similar response to 2-DG, oxamate, DCA and BPTES. A375-R showed significant resistance to MTX and 6-AN compare to A375 (IC50s: >10 *vs* 0.73 μM, p< 0.001). Interestingly, A375-R showed a higher sensitivity to MET and antimycin A than A375 (IC50s: 2.35 *vs* 4.22, p<0.001 and 0.005 *vs* 0.022 μg/mL, p<0.001, respectively). In other words, acquired resistance to GSK2118436 favored OXPHOS inhibitors treatment such as MET and antimycin A. Despite this sensitivity, the combination of MET and GSK2118436 did not present synergistic effect when there were incubated simultaneously. However, sequential treatment with these drugs at high concentrations showed a potentiating effect. Thus, further *in vitro* and *in vivo* combinatory treatments are required to identify the potential benefit of this combinatory strategy.

258. (147) **VALPROIC ACID RADIOSENSITIZE ANAPLASTIC THYROID CANCER CELLS THROUGH A DECREASE OF THE REPARATION CAPACITY AT LOW DOSES OF RADIATION**

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Introduction: Histone deacetylase (HDAC) inhibitors have emerged recently as promising anticancer agents. The antitumor activity of HDAC inhibitors has been linked to their ability to induce gene expression through acetylation of histone and nonhistone proteins. Anaplastic thyroid cancer (ATC) is a rare and aggressive malignancy. Radiotherapy (RT) is one of the main modalities of treatment for ATC. In most patients, surgical resection is not possible. Therefore, RT either as altered fractionation or in combination with chemotherapy has an important role in achieving local control. **Objectives:** The objective was to study the radiosensitizing effect of valproic acid at different radiation doses in an anaplastic thyroid cancer cell line (8505c). **Methods:** Cells were incubated with 1 mM VA and irradiated with a source of gamma rays at different doses. Radiation response was analyzed by clonogenic assay. Cell cycle and cell death were measured 24 and 48 h after irradiation at 2 and 5 Gy. DNA damage was evaluated 30 min and 24 h after irradiation at 2 and 5 Gy. Ku80 expression was assessed by Western Blot. **Results:** A radiosensitizing effect was observed with a reduction of survival fraction at 2 Gy from 0.28 to 0.20 in the treated cells ($p<0.05$). VA treatment increased apoptotic cell death 24 and 48 h after irradiation at 5 Gy ($p<0.001$ and $p<0.05$, respectively). Average γH2AX foci number was increased at 30 min ($p<0.01$ for 2+VA and 5+VA Gy). Average foci size was increased at 24 h at 2+VA Gy ($p<0.01$). AV enhanced the frequency of foci larger than 1.0 μm^2 in cells irradiated with 2 Gy at 24 h ($p<0.01$). Ku80 expression was reduced in the 2+AV group at 30 min compared to 2 Gy. **Conclusion:** These results suggest that VA pretreatment could have an important role in enhancing the effect of radiation in anaplastic thyroid cancer cells. Particularly, the radiosensitizing effect could be mediated by a reduction in the DNA damage repair capacity at low doses (2 Gy).

259. (151) **CRITICAL MOLECULAR MECHANISMS THAT MODIFY BONE MARROW-MESENCHYMAL STEM CELL BEHAVIOUR IN ADVANCED BREAST CANCER PATIENTS**

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Most of untreated advanced breast cancer patients (**BCP**, invasive ductal, stage IIIB) develop osteolytic bone metastasis, due to the existence of a pre-metastatic niche (**PMN**) that favours extravasation, migration, and proliferation of tumor cells. We found that bone marrow (**BM**) mesenchymal stem cells (**MSC**) from BCP have lower cloning, proliferation, and osteogenic differentiation capacities than healthy donors (**HD**) MSC. A conserved pool of functional MSC is essential for preventing the PMN formation.
Thus, our aim was to identify the molecular mechanisms responsible for the loss of function in MSC from BCP. For this purpose, we studied the expression of stemness markers (OCT4, SOX2, hTERT, CD49b, CD146, telomere length), triggers osteogenic differentiation (RUNX2 and BMP2) [qPCR], and the cellular oxidative state (ROS levels) [FACS] in MSC from enriched cultures of BM aspirates from BCP vs HD. We also evaluated the morphologic characteristics [Optical Microscopy], proliferation capacity [Proliferation Assay], and cell cycle profile of MSC [FACS]. Besides, we compared the pool of MSC in BM aspirates [RosetteSep™].
The results indicated that BCP-MSC (n=7) vs HD-MSC (n=7) had: lower expression levels of OCT4 (p=0.026), SOX2 (p=0.008), hTERT (p= 0.004), CD146 (p=0.041) and higher levels of CD49b (p=0.028), RUNX2 (P=0.039) and BMP2 (P=0.033); shortened telomere length (p=0.002); increased ROS levels (p=0.036); decreased proliferative capacity (p=0.03); higher relative proportions of cells in G0/G1 phase (p=0.011) and lower in S phase (p=0.033); lower number of CFU-F (p=0.01); increased area (p=0.001), higher long (p=0.02) and short axis (p=0.001). In addition, BCP vs HD had a poor pool of BM MSC (p=0.006).
In conclusion, the lower expression of OCT4 and SOX2 in MSC, would lead to an ineffective self-renewal, proliferation, and differentiation of these stem cells, providing insights about the molecular alterations in the BCP-MSC, key players in the development of the PMN.

260. (156) **P300 INVOLVEMENT IN APOPTOSIS AND METASTASIS IN TRIPLE NEGATIVE BREAST CARCINOMA (TNBC)**

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Triple-negative breast cancer (TNBC) is a heterogeneous group of tumors that lack specific molecular targets. Therefore, it is necessary to investigate potential tumor markers for this subtype of BC. There are few studies in TNBC on the role of p300 in apoptosis and they are contradictory. Previously, we published that pharmacological inhibition of p300 increases apoptosis in murine BC cells, however, it is necessary to check whether the same effect occurs in human TNBC cells. Recently a prometastatic role of p300 in BC has been found. In addition, we demonstrated that pharmacological inhibition of p300 decreases migration and adhesion in TNBC, showing the need to delve into the molecules involved. Hence, the aim of this work was to study the effect of the inhibition of the acetylase function of p300 in the processes of apoptosis and metastasis in human TNBC cells. The MDA-MB-231 cell line was treated with VV59 (inhibitor of the acetylase function of p300) or its vehicle (DMSO). In a cell viability assay, we observed that the pharmacological inhibition of p300 produced a decrease in the number of cells compared to vehicle ($p<0.001$). When we analyzed the cell cycle by flow cytometry, we detected an increase in the sub G0 phase and a decrease in the G0/G1 phase in the cells treated with VV59 compared to those treated with the vehicle ($p<0.001$). We also observed an increase in apoptotic morphology in VV59-treated-cells compared to vehicle-treated cells by dapi staining ($p<0.001$). On the other hand, by immunofluorescence assay we detected that pharmacological inhibition of p300 induced an increase in the levels of E-cadherin and β-catenin in the membrane, and a decrease in the number of stress fibers compared with the control cells ($p<0.01$). Taken together, these results demonstrate an antitumor role for pharmacological inhibition of p300 acetylase function in TNBC.

261. (157) **QUERCETIN INDUCES CELL DEATH OF COLORECTAL CANCER CELLS OVEREXPRESSING RAC3**

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Cancer Stem Cells (CSC) are the responsible of colorectal cancer (CRC) persistence. We have previously demonstrated that RAC3, a transcription coactivator usually overexpressed in CRC is required for maintaining CSC, with anti-apoptotic and anti-autophagy effect. Moreover, *Aloysia polystachya* (AP) extracts induce the cell death of CRC, *in vivo* and *in vitro.* Quercetin, an agonist of the AHR, was a flavonoid detected at high level as one of the AP components.
In this work, we investigated the effects of quercetin and the probable AHR/CSC/RAC3 relationship in CSC of CRC. The effect of the

flavonoid was investigated performing cytotoxicity assays in the human CRC HCT116wt cell line (overexpressing RAC3) or shRNA-RAC3 stimulated with different concentrations of quercetin for 24h. HEK293 cells (low levels of RAC3) were used as a non-tumoral control. The quercetin pathways and AHR/CSC/RAC3 relationship were investigated by bioinformatics studies using public repository microarrays data from the human CRC CaCo-2 cells, CD133+ or CD133– side populations, from rat CRC and from human CRC samples from TCGA, using Xena, ConsensusPathDB and STRING platforms. We found that quercetin induced a significant increased cytotoxicity of HCT116wt respect to shRNA-RAC3 HCT116 and HEK293 ($p<0.05$; Tukey test). High expression levels of AHR and RAC3 were mainly associated to the CD133+ side population ($p<0.01$; Tukey test). The AHR expression was associated to CD133+ and both were significantly increased in CRC (598 samples) respect to normal tissues (GTEX, 308 samples) (Welch´s t-test p=7.14e-20, and p=2.297e-26, respectively). The overrepresentation analysis of genes induced by quercetin demonstrated that at least apoptosis and autophagy pathways were significantly affected ($p<0.01$). Therefore, the increased sensitivity of CSC to cell death induced by quercetin could be a consequence of enhanced AHR expression, where perhaps RAC3 could be an AHR coactivator.

262. (171) **OPTIMIZATION OF THE BLOOD VESSEL NORMALIZATION PROTOCOL USED FOR BNCT (BORON NEUTRON CAPTURE THERAPY) STUDIES IN AN EXPERIMENTAL ORAL CANCER MODEL**

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Introduction: BNCT combines preferential tumor uptake of ^{10}B compounds and neutron irradiation. Homogeneous boron targeting to all tumor cell populations contributes to BNCT success. However, tumor blood vessels are structurally abnormal, affecting the distribution of the boron agent in the tumor. We demonstrated, in the hamster cheek pouch oral cancer model, that thalidomide (Th) induced a transient aberrant blood vessel normalization, improved boron distribution in tumor and BNCT therapeutic effect. However, Th is highly soluble in dimethilsulfoxide (DMSO) which negatively affects animal´s welfare. In this study we aimed at optimizing the previously published Th protocol, increasing the animal´s welfare. **Materials & methods:** Cancerized hamsters were subjected to 2 intraperitoneal injections on 2 consecutive days of: (A) Th as previously published: 112 mg/ml DMSO (n=3 animals); (B) 100 mg Th/250 μl DMSO+meloxicam (anti-inflammatory drug)+lidocaine (local anaesthesia) (n=4); (C) same as (B), no meloxicam, no lidocaine (n=3). Aberrant blood vessel normalization was assessed by a double-blind macroscopic study in the precancerous tissue surrounding tumors. Animal clinical signs and tumor response were evaluated. **Results:** All protocols exhibited transient blood vessel normalization in the precancerous pouch tissue. 1 out of 3 animals died in (A). The animals exhibited pain, internal bleeding, intestinal inflammation and adherences. In (B), meloxicam reduced the intestinal inflammation and lidocaine reduced the pain due to Th injection vs (C) (p=0.0286). Ongoing studies (n=10) showed 10% mortality rate and 84% of tumor reduction after (B). **Conclusion:** We optimized the Th protocol used for our BNCT studies in terms of the animal's welfare. It induced blood vessel normalization in precancerous tissue and reduced tumor volume. This would improve boron distribution and benefit BNCT therapeutic effect. **Acknowledgments:** To Med. Vet. P. Oña; Dr. E. Kreimann; Lazar laboratory.

263. (180) **RUNX2 OVEREXPRESSION GENERATES ENDOCRINE RESISTANCE IN HUMAN BREAST CANCER CELLS GROWING *IN VIVO*.**

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It has been hypothesized that FGF2 increases RUNX2 and RUNX2, in turn, increases FGF2 expression, maintaining a positive loop. We have shown that T47D and IBH6 cells overexpressing RUNX2, express high levels of FGFR2 and FGF2, supporting this hypothesis. However, RUNX2 tumors are resistant to FGFR inhibitor therapy showing a more aggressive phenotype compared with control tumors. We are exploring the role of FGF2-FGFR-RUNX2 axis and its relationship with hormone receptors in BrCa.

The aim of this work was to evaluate endocrine therapy in RUNX2 overexpressing tumors.

T47D and IBH6 cells express ER, PR and FGFR1-4. RUNX2 and control cells (C, empty vector) were injected into the flank of NSG mice. Animals were treated for 3 weeks with an antiestrogen (Fulvestrant FUL, 0.5mg/week) or an antiprogestin (Mifepristone MFP, 6mg pellets). Control tumors showed a significant growth inhibition with the therapy (C-T47D $p<0.0001$ C vs FUL and MFP; C-IBH6 $p<0.0001$ C vs FUL), a lower Ki67 index (C-T47D: $p<0.0001$ C vs FUL, $p<0.05$ C vs MFP,) and higher stromal remodeling compared with untreated tumors. RUNX2-T47D and -IBH6 tumors were resistant to endocrine therapy and all animals bearing RUNX2-T47D tumors developed lung metastasis. RUNX2-IBH6 responses to MFP remains to be assessed.

We conclude that RUNX2 promotes BrCa progression and plays a role in the acquisition of endocrine resistance. Further studies are required to elucidate the role of FGF2/FGFR2 in the establishment of this phenotype. Our results emphasize the development of RUNX2 inhibitors to use in combination with standard therapy for BrCa treatment.

264. (184) **GLUTAMINE ADMINISTRATION COMBINED WITH BORON NEUTRON CAPTURE THERAPY MEDIATED BY BPA IMPROVED TUMOR CONTROL IN AN EXPERIMENTAL MODEL OF ORAL CANCER**

Santa Cruz IS[1], De Leo LN[1], Ramos PS[1], Goldfinger JA[1], Thorp S[2], Curotto P[3], Pozzi ECC[3], Hsiao MC[4], Palmieri MA[5], Garabalino MA[1], Trivillin VA[1;6], Schwint AE[1;6], Monti Hughes A[1;6]

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Introduction Head and neck cancer patients are naturally depleted of Glutamine (GLN) and this deficit is accentuated by radiotherapy/radiochemotherapy side effects. Other authors previously demonstrated that GLN significantly reduced mucositis in patients treated with chemotherapy and/or radiation, accelerated the healing process in the hamster oral mucositis model and prevented DMBA-induced squamous cell cancer in the hamster cheek pouch. BNCT combines preferential tumor uptake of ^{10}B compounds and neutron irradiation, selectively destroying the tumor cells while preserving the normal cells. Our group assessed different compounds as an adjunct to BNCT to improve therapeutic efficacy and/or reduce toxicity in the hamster cheek pouch oral cancer model. The aim of the present study was to evaluate the potential capacity of GLN to reduce BNCT-induced mucositis and/or enhance therapeutic efficacy in

this model. **Materials and methods** Tumor bearing hamster cheek pouches were exposed to BNCT mediated by boronophenylalanine (BPA) at 2.6 Gy absorbed dose to precancerous tissue +/- GLN (joint topical and oral administration; 1 g/kg body weight per day during follow-up) at two different pH (pH 5 and pH 7). The animals were followed during 28 days after BNCT. **Results and Conclusion** In this pilot study GLN was not toxic for the animals. Although GLN did not reduce mucositis, we observed a tendency for both GLN protocols to improve BPA-BNCT tumor complete response (CR) (reaching statistical significance for GLN-BNCT pH 7 at 7 days, Fisher's exact test) with similar overall responses (OR) vs BPA-BNCT alone, at 7 and 14 days after BNCT. At 21 and 28 days post irradiation, this tendency was still exhibited by BNCT+GLN at pH 7, showing a higher CR (60%) vs BNCT alone (45%) and BNCT+GLN pH 5 (53%), with similar OR (90% and 80%) vs BNCT alone (93%). Future studies will assess if GLN increases BPA uptake in tumor and the role of the pH of the GLN solution in BNCT+GLN tumor response.

265. (195) **BORON NEUTRON CAPTURE THERAPY (BNCT) EFFECT IN NADPH OXIDASES NOX1-NOX5 AND GALECTIN-1.**

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Boron neutron capture therapy (BNCT) is based on the radiation from the nuclear reaction ^{10}B (n, α) ^{7}Li and there is not much known about direct or indirect effects of the therapy that contribute to tumor cell survival, for example, ROS production or the immune system response. The aim of this work was to study the contribution of NADPH oxidases (NOX) in the generation of ROS and the expression of galectin 1 in HT-29 colon carcinoma cells after the irradiation with a neutron beam without (N) and with boron (BNCT). **Metodology and results**: HT-29 colon carcinoma cells incubated with 50 ppm of BPA (BNCT) or without BPA were irradiated with a neutron beam (3 and 8 Gy) at the RA-3 nuclear reactor. After 24 hours or 4 days, the supernatants of the irradiated cells were collected and used as conditioned medium for migration (wound) and cell proliferation (MTT) assays. Total RNA was extracted from the irradiated cells and the expression of NOX 1 and 5 and galectin-1 was measured (PCR). **Results:** Irradiation with 3 Gy of neutrons (3N) after 24 hours inhibited the migration of non-irradiated cells while at 8 Gy (8N) migration was induced. After 4 days, neutron irradiation at both doses induced migration and pre-incubation with BPA reduced this effect (3 and 8 BNCT). BNCT treatment (8 Gy) induced proliferation after 24 hours while 4 days later there was no effect. We observed a decrease in galectin-1 expression in the first 24 hours post-irradiation while after 4 days the expression increased in 3 BNCT and 8N groups. The expression of NOX1 and NOX5 enzymes increased at both doses, with or without BPA. **Conclusion:** Increasing the dose radiation will not necessarily lead to a therapeutic improvement as it is demonstrated here that pro or antitumor effects are achieved independently.

266. (199) **6-IODOLACTONE INHIBITS ANGIOGENESIS IN COLORECTAL CANCER**

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Several studies have shown the beneficial potential of iodine and 6-iodolactone (IL-δ) in different cancer pathologies. It was demonstrated that molecular iodine (I_2), but not iodide (I) and IL-δ exerts anti-neoplasic actions on diverse tissues. The anti-neoplasic effect of iodine may be due to the synthesis of intracellular iodolipids. The underlying mechanism through which IL-δ inhibits tumor growth remains unclear. The aim of this study was to analyze the effect of IL-δ on tumor growth and angiogenesis in human colorectal cancer HT-29 xenograft. **Methodology and results**: HT-29 cells were injected subcutaneously into the flanks of nude mice and IL-δ was i.p. injected daily at a dose of 15 µg. IL-δ treatments in HT-29 xenograft showed time-dependent inhibition of tumor growth. IL-δ induced a significant decrease of PCNA ($p<0.05$) and an increase of p27 expression after 18 days of treatment ($p<0.05$). To assess tumor microvessel densities we analyzed CD31 staining by immunohistochemistry. IL-δ treatment decreased microvessel density by 17 % and 30% ($p<0.01$) after 18 and 30 days respectively. We analyzed VEGF and VEGF R2 mRNA and protein expression by real time PCR and immunohistochemistry respectively. IL-δ decreased VEGF-R2 mRNA by 23 % ($p<0.01$) after 30 days of treatment and also decreased the percentage of VEGF and VEGF-R2 stained cells by 73 and 70 % respectively after 30 days of treatment ($p<0.01$). Real time PCR revealed that IL-δ treatment increased VEGF-R1 and Ang-1 mRNA expression ($p<0.01$). **Conclusion:** these results demonstrated the anti-angiogenic effect of IL-δ. The mechanism involves a combination of VEGF and VEGF-R2 expression decrease with Ang-1 increase that would contribute to mature vessels stabilization and maintenance while VEGF R1 increase would produce anti-proliferative effect on endothelial cells.

267. (200) **PROGESTERONE PROMOTES TUMOR METASTATIC DISSEMINATION THROUGH RANKL+ FOXP3+ REGULATORY T CELLS IN TRIPLE-NEGATIVE BREAST CANCER**

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Hormone supplementation with progestins has shown a higher incidence and aggressiveness of mammary tumors. In this work, we set out to address the effect of progesterone (Pg) and its synthetic analogues on tumor progression. For this we used the 4T1 triple negative breast cancer tumor model in BALB/c mice. The administration of Pg, Medroxyprogesterone (MPA) and Norgestrel (Ng) did not impact tumor growth kinetics. However, we observed an increase in metastatic spread ($p<0.05$) that was not reversed by the antiprogestin Mifepristone. FACS analysis of the tumor microenvironment revealed an increased frequency of Foxp3 + regulatory T cells ($p <0.05$) and CD8 T lymphocytes with dysfunctional PD-1 + TIM3 + phenotype ($p <0.05$). In vitro tests showed that progestins favored the differentiation and expansion of Tregs independent of the nuclear Pg receptor ($p <0.01$). In vivo Tregs depletion using DEREG mice drastically suppressed lung metastasis formation and the prometastatic effect of Pg ($p<0.01$). In contrast, adoptive transfer of Pg-educated Tregs to DEREG mice favored lung metastases ($p<0.05$). Mechanistically, we observed that progestins induced RANKL expression in Tregs cells, which increased the invasiveness of 4T1 cells ($p<0.05$) and a RANKL-dependent epithelial-mesenchymal transition transcriptional program, with increased expression of Snail and Vimentin and a decrease in E-cadherin and Maspin ($p <0.05$). Finally, treatment with a RANKL blocking antibody decreased Pg-induced metastatic spread ($p <0.01$). These results describe an unknown mechanism by which Tregs cells stimulate metastatic spread through RANKL, induced by progestins. Finally, we also identified RANKL as a mediator of the metastatic cascade in breast cancer, which represents a novel therapeutic approach.

268. (206) **DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES INTO BEIGE ADIPOCYTES CONTRIBUTE TO BREAST CANCER PROGRESSION**

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Crosstalk between breast cancer cells and adipose tissue in the mammary microenvironment plays a key role in the regulation of tumor behavior. We previously demonstrated morphologic and metabolic changes in adipocytes indirectly cocultured with breast cancer cells, suggesting a first step towards browning of adipose tissue, which is an important contribution to the hypermetabolic state of breast cancer. Thus, the aim of study was to obtain insight into the effect of epithelial cell-beige adipocyte communication on tumor progression. Here, we characterized components present in conditioned media (CMs) from beige adipocytes (BA) or white adipocytes (WA) achieved upon differentiation of 3T3-L1 preadipocytes, and evaluated the effects of BA- and WA-CMs on both adhesion and migration of tumor (LM3, 4T1 and MC4-L1) and non-tumor (NMuMG) mouse mammary epithelial cell lines. Additionally, we analyzed the expression of ObR, CD44, vimentin, MMP-9, MCT1 and LDH on tumor and non-tumor mouse mammary epithelial cell lines incubated with BA-, WA-CMs or Ctrol-CMs (control). 3T3-L1 preadipocytes differentiated into beige adipocytes upon PPARγ activation (rosiglitazone), displaying characteristics that morphologically resembled brown/beige adipocytes. Levels of UCP1, CIDEA, GLUT4, leptin, MCT4 and FABP4 were increased while adiponectin, caveolin 1, and perilipin 1 levels were decreased in BA with respect to WA. Tumor cell lines revealed lower cell adhesion ($p<0.01$) and increased cell migration ($p<0.05$) after incubation with BA- and WA-CMs vs Ctrol-CMs. ObR and MMP-9 protein levels in MC4-L1 cells were significantly increased ($p<0.05$) after incubation with BA-CMs vs WA- and Ctrol-CMs. In addition, MC4-L1 and LM3 cells significantly increased their migration ($p<0.05$) in the presence of beige adipocytes. These results suggest that beige adipocytes secrete soluble factors that regulate the behavior of both tumor and non-tumor mouse mammary epithelial cells, favoring tumor progression.

269. (212) **PERIRENAL WHITE ADIPOSE TISSUE BROWNING, CONTRIBUTES TO TUMOR DEVELOPMENT IN KIDNEY CANCER**

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There are white, brown and beige adipocytes with different functions. Beige adipocytes are located in white adipose tissue (AT) and could be derived from pre-existing adipocytes that, when properly stimulated, undergo a process known as browning. Recent studies shows that AT associated with breast cancer suffer browning and contribute to tumor development. The factors and signaling pathways responsible for the browning process are unknown, as well as the role of the adipose tissue browning could have on the tumor. We recently demonstrated that human AT around kidney tumor (hRAT), showed a differential protein expression profile respect to AT from normal kidney (hRAN). In this work, we study the browning process in AT in hARTh and hRNA, evaluating the white and brown AT markers expression. The ATs were obtained from patients with tumor kidney (hRAT, n=21) and kidney donors (hRAN, n=20). The AT fragments were lysed and total proteins were obtained and 1) UCP1, TBX1, PPARγ, c/EBPα, PGC1α, adiponectin and leptin were quantified by western blot; and 2) UCP1, PGC1α and HSP by immunohistochemistry. In addition, we incubated hRNA fragments with conditioned media (CMs) from tumor and non-tumor human renal epithelial cell lines for 48h, the tissues were lyzed and UCP1, TBX1 and PGC1α proteins were measured. Statistical differences among the groups were evaluated by one-way ANOVA with Tukey´s *post hoc* tests. We found UCP1, TBX1, PPARγ, c/EBPα, PGC1α and leptin significantly increased in hRAT *vs.* hRAN explants ($p<0.05$). In addition, perilipin (mature adipocyte marker) was significantly decreased in hRAT *vs.* hRAN ($p<0.05$). Furthermore, UCP1, TBX1 and PGC1α expression were significantly increased in the hRAN incubated with tumor cells-CMs *vs.* hRAN incubated with non tumor cells-CMs ($p<0.05$). This would indicate that the renal tumor cells secrete soluble factors stimulating the surrounding adipose tissue browning. The AT browning could fulfill a regulatory function on the tumor.

270. (213) **BRACHYURY (BRACHY) AND INSULIN LIKE GROWTH FACTOR RECEPTOR I (IGF1R) EXPRESSION IN THYROID NODULAR PATHOLOGY**

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In pediatrics, thyroid tumor stratification is difficult to assess. Epithelial-mesenchymal transition (EMT), plays a role in tumor development. In human carcinomas Brachy has been identified as a regulator of EMT associated to malignancy. To date, no information about Brachy and IGF1R expression in pediatric thyroid nodular disease is available. **Aim:** To evaluate Brachy and IGF1R expression in thyroid nodular samples from pediatric patients and to study the effect of Brachy overexpression in a thyroid papillary carcinoma (TPC) cell line in vitro. **Methods**: Paraffin-embedded samples from pediatric patients with Thyroid Papillary Carcinomas (TPCa), Follicular Adenomas (FA) or Benign Thyroid Nodular disease (BTN) were processed for Brachy and IGF1R immunostaining. TPC cells were used to obtain clones overexpressing Brachy (TPC. 50 and TPC. 150). Gene expression was quantified by rqPCR. Proliferation assays (6 days) and wounding assays (24h) were carried out. Protein extracts were obtained from whole lysates and processed by western blot (WB). **Results**: 43 samples were analysed, 10 from BTN. Only TPCa and FA showed positive staining for Brachy (16/25TPCa;5/8FA) and IGF1R (12/25TPCa;3/8FA). In carcinomas, positivity for IGF1R was only detected when Brachy was present. Brachy overexpression in TPCcells (TPC50) increased proliferation compared to parental cells (**$p< 0.005$). Cell migration was also higher for Brachy overexpressing cells (**$p< 0.005$ TPC50 vs TPC), e-cadherin expression was diminished, and mesenchymal markers (vimentin-fibronectin) were increased. Finally, WB studies showed that Brachy overexpression was related to a higher IGF1R expression in TPC50 cells in comparison to parental cells. **Conclusion:** Brachy expression was associated to IGF1R expression in thyroid carcinomas. In vitro, Brachy overexpression had an impact on IGF1R expression, increased proliferation and migration. These results suggest a potential role for Brachy in the biology of thyroid tumors.

271. (216) **UNDERSTANDING THE REGULATORY CONTEXT OF FGFR3 ALTERED BLADDER TUMORS, KEY TRANSCRIPTION FACTORS AS NEW POSSIBLE THERAPEUTIC TARGETS**

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Currently bioinformatic studies aim to understand the underlying mechanisms in bladder cancer (BC). FGFR3 is the most common alteration in BC, its oncogenic potential has been demonstrated and target therapies have been developed. However, like other target therapies it is expected that patients develop resistance. The identification of additional therapeutic options to use concomitant or se-

quentially with these ones could help in this matter. In this context, our objective is to identify transcription factors (TFs) that could be new potential targets in FGFR3-altered (FGFR3*) bladder tumors. Using a BC gene regulatory network, publicly available data and our transcriptomic data we obtained the activation profile of the TFs activated in an FGFR3* context, we identified FOXM1 and p63 as essential and highly active TFs. We generated a p63 target gene signature from MGHU3 cells by ChIP-seq integrated with the siTP63 RNA-seq expression profiling. Consistently with our previous experimental results, gene ontology enrichment analysis revealed that p63 positively mediates cellular processes such as migration, invasion, proliferation, and represses cell death. We demonstrated that altered FGFR3 regulates p63 at mRNA and protein level by using a panFGFR inhibitor (PD173075) in human FGFR3* BC cell lines. Analysis of mRNA levels in human bladder tumors showed a correlation between FGFR3 and TP63 expression in both Non muscle invasive BC (r=0.57,p=6.59e-10) and muscle invasive BC (r=0.50,p=1.34e-07) FGFR3*. We also probed that inhibition of FGFR3 blunted the carcinogenic program carried out by p63 diminishing migration and proliferation at equivalent levels than specific silencing of p63 in BC cells. In summary, we identified p63 a new possible target in FGFR3* BC, where we demonstrated the FGFR3 regulation over the protumoral TF p63. Additionally, we validated the gene regulatory network through experiments and reinforced our previous results with bioinformatics.

272. (217) **EFFECT OF YERBA MATE IN A MOUSE MODEL OF COLORECTAL CARCINOGENESIS**

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Colorectal carcinogenesis (CRc) is a process that stems from genetic mutations induced by physical or chemical agents. Epidemiological studies suggest that the consumption of some phytochemicals of the diet could directly affect the development of the disease. Historically, there have been mixed positions in what concerns yerba mate's pro or anti carcinogenic role. In 1991, the IARC classified hot mate as a probable carcinogenic beverage. In 2016, IARC again evaluated the carcinogenicity of mate under the hypothesis "It is the temperature of the water and not the plant that is associated with cancer". Due to insufficient evidence, cold mate could not be classified. There are no studies that evaluate the relation between consumption of cold mate and the carcinogenesis process. The aim of our research was to evaluate whether the consumption of cold mate modulated CRc.

To establish the model, we used 1,2-Dimethylhydrazine (DMH). Cold beverages of yerba mate extract (YMe) in a dose of 1.6 gr/kg/day and Maltodextrin (MD - excipient of extract), were administered via the drinking water. Male and female BALB/c mice were injected with DMH (20 mg/kg/week) subcutaneously once a week for 14 weeks. Fourteen weeks after the last DMH injection, the animals were sacrificed. After extracting the colons, polypoid lesions were counted. Then, colon´s mice were examined histologically. There was a correlation between macroscopic and histologic results. In female mice we observed that 25% of the control group (MD + DMH) developed tubulovillous adenomas while, in the treated group (YM + DMH) all animals presented normal mucosa. In male mice, 37.5% of the MD + DMH group and 42% of the YM + DMH group showed adenomas. It is the first study that reports, on the one hand, that cold mate is not a carcinogenic beverage and on the other hand that chronic consumption of YMe would have a preventive role in CRc in female mice. Further studies are needed to understand sex-related difference.

273. (219) **YERBA MATE (*Ilex paraguariensis*) INDUCES APOPTOSIS IN MURINE COLON CANCER MODELS; THE INTRINSIC PATHWAY AS A POSSIBLE MECHANISM**

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Colorectal cancer (CRC) is one of the so-called westernized diseases and is the third most common cancer in both men and women. In a previous work, we reported that yerba mate extract (YMe) from *Ilex paraguariensis,* a native South American tree which has a large amount of bioactive compounds, inhibits CT26 cell proliferation by induction of apoptosis. The aim of this research was to determine the mechanism by which apoptosis is induced by YMe. To this end, *in vitro* and *in vivo* experimentation was carried out using CRC models.

The mechanism of cellular apoptosis has an initiation and an execution phase. The initiation phase has two possible origins: the extrinsic or intrinsic pathway. It is reported that a key point in the intrinsic pathway of cellular apoptosis are mitochondrial permeabilization processes, which are regulated by members of the Bcl-2 protein family. *In vitro*, we investigated the expression of the Bcl-2 protein using CT26 cells. Western blot analysis showed that the level of anti-apoptotic protein Bcl-2 was decreased in YMe treated cells. Immunofluorescence analyses also revealed a similar result. *In vivo*, using a murine syngeneic tumor model, we showed that oral administration of YMe significantly inhibited tumor growth. The tumor growth rate is the result of the balance between the proliferation and the induction to apoptosis of the neoplastic cells. Therefore, to determine whether this reduction in tumor growth was due to an increase in cell apoptosis, a TUNEL assay on the tumor section was performed. In agreement with what was observed *in vitro*, the TUNEL assay demonstrated that consumption of YMe increased apoptosis in cells in tumor tissues. *In vitro* and *in vivo* results suggest that YMe induces apoptosis by the intrinsic pathway; however, further studies are needed to confirm this assumption.

274. (228) **META-ANALYSIS OF MRP4/ABCC4 TO DETERMINE HOT SPOTS FOR CRISPR-Cas9 EDITING DESIGN TO STUDY THE REGULATION OF ITS NUCLEAR LOCALIZATION.**

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Recent findings show that high MRP4 levels are critical for pancreatic ductal adenocarcinoma (PDAC) cell proliferation and associated with a more aggressive phenotype. We have demonstrated the presence of MRP4 in the nucleus in pancreatic human PDACs tissues. Also, we have observed this MRP4 novel localization in both hepatic and pancreatic cancer cell lines, using techniques such as western blot prior to subcellular fractionation and confocal microscopy. The aim of the present work is to make a computational analysis to study possible sequences to be targeted with CRISPR-Cas 9 to study the mechanism underlying this novel localization. Our analysis of the complete protein sequence, consisting in 1325 amino acids, using cNLS Mapper SOFTWARE found a nuclear localization sequence (NLS) located from I33 to D42 (IGHKRRLEED) with a score of 7 that corresponds to proteins partially located in the nucleus. Bibliographic recopilation describes post-translational modifications, as phosphorylations, near NLS regions that could regulate the possibility of interaction between NLS and the proteins involved in the transporting. The analysis of the sequence described that the Y45 is a phosphorylation site linked evolutively with a conserved site of ubiquitination located in K1278, closely to the previously described PDZ domain (ETAL[1325]). It is known that this domain is decisive for its location in the plasma membrane due to its interaction with adapter proteins such as NHERF1, NHERF3 or MPP1. In conclusion, we propose to explore the effect of three different CRISPR cas9 constructions on the transport of MRP4 to the nucleus. The first one

will be focused on producing changes in the NLS sequence without changing the reading frame and the other two to modify punctually Y45 near NLS and the K1278 near PDZ domain.

275. (235) **COMPARATIVE EFFICACY OF COMBINED TREATMENTS USING METRONOMIC CHEMOTHERAPY (MCT) PLUS REPOSITIONED DRUGS (RD) ON A MURINE TRIPLE NEGATIVE MAMMARY ADENOCARCINOMA**

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MCT consists of the chronic, equally spaced administration of drugs at low doses without extended rest periods. RD are compounds originally formulated for other indications that showed antitumor potential. Previously, we inoculated the triple negative (TN) mammary tumor M-406 in syngeneic CBi mice and studied, separately, the effect of four combined MCT schemes administered in the drinking water: **A)** Cyclophosphamide (Cy)30 mg/kg/d + celecoxib 30 mg/kg/d, **B)** Cy 20 mg/kg/d + metformin (M) 400 mg/kg/d **C)** M 400 mg/kg/d + propranolol 7 mg/kg/d, **D)** Cy 25mg/kg/d + losartan 150 mg/kg/d. Our aim was to compare the results of MCT therapeutic effect for all the drugs combinations, in order to identify the most effective treatment. The % of reduction of tumor volume respect to the control group without treatment [median (range), **A:**77.9(50.9-89.9), **B:**82.3(59.9-93.4), **C:**61.4(31.9-70.8), **D:**94.9(80.5-99.8)] differed significantly among treatments (nonparametric ANOVA P<0.001); **C <B** and <**D** (Dunn's test P<0.05 and P<0.001 respectively). The % of reduction of the total lung metastatic volume [**A:**98.8 (97.1-99.2) **B:**72.9 (50.6-100), **C:**60.0 (16.5-88.2, **D:**76.5 (76.5-100)] differed among treatments (P<0.05), **A** >**C** (P<0.05). Besides, the difference in the % of increase of survival [**A:**56.5 (-4.3-56.5) **B:**66.7 (50.0-66.7), **C:**41.7 (12.5-58.3), **D:**77.8 (17.3-149.7)] was significant (P<0.05), **B** <**D** (P<0.05). The four treatments did not cause weight loss and showed normal toxicity related signs. As a result, **B** and **D** showed the bests antitumor effects, the antimetastatic effect was similar for **A**, **B** and **D**. Moreover, **D** produced the higher effect on increasing survival. We conclude that cyclophosphamide + losartan was the most effective of the four schemes. The other advantages of this drug combination as lack of toxicity, oral administration and low cost, favors its quick translation to the clinic for the treatment of the TN mammary cancer, an aggressive subtype with scarce therapeutic options.

276. (236) **ROLE OF HYDROXYMETHYLGLUTHARYL-COENZIME A REDUCTASE (HMGCR) IN THE GENERATION OF STEM CELL STATES IN BREAST CANCER.**

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Alterations of lipid metabolism are important players in tumor progression, including the generation and maintenance of cancer stem cell (CSC) states. Here, we addressed whether the rate limiting enzyme in cholesterol biosynthesis, HMGCR, was associated with stem cell phenotypes. First, we analyzed HMGCR expression by RT-qPCR in the pluripotent stem cells lines WA-09 and FN2.1, in several CSCs lines derived from glioblastomas and in a breast cancer (BC) stem-like state with HMGCR overexpression, generated in our lab (MCF-7/CR). With the exception of two of the glioma CSCs, the rest of the cell lines showed increased levels of HMGCR when compared to MCF-7 BC cell line. To further determine the role of HMGCR in the generation of stem states in BC, we infected MCF-7 cells with lentiviral vectors expressing the Yamanaka reprogramming factors and obtained four clones, termed MCF-7/Rep clones #3, #5, #6 and #9. We analyzed the expression of the pluripotency factors Oct4, Sox2 and Nanog by qRT-PCR, using an iPSCs cell line (FAD) as a positive control. All MCF-7/Rep cells showed increased levels of Sox2, up to 10-fold (MCF-7/Rep clone #3) when compared to MCF-7 parental cells. Oct4 was increased up to 10-fold in MCF-7/Rep #9 cells to levels comparable to those observed in FAD iPS cells. Immunofluorescent detection of Sox2, Oct4 and Nanog in two clones (MCF-7/Rep #3 and #9) corroborated the observations found at the transcriptional level. There was no expression of the pluripotency surface markers SSEA-4, TRA-1-60 and the alkaline phosphatase assay was negative. Interestingly, HMGCR expression in the MCF-7/Rep cells was decreased when compared to their parental counterpart. These results suggest that the MCF-7/Rep clones may be intermediate states between a cancer cell and a *bona fide* pluripotent cell, and that, while HMGCR expression is unstable during reprogramming, is associated with well-established stem cells phenotypes in transformed and non-transformed cells.

277. (253) **TRANSLATIONAL STUDY OF BNCT MEDIATED BY BPA+GB-10 USING OLIGO- FUCOIDAN AND GLUTAMINE AS ADJUNCTS TO IMPROVE EFFICACY AND REDUCE RADIOTOXICITY**

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Introduction Boron Neutron Capture Therapy (BNCT) combines selective tumor uptake of ^{10}B compounds and neutron irradiation. Oligo-Fucoidan (O-Fuco), a seaweed extract, has anti-inflammatory and anticancer activities. Glutamine (GLN) reduced the radio/chemotherapy induced dermatitis in patients and inhibited tumor development in an experimental oral cancer model. The aim of the present study was to evaluate the therapeutic efficacy and radiotoxic effects of (BPA+GB-10)-BNCT alone or combined with Oligo-Fucoidan or Glutamine. **Materials and methods** 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 (BPA+GB-10)-BNCT (Comb-BNCT) at the RA-3 Nuclear Reactor. Experimental groups:
a- **Comb-BNCT**: borophenylalanine (BPA) 31 mg ^{10}B /kg bw + Decahydrodecaborate (GB-10) 34 mg ^{10}B/kg bw, i.v.
b- **Comb-BNCT+O-Fuco:** same as (a) + Oligo-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- **Sham:** same manipulation, no treatment.
Results and Conclusions 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 57% for Comb-BNCT alone, 80% for Comb-BNCT + GLN and 100% for Comb-BNCT + O-Fuco. The incidence of severe dermatitis at two weeks (when the peak occurs) was 100% for Comb-BNCT alone, while O-Fuco reduced incidence to 80 % and GLN reduced incidence to 40%, the latter reduction being statistically significant vs. Comb-BNCT alone (p<0.05).
(BPA + GB-10)-BNCT is therapeutically effective. Oligo-fucoidan and GLN used as adjuvants would improve therapeutic efficacy and reduce radiotoxicity.

278. (255) **EFFECT OF PACLITAXEL IN COMBINATION WITH THE BETA2 ADRENERGIC AGONIST SALBUTAMOL ON**

BREAST CANCER MDA-MB-231 CELL LINE GROWING IN VIVO. MECHANISM OF ACTION

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Breast cancer is the leading cancer death cause of women in Argentina. Beta2 adrenergic receptors have been described in several breast cancer cell lines and tumors. We have previously described (SAIC 2016, abstract 723) that the beta2 adrenergic agonist salbutamol exerted in vitro a synergic effect with paclitaxel inhibiting cell proliferation. The aim of the present study was to assess the in vivo effect of the combination of paclitaxel and salbutamol in the triple-negative MDA-MB-231 cell line growing in nude mice. Both salbutamol (daily sc 1.2 mg/kg/day) and paclitaxel (10 mg/kg/week, ip in two injections) significantly inhibited tumor growth. Moreover, their combination inhibited very significantly this parameter. For example, day 63 (mean ± SEM in mm2): control tumors: 46,5 ± 9,70, n=8; paclitaxel: 32.4 ± 6,40, n=7, $p<0.05$; salbutamol 29.3 ± 7.58, n=7, $p<0.05$. Paclitaxel + salbutamol 9.33 ± 2.45, n=9, $p<0.0001$ against control, $p<0.05$ against each individually. The coefficient of drug interaction indicated synergism since the value obtained is < 1 (0.010, $p<0.0005$ in interaction by two-way ANOVA).

In order to assess the mechanism of action, several in vitro experiments were performed. Paclitaxel diminished cell viability (trypan blue essay), while salbutamol diminished pERK/ERK ratio (using WB) and the expression of cyclin D1 gene (RT-qPCR). Moreover, both drugs enhanced apoptosis (acridine orange ethidium bromide staining assay). In preliminary results, salbutamol completely reversed the increase of MDR1 (ABCB1) expression induced by paclitaxel. This could account for the synergic effect of both drugs. These results suggest that the doses of chemotherapeutic drugs administered to patients in some triple negative (claudin-low) tumors could be reduced in the presence of salbutamol, diminishing their toxicity.

279. (264) **DIRECT AND VASCULATURE-MEDIATED MECHANISMS OF ACTION OF [V^{4}Q^5]DDAVP VASOPRESSIN ANALOG: IMPLICATION OF VON WILLEBRAND FACTOR (VWF) IN BREAST AND COLORECTAL CANCER GROWTH**

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[V^{4}Q^5]dDAVP is a second generation vasopressin analog which acts as a selective agonist of AVPR2 receptor present in tumor and endothelial cells. Besides triggering cytostatic mechanisms in malignant cells, AVPR2 stimulation in microvascular tissue favors the release of VWF, a multifunctional protein involved in hemostasis and different aspects of tumor biology. Our aim was to explore the potential implications of VWF in the antitumor activity of [V^{4}Q^5]dDAVP using breast and colorectal cancer models. First, *in vivo* hemostatic activity of [V^{4}Q^5]dDAVP was assessed in New Zealand rabbits, in which administration of clinically relevant single doses of the compound (0.3-5.0 μg/kg i.v.) was associated to a 45% reduction in aPTT clotting times and a 20-70% increase in VWF:Ag and tPA plasma levels ($p<0.05$). Breast (MCF7, MDAMB231) and colorectal (COLO205, CT26) AVPR2-expressing cancer cell lines were used for *in vitro* and *in vivo* assays. All tested cell lines showed similar *in vitro* sensitivity to [V^{4}Q^5]dDAVP (IC50 about 1 μM) as assessed by colony formation assays. However, using different syngeneic and xenogenic mouse tumor models, sustained [V^{4}Q^5]dDAVP treatment (0.3 μg/kg i.v. thrice-weekly) had highly variable and model-dependent effects on primary tumor progression (70, 25 and 0% tumor growth rate reduction for MDAMB231, COLO205 and CT26, respectively; $p<0.05$). To evaluate whether differential tumor response to [V^{4}Q^5]dDAVP could also depend on the activity of vasculature-derived VWF, 72-h cell growth assays were conducted using pharmacologically achievable concentrations of VWF (2 UI/ml). Sensitivity of tumor cells to VWF were found as MCF7>MDAMB231>COLO205>CT26, correlating with *in vivo* results and with tumor cell expression of integrin αvβ3, a VWF receptor. In conclusion, response to [V^{4}Q^5]dDAVP therapy by primary tumor may depend on direct AVPR2-mediated cytostatic effects as well as on the sensitivity of cancer cells to VWF released from the vascular milieu.

280. (287) **MODULATION OF PHOTODYNAMIC THERAPY EFFECT BY STROMAL HIF-1**

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Tumor microenvironment (TME) is a unique interactive ecosystem, where fibroblasts represent the most abundant stromal population that supports tumor growth. Due to aberrant and disorganized tumor proliferation, another common feature of TME is hypoxic regions development, in which HIF-1 acts as the main molecular mediator of adaptability. We have previously shown that photodynamic therapy (PDT), an antitumor therapy based on the combination of light, oxygen and a photosensitizer (PS), promoted HIF-1 activation on tumor cells. Concomitantly, we also demonstrated a direct association of tumoral HIF-1 and therapeutic resistance to PDT.

However, it is unknown whether this transcription factor modulates surrounding stroma response to photo-intervention. In this study, we investigated the contribution of stromal in PS generation and its involvement on photo-cytotoxicity.

TME was mimicked using homotypic spheroids of fibroblast (MRC-5 wild type or shHIF-1) or colorectal cancer cells (SW480 shHIF-1), and heterotypic spheroids composed by 1:1 mixed fibroblast/tumor cells. Stromal HIF-1 status was sensed through a reporter gene construct. The production of PS: Protoporphyrin IX (PpIX) was quantified by fluorescence microscopy. Cell viability was analyzed through MTT assay.

Our findings determined that HIF-1 silencing conferred resistance to PDT in stroma 3D monoculture, without modifying generation of PpIX. On the other hand, in heterotypic spheroids, the profile of PpIX production was associated to the hypoxic-preferential distribution of the stroma. Surprisingly, whereas stromal HIF-1 status did not impact on therapeutic resistance, the cross-talk between fibroblasts and cancer cells improved the effect of PDT.

Overall, these results suggest that response to PDT differs across hypoxic populations within TME.

As a consequence, the overall effectiveness of PDT is not defined only by tumor sensitivity, there by stroma behavior should also be considered to certainly predict therapeutic outcome.

281. (295) **PARADOXICAL ANTINEOPLASTIC EFFECT OF SHIGA TOXIN 2 FROM ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN TRIPLE-NEGATIVE BREAST CANCER CELLS**

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Shiga toxin 2 (Stx2) is a virulence factor responsible for hemolytic uremic syndrome. Classically, the Stx2´s cytotoxic effect is medi-

ated by its receptor globotriaosylceramide (Gb3). Furthermore, it has been observed that malignant cells represent a great source of Gb3. One of these Gb3-producing malignant tumors is breast cancer, which is the most common cancer type in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive and difficult to treat from all breast tumors. Our goal is to determine the antineoplastic effect of Stx2 in the TNBC cell line MDA-MB-231. The non-tumorigenic mammary epithelial cell line NMuMG, which lacks Gb3 (negative control), and VERO cells (positive control) were used. Gb3 expression was immunolocalized in MDA-MB-231 and VERO cells, and Stx2 uptake was also observed in both cell lines, with higher levels in VERO cells ($p<0.0001$). Besides, Gb3 levels were increased after Stx2 treatment in MDA-MB-231 cells ($p<0.007$). MTT results showed that 1 and 10 ng/ml Stx2 reduced cell viability after 12, 18, 24 and 48h ($p<0.05$). A Stx2 dose-dependent cytotoxic effect was found in MDA-MB-231 cells after 48h ($p<0.05$). This action was accompanied by an increase of karyorrhexis ($p<0.0001$) and a reduction of mitosis only in MDA-MB-231 and in VERO cells ($p<0.0004$), analyzed by immunofluorescence. With the purpose of evaluating whether an anti-Gb3 antibody would be able to trigger a cellular response, 10 ng/ml of anti-Gb3 or combined with Stx2 was assayed. A cytotoxic effect of anti-Gb3 was observed by MTT in MDA-MB-231, but with less potency than that produced by Stx2 itself ($p<0.05$) and no synergism was found between them. These results suggest that MDA-MB-231 cells are susceptible-dose dependent to Stx2 and sensitive to anti-Gb3 antibodies. Further studies are necessary to evaluate the safety and effectiveness of Stx2 or anti-Gb3 as a potential treatment in TNBC.

282. (316) **THYROID HORMONE (TH) IMPACT ON THE PROLIFERATION OF ON HUMAN MELANOMA (ME) CELLS AND TREATMENT WITH THE SYNTHETIC REXINOID BEXAROTENE.**

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With a constantly increasing worldwide incidence, melanoma (ME) represents the most lethal tumor among all skin cancers. Despite novel target therapies and immunotherapies improve overall survival rates, only a part of ME patients benefits from this therapies and others develops drug resistance, making crucial the study of new compounds for ME treatment.

Bexarotene (Bex), an RXR agonist used for cutaneous T cell lymphoma treatment, is currently being studied as alternative therapy for other types of cancer. The first aim of this work was to evaluate Bex effect on A375 and WM35 human melanoma cell lines *in vitro*. We found that Bex significantly decreases cell viability after 48h of both ME cell lines in a dose dependent manner ($p<0.05$). On the other side, Bex is associated with hypothyroidism so patients require the concomitant administration of a replacement therapy with the thyroid hormone (TH) levothyroxine. We previously found that TH, mostly through the action on integrin αVβ3, contribute to the malignant phenotype of T cell lymphomas and other tumor cells. In this sense, we then analyzed TH effect on the proliferation of ME cells and found that physiological levels of T3 and T4 (1nM and 100nM, respectively) induce 15 to 30% ME cell proliferation compare to control ($p<0.05$). We then evaluated if the TH proliferative effect influence Bex antineoplastic activity on ME cells. We treated cells with Bex for 48h with or without TH and found that Bex activity on cell viability was higher in the absence of TH *($p<0.05$)*. Importantly, we found in a skin cutaneous melanoma project from The Cancer Genome Atlas portal (TCGA-SKCM) that ME patients expresses RXR genes. We are now studying the inhibition of the TH membrane receptor, integrin αVβ3, as possible adjuvant for Bex antiproliferative actions on ME cells. Despite it should be studied more deeply, our preliminary results point Bex as a new therapeutic option that could be considered in the future for ME treatment.

283. (325) **CHARACTERIZING THE ROLE OF ALPHA-SYNUCLEIN IN MELANOMA**

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The amyloid protein alpha synuclein (αS) is the main component of Lewy bodies, the neuropathological hallmark of Parkinson's disease (PD). The mechanisms underlying αS aggregation, neurotoxicity and cell-to-cell transmission were explored in the context of PD. Recent evidence suggests that αS may also play a putative role in melanoma, the most dangerous form of skin cancer. Current studies suggested that αS could be protective for advanced melanoma but its role in this type of cancer was not deeply explored yet.

Previously, we demonstrated by *in vitro* studies that melanoma cells are able to uptake different aggregation species of αS. These species were not toxic for melanoma cells. Instead, they promoted proliferation, cytoskeleton rearrangement and migration. Here, we evaluated the ability of human SK-MEL 28 and mouse B16-F10 melanoma cells to form clones in the presence of αS fibers by standard colony forming assays. Cells were incubated (or not) with αS fibers for 24 hours and 300 cells were plated to form colonies. For both types of cells, incubation with αS promoted a higher number of clones ($P<0.01$) of increased size ($P<0.05$). We corroborated this result by an *in vivo* experiment; subcutaneously injecting B16-F10 cells below the minimal tumorigenic dose in C57BL/6 mice ($7.5x10^4$ cells; n=5/group). By this approach, we observed that animals injected with αS-treated cells developed tumors after 4 weeks (no tumor observed in control animals at this time). To further confirm αS role in melanoma progression, $2x10^5$ B16-F10 cells (treated or not) were subcutaneously injected in the right flank of C57BL/6 mice (n=7/group). Tumor volume was measured during 3 weeks. Growth kinetics indicated that αS treatment promoted melanoma growth (doubling time 2.34±0.24 vs 2.74±0.59 for control cells; $P<0.05$).

Altogether, our data indicate that αS fibers are able to promote growth and clonogenicity of melanoma cells, suggesting a role for αS and αS-transmission in melanoma progression.

284. (332) **ENDOCRINE THERAPY WITH MIFEPRISTONE PROMOTES THE DEVELOPMENT OF AN ANTITUMOR RESPONSE AND IMMUNOLOGICAL MEMORY AND SENSITIZES TUMORS TO PD-L1 BLOCKADE IN LUMINAL BREAST CANCER.**

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In recent years, interest has been renewed regarding the role of the immune system on tumor growth in breast cancer. In this work we set out to determine the contribution of the immune system to antiprogestin-mediated tumor inhibition in ER+ PR+ (HR+) luminal breast cancer. To do this, we transferred whole bone marrow from BALB/c-GFP mice to immunodeficient NSG mice and generated an immunocompetent model (NSG-R). We observed that mifepristone (MFP) inhibited HR+ 59-2-HI tumor growth in NSG and NSG-R mice, similarly. However, MFP remodeled the immune landscape of NSG-R mice-treated tumors, favoring infiltration of MHC-II+ PD-L1+ macrophages, CD103+ dendritic cells (DCs) ($p<0.05$), NK, T lymphocytes and decreasing the frequency of Tregs Foxp3+ ($p<0.01$) and MDSCs ($p<0.001$). At the transcriptomic level, it favored the expression of pro-inflammatory programs, with high levels of cytokines and chemokines ($p<0.01$) and particularly of an immunogenic death program (GSEA). We confirmed by IHC that MFP induced subcellu-

lar relocation of alarmines HMGB-1 and Calregulin in MFP-sensitive breast tumors ($p < 0.01$), which induced the activation of DCs, by increasing the expression of MHC-II and CD86 ($p<0.05$). Treatment with MFP favored the development of an immunological memory capable of protecting against tumor re-challenge ($p<0.001$). Finally, we observed that changes at the transcriptomic level and tumor microenvironment immune composition induced by MFP sensitized the tumors to PD-L1 blockade ($p<0.05$). These results shed light on the underlying mechanisms triggered by hormonal treatment on antitumor responses and to the rational design of new therapeutic combinations in luminal-type breast cancer.

285. (341) **MICRORNA EXPRESSION PROFILE IN PLASMA OF PATIENTS WITH CLINICAL FEATURES RELATED TO METABOLIC SYNDROME AND THEIR IMPLICATIONS IN BREAST CANCER.**

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Metabolic Syndrome (MeS) is a risk factor for breast cancer (BCa). Molecular mechanisms underlaying this association have not been fully elucidated. MiRNAs are small non-coding RNAs that regulate gene expression. Circulating miRNAs can be detected in body fluids. Aberrant expression of miRNAs in both tissues and fluids are linked to several pathologies. Might plasma miRNAome associated to MeS influence BCa development and progression? The aim of this work was to identify the circulating miRNAs in plasma of patients with clinical features linked to MeS and their role in BCa.
Healthy women from Hospital Militar Central (CABA) were recruited and divided in two groups, control or MeS-linked disease (MeSL) when presented two or more of these conditions: BMI≥25.00 kg/m^2, waist diameter≥82 cm or high blood pressure (systolic≥120, diastolic≥80). MiRNAs were isolated from plasma and a pool of each samples were hybridized to GeneChip® miRNA 4.0 Array (Affymetrix). We found 24 miRNAs (FSG<0.05%, Log FC>1.5 and p value<0.01) altered in plasma of MeSL women and analyzed them by KEGG using DIANA miRPath. These miRNAs were involved in several processes related to cancer including proteoglycans and transcriptional misregulation in cancer. In particular, MeSL-downregulated miR-23a-3p, -19b-3p, -181a-5p, -122-5p, -425-5p, -28-3p, -146a-5p, let-7b-5p and MeSL-upregulated miR-101-5p and -877-5p showed the strongest association. We determined their expression levels in primary tumors (PT) and adjacent normal tissue of patients from TCGA BRCA data set. The miR-28-3p, -101-5p and let-7b-5p were diminished while miR-181a-5p, -425-5p and -877-5p were increased in PT. Analysis using UCSC Xena tool showed that some miRNAs expression were dysregulated in a tumor subtype related manner. Interestingly, miR-877-5p correlated with decreased survival of BCa patients.
Our results suggest that MeSL condition modifies miRNAs expression profile from plasma which might impact on BCa.

286. (360) **UNEXPECTED TUMOR SUPPRESSOR ROLE FOR Vav3 IN MELANOMA**

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Melanoma is the most dangerous form of skin cancer, accounting for the third highest number of lives lost among all cancers. Since in our country the mortality has doubled in last decades, finding prognostic and therapeutic targets appears a key task.
Vav proteins are guanosine nucleotide exchange factors (GEFs) for the Rho GTPase family. They modulate processes highly associated to the development of cancer and metastasis. Classically the members of Vav family are considered proto-oncoproteins. However their involvement in melanoma is unknown.
We previously characterized the role of Vav2 in melanoma; now we began to study the putative involvement of Vav3. First, by bioinformatic approaches with human patients databases, we found that Vav3 expression correlated with patient survival ($p\leq0.0001$). Second, we modulated we modulated Vav3 expression in B16-F0 cells by transfection methods, generating cells with reduced and increased expression of Vav3. By MTT assays we explored proliferation, noting that decreased expression of Vav3 promotes proliferation ($p\leq0.001$) whereas increased levels of this protein affected negatively cell growth ($p\leq0.001$). By fluorescence studies with rhodamine-phalloidin staining we observed that Vav3 affected actin cytoskeleton and cell morphology. Cells with decreased Vav3 expression showed an improved migratory behavior as observed by wound healing assays ($p \leq 0.05$).
Finally, we injected cells subcutaneously in female 8-week old C57BL/6 mice (n=6/group). Tumor volume was measured biweekly for 3 weeks. Growth kinetics indicated that decreased Vav3 expression promoted increased tumor proliferation ($p\leq0.01$) and lung metastasis development. Increased Vav3 expression affected negatively melanoma growth ($p\leq0.05$).
Altogether, our results suggest a tumor suppressor role for Vav3 in melanoma, in contraposition with the pro-tumoral function reported for Vav2 in this tumor type and the classical role reported previously for Vav3 in other tumors.

287. (376) ***IN VITRO* AND *IN SILICO* EVALUATION OF A NOVEL INHIBITOR OF TELOMERASE COMPLEX ASSEMBLY IN MDA MB 231 CELL LINE**

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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 diskeryn (DKC1), the catalityc retrotranscriptase (hTERT) and the RNA template (hTR). Telomerase complex is regulated in many levels, such as epigenetic regulation, transcriptional and post-traductional processing, intracellular compartmentalization, and recruitment and substrate accessibility. Considering these processes, so far different strategies have been adopted to carry out novel telomerase inhibitor therapies. This work aims to the development of new inhibitors of the telomerase, selecting as target the interaction between hTR-DKC1. We designed a model of the human protein DKC1, which was tested against a library of 300.000 drug-like molecules by virtual screening. We selected the first 20 molecules that showed the highest affinity values to test it inhibitory activity on the mammary adenocarcinoma cell line MDA MD 231. After 48 hs of treatment with compound N°10 at a dose of 2μM, we found that telomerase activity diminished a 57% ($p < 0.05$). We continued with cell treatment for 55 passages, resulting in telomere shortening (66%, $p < 0.001$), a rise in the expression of *p16ink4a* gene (400%, $p < 0.05$) and a positive staining of SA-β-galactosidase, which strongly confirms that cells are going through a senescence stage. Furthermore, we evaluated parameters related with the apoptosis process, obtaining an increase both in *bax*/*bcl2* ratio (>2.3, $p<0.001$) and in caspase 3 activity (155%, $p<0.01$). In order to improve the properties of the leader compound, we designed 100 analogues and studied their affinity profile. We ranked all the candidates and selected the 10 analogues with major affinity to carried out an *in silico* prediction of ADME/Tox properties. These results allow us to postulate compound 10 and its analogues as novel inhibitors of telomerase activity with a potential clinical use

as an antitumoral drug.

288. (377) **DIFFERENTIAL EXPRESSION OF CPB1 AND CRISP3 IN LUMINAL BREAST CANCER MODELS TREATED WITH MIFEPRISTONE AND MEDROXYPROGESTERONE ACETATE.**

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Several epidemiological studies, supported by experimental research, suggest that progestins exert a relevant role in breast carcinogenesis. Thus, there is an increased interest in targeting progesterone receptors (PR) for breast cancer treatment. We have recently shown that the antiprogestin mifepristone (MFP) inhibited cell proliferation of breast cancer tissue cultures with higher levels of PR isoform A (PRA) than B (PRB), highlighting the relevance of identifying markers to monitor antiprogestin responsiveness. Using RNA-seq we have previously identified 139 genes differentially expressed in breast carcinomas with high or low PRA/PRB ratio (PRA-H and PRB-H, respectively). The aim of the study was to investigate the expression of genes that could be used to monitor treatment responsiveness. We focused in CPB1 and CRISP3, since both are secreted proteins that might be detected in serum. We used two PRA-H (C4-HI and C7-2-HI) and two PRB-H (C42-HI and C7-HI) tumors from the medroxyprogesterone acetate (MPA)-induced murine breast cancer model, and the human breast cancer cell lines, T47D-YA and T47D-YB. A statistically significant interaction was observed when the expression of CPB1 and CRISP3 was studied by qPCR in tumors treated with MFP or the progestin MPA ($p<0.05$). MFP down regulated CPB1 and CRISP3 expression in PRA-H tumors ($p<0.05$) and an opposite trend was observed in PRB-H tumors. We conclude that CPB1 and CRISP3 might be good biomarkers to monitor MFP response.

289. (380) **ADIPOCYTES IN BREAST CANCER: LIPOLYTIC AND MITOCHONDRIAL ALTERATIONS IN THE TUMOR MICROENVIRONMENT**

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Breast cancer cells induce metabolic reprogramming of peritumoral adipocytes, promoting adipocyte delipidation and atrophy/regression. We have previously reported that peritumoral adipocytes showed a decrease in the expression of Plin1, which together with HSL and ATGL regulate the lipolytic process, and smaller lipid droplet size. In this study we analyzed the effect of breast cancer cells on expression of several proteins involved in the lipolytic process in explants of human adipose tissue of tumor (hATT) and non-tumor (hATN) breasts. Also, we evaluated the effect of conditioned media (CMs) from hATT and hATN on the lipolytic process of 3T3-L1 adipocytes. The expression of ATGL and HSL was analyzed by immunohistochemistry and Western blot. Levels of both proteins were increased in hATT with respect to hATN ($p <0.05$). Interestingly, 3T3-L1 adipocytes showed an increase in the average number of lipid droplets (LD) per cell ($p <0.01$), and a decrease in LD size and triglyceride accumulation after incubation with hATT-CMs vs hATN-CMs. HSL and Plin1 in 3T3-L1 adipocytes were increased after incubation with hATT- vs hATN-CMs and Ctrol-CMs. In addition, morphological changes in mitochondria were observed in adipocytes because of CMs treatment. The results of indirect immunofluorescence revealed that about 90% of adipocytes incubated with hATT-CMs showed fragmented mitochondria, whereas about 60% of adipocytes incubated with hATN-CMs showed this change in their mitochondria. The highest percentage of mitochondrial fragmentation per cell was more frequent in adipocytes treated with hATT-CMs vs hATN-CMs.
In summary, these findings suggest that peritumoral adipocytes underwent morphological and protein expression changes consistent with activated lipolysis and this phenomenon could, in turn, promote lipolytic and mitochondrial alterations in adjacent adipocytes by paracrine signaling.

290. (382) **TUMOR-SUPPRESSIVE FUNCTIONS OF 4-METHYLUMBELLIFERONE ON HUMAN AML CELLS: STUDY OF HYALURONAN-SYNTHESIS-INHIBITION INDEPENDENT MECHANISMS.**

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Despite continuous improvement in the treatment for acute myeloid leukemia (AML), new therapies are still needed to overcome resistance and reduce adverse effects. We previously proposed that 4-methylumbelliferone (4MU), known as an inhibitor of hyaluronan (HA) synthesis, would be an interesting new drug for leukemia treatment. Previous results in our lab, showed that 4MU inhibited cell proliferation in a dose-dependent manner in human AML cell lines. Moreover, this drug was able to modulate mitochondrial status and ROS production. However, it remains to assess whether the observed effects are explained by the inhibition of HA synthesis. The aim of this work was to analyze if the anti-tumor activity of 4MU on U937 and THP-1 AML cells could be explained by HA synthesis inhibition. Results showed, that both AML cell lines were able to produce significant quantities of AH (121.0 ± 0.6 ng/ml and 107.6 ± 0.5 ng/ml, respectively) as assessed by ELISA. Surprisingly, 4MU was able to partially inhibit HA synthesis in THP-1 cells ($p< 0.001$) but not in U937 cells at the doses tested. The addition of HA failed to prevent the effects of 4MU on metabolic activity and cell proliferation in both cell lines, evaluated by XTT and ^{3}H-T uptake, respectively. Moreover, 4MU+HA co-treatment failed to prevent the increase in the mean of fluorescence of NAO by FC, as well as the increase in ROS production, as it was evaluated also by FC with DCF-DA and MitoSox staining, in both cell lines. These results suggested that there would be 4MU mechanisms independent of HA synthesis inhibition. To delve further into these mechanisms, we conducted a proteomic study in U937 cells after 4MU treatment by nano LC-MS/MS. Data analysis with free software RStudio resulted in 15 proteins modulated by 4MU ($p<0.05$ and power>80), mainly related with cell metabolism signatures, but not directly linked to HA synthesis mechanisms. This finding expand the knowledge of 4MU for its potential use in AML treatment.

291. (387) **EXPERIMENTAL STUDIES FOR THE PERSONALIZED APPLICATION OF BORON NEUTRON CAPTURE THERAPY (BNCT) TO THE TREATMENT OF CUTANEOUS MELANOMA (CM)**

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Boron Neutron Capture Therapy (BNCT), based on the nuclear reaction 10B (n, α) 7Li, has been used to treat cutaneous melanoma in different countries including Argentina. In previous studies in agreement with the personalized oncology we have demonstrated that the response to BNCT might be correlated with tumor temperature and viability. **The aim of these studies** was to develop a model of heat transport that allows predicting the tumor response to BNCT. **Methods and Materials**: Nude mice implanted with human melanoma cells Mel J were divided into different groups (Control, beam only, BNCT) and exposed to the thermal neutron beam of the RA 6 nuclear reactor clinical facility (4.96 x108 n/cm2sec). Tumor and body temperatures were measured by Static Infrared Imaging (SIRI), and the follow up of the animals was performed. A heat transport model was developed, considering conduction, convection and an internal heat source. From this model, the thermal conductivity of the tumor was derived taking into account the internal and external temperatures, plus the ambient temperature and the size of the tumor. Then the conductivity was correlated with the boron concentration between tumor and blood (T/B) previously obtained by ICP OES measurements. **Results:** Tumor growth showed a complete growth inhibition during the first 20 days after BNCT ($p<0.001$). It was observed that tumors with higher temperatures corresponded to a greater degree of tumor growth control. Importantly, the results showed that tumors with higher calculated thermal conductivity (between 0.4 and 0.5 W / mK) and a higher derived boron concentration ratio between tumor and blood showed a better response to BNCT. On the other hand, tumors with lower thermal conductivity presented different degrees of tumor inhibition (R= 0.8). **Conclusions:** tumor characteristics, specially the temperature and the associated heat transport model, could be used to plan a personalized BNCT treatment for each patient.

292. (396) **EVALUATION OF PHARMACOKINETIC PARAMETERS OF THE NON-HYPERCALCEMIC CALCITRIOL ANALOGUES EM1 AND UVB1**

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Vitamin D analogues EM1 and UVB1 have demonstrated antitumor effects in preclinical studies employing cell lines, animal models and patient-derived xenograft cells. In the current work we focused on the study of Absorption, Distribution, Metabolism, and Excretion (ADME) properties of these analogues by SwissADME software, an in silico tool. The results showed that the analogues have similar lipophilicity to calcitriol. The resulting log $P_{o/w}$ values were 5.02, 6.27 and 5.03 for EM1, UVB1 and calcitriol, respectively. In addition, the polar surface area (PSA) values obtained to EM1 (85.80 Å²), UVB1 (80.92 Å²) and calcitriol (60.69 Å²) suggest the ability of the analogues to cross cell membranes such as the blood-brain barrier. In accordance with these results, BOILED-egg plots predict that both analogues have high brain penetration, however they could be efflux by p-glycoprotein as the analogues are substrates of this pump. Regarding transdermal absorption, skin permeability coefficients (log Kp) for EM1, UVB1 and calcitriol were -5.99 cm/s, -4.95 cm/s, -5.24 cm/s, respectively, suggesting the potential to administer these compounds by this route. Moreover, Bioavailability Radar plots indicate that EM1 has similar properties to calcitriol for oral bioavailability while UVB1 needs to decrease its flexibility, lipophilia and size to be orally administered. Finally, the in silico prediction of genotoxicity indicates that the analogues have non-mutagenic and non-carcinogenic properties in AMES tests, the probabilities were 0.8069 for EM1 and 0.94 for UVB1. Altogether, these in silico analyses complement the reported in vitro and in vivo studies to reinforce the use of these analogues as chemotherapeutic agents.

293. (404) **INTEGRATIVE TISSUE "OMICS" UNVEIL NOVEL MARKERS AS PREDICTORS OF AGGRESSIVENESS THAT OUTPERFORM GLEASON SCORE IN PROSTATE CANCER**

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Prostate cancer (PCa) is the second most frequent cancer and the sixth leading cause of cancer-related death in men worldwide. Widespread use of prostate-specific antigen (PSA) levels for screening has led to a large increase in the incidence of diagnosed PCa and a reduction in both, the advanced disease and PCa mortality rates. However, the overtreatment of PCa is widely recognized. For men with newly diagnosed PCa, besides from stage, the strongest predictor of lethal PCa is the Gleason score (GS). PCa grading has significantly improved since Gleason's original description in the mid-1960s. Literature reflects that, although some prostate cancers (PCas) are histo-pathologically grouped within the same GS, they can differ significantly in aggressiveness. In this work, we aimed at identifying molecular biomarkers that could improve risk prediction in PCa. In-depth proteomics analysis was performed on human PCa and Benign Prostatic Hyperplasia (BPH) tissues. We then validated the clinical significance of these peptides through an integrative bioinformatics analysis using public database repositories. We identified high expression of *YWHAZ* and *NDRG1* to be strongly associated with poor PCa prognosis considering all Gleason scores. *YWHAZ* and *NDRG1* expression defined two clearly and distinct subpopulations of patients with high and intermediate risk of disease progression. Adjusted multivariable analyses confirmed their independence from GS, patient age and TMPRSS2-ERG fusion. Further, ROC analysis unveiled that *YWHAZ* outperformed GS beyond 60 months post-diagnosis. We hereby state the relevance of *YWHAZ* in PCa, showcasing its role as an independent strong predictor of aggressiveness, making it a potential prognostic tool when taking into consideration the difficulties that PCa presents at the time of decision making.

294. (408) **PTHrP DIFFERENTIALLY REGULATES MIR-423 AND MIR-27a IN NORMAL INTESTINAL CELLS AND COLORECTAL CANCER CELLS**

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MicroRNAs (miRs) are small non-coding RNAs that participate in the regulation of mRNAs at the post-transcriptional level in physiological and pathological processes such as cell differentiation, migration, and invasion. The differential presence of the 3p and 5p isoforms originating from the pre-microRNA precursor has recently been correlated with cancer initiation and progression. Previously, we obtained evidence that parathyroid hormone-related peptide (PTHrP), which is overexpressed in colorectal cancer (CRC), induces molecular changes promoting angiogenesis, Epithelial-Mesenchymal Transition (EMT), and cancer stem cell features in CRC cells. The objective of this work was to explore the potential role of PTHrP in the post-transcriptional regulation of mRNAs, evaluating the expression of miR-423 and miR-27a isoforms in the normal intestinal

CCD841 CoN cell line and in HCT116 cells derived from colorectal cancer. miR-423 is increased in the plasma of CRC patients, while miR-27a was identified as a tumor suppressor. Employing TaqMan MicroRNA assays by qPCR, we observed that PTHrP treatment for 5 hours significantly increases the miR-423-3p and miR-423-5p transcripts levels in HCT116 cells; however, no changes were evidenced in CCD841 CoN cells at the studied times. In HCT116 cells, the transcription of miR-27a-5p increased at 5 hours of PTHrP exposure with a return to control levels within 24 hours. Differently, PTHrP induced a consistent decrease in miR-27a-3p transcription in HCT116 cells and CCD841 CoN cells at the same times. These results highlight the relevance of the temporal coexistence of the two functional isoforms of these miRs and propose that miR-27a-3p isoform and miR-423 isoforms could be possible post-transcriptional regulators modulated by PTHrP in intestinal tumor cells.

295. (415) **FURTHER CHARACTERIZATION OF FXYD5/ DYSADHERIN EXPRESSION IN ENDOMETRIAL CANCER AND ITS ASSOCIATION TO TUMOR PROGRESSION AND TREATMENT**

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IBYME

Aim:Endometrial cancer (EC) is 6th most frequent women cancer worldwide. Stage IA endometroid EC (EEC) patients have good prognosis, and a conservative treatment of Medroxyprogesterone acetate (MPA) is applied to patients attempting fertility preservation or unsuitable candidates for surgery. However, MPA response is partial or nule, and recurrence is reported. Using cell models and patient samples, we demonstrated an increased expression of FXYD5/ Dysadherin (Dys) in ECC. In cell models, we found a negative association between Dys and E-cadherin/CDH1, activation of TGF-β and NF-κB pathways, and cytokine expression. In this study, we further evaluated Dys expression, association with ECC progression and with MPA response.
M&M:mRNA expression of Dys and related molecules was analyzed in EC patient datasets, and in MPA-resistant Ishikawa EEC cells and control (GSE121367).
Results:A negative ($p<0.05$; Spearman) association was confirmed between CDH1 and Dys mRNA expression (TCGA total dataset n=388, and Stage IA samples n=140). Dys expression was higher in Stage IA EC samples than in atrophic controls (GSE17025; $p<0.05$; Mann Whitney). A positive ($p<0.05$) association was found between Dys expression and markers of TGF-β (TGF-beta1, ZEB1, ZEB2, SNAI1, SERPINE1, SMAD3) and NF-κB (CCL2, TNF-alpha, CCL17, CCL22, IL6, IL10) pathways. A positive correlation was found between Dys levels and CD68 (monocyte lineage), CD163 (macrophage) and FOXP3 (Treg) markers ($p<0.05$). MPA-resistant Ishikawa cells showed lower ($p<0.05$) expression of CDH1 mRNA, and higher ($p<0.05$) expression of Dys and related markers (ZEB1, ZEB2, SAMD3, CCL22, CD68, TNFRSFB) than control cells.
Conclusion:Results confirm Dys mRNA expression in ECC patients, and its negative correlation with CDH1 expression. Findings also reveal a possitive association of Dys with molecular markers of immune system and inflammatory response in EC, and give new insights into potential Dys role in EC MPA treatment resistance.

296. (422) **FURTHER CHARACTERIZATION OF EPHRINB1 AND EPHB2 EXPRESSION IN BLADDER CANCER AND ITS ASSOCIATION WITH TUMOR PROGRESSION AND AGGRESSIVENESS**

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IBYME

Aim: Bladder cancer (BC) is one of the ten solid tumors with highest incidence/mortality worldwide. Clinically, it is classified as non-muscle-invasive (NMIBC; Ta-T1 stages) and muscle-invasive (MIBC; T2-T4 stages). Samples are also classified by tumor grade (G1 to G3, G3 most aggressive). BC molecular classification has also been reported. In a murine model of BC progression/aggressiveness, we reported β-catenin nuclear translocation, higher expression of Ephrin B1 (EFNB1) ligand and its EphB2 (EPHB2) receptor, and STAT3-related pathway activation. Also, increased expression of EFNB1 protein was shown in BC patients (Mencucci et al, 2020). This study aimed to further characterize EFNB1 and EPHB2 expression in BC.
M&M: Datasets were queried to analyze EFNB1 and EPHB2 mRNA levels in BC patients (GSE13507, GSE32894). To explore binding sites of TCF4 transcription factor to EFNB1 and EPHB2 promoters, a set of programs were used (Ensembl, Methyl Primer Express v1.0, PROMO).
Results: EFNB1 mRNA levels were higher in tumor samples (n=165) than in mucosa surrounding cancer (n=58); Ta and T1, and G1 tumors had higher expression levels than controls ($p<0.005$). Higher EPHB2 transcript was also found in tumor samples compared to controls ($p=0.0106$), and in T3 tumors depicted higher mRNA levels ($p=0.0130$). EPHB2 mRNA levels were higher in G1 and G2 tumors (GSE13507), and in G3 compared to G1 and G2 tumors (GSE32894). A positive correlation was found between EFNB1 and EPHB2 mRNA levels ($r=0.2775$; $p=0.0074$; Spearman). Survival analysis revealed an association between EPHB2 expression and Disease Free Survival ($p=0.0158$). Using molecular classification reported by Sjödahl (2012), EPHB2 mRNA was higher in SCC-like (worse prognosis) molecular subtype than in all others ($p<0.05$). Promoter analysis identified TCF4 binding sequences in EFNB1 and EPHB2 promoters.
Conclusion: EFNB1 and EPHB2 mRNA expression was validated, showing an association with BC progression disease/aggressiveness.

297. (434) **DEVELOPMENT OF NEW THERAGNOSTIC RADIOPHARMACEUTICALS FOR EGFR (+) AND *KRAS* MUTATED TUMORS**

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The epidermal growth factor receptor (EGFR) is one of the most explored cancer associated molecules due to its overexpression in various types of cancers. Nowadays, there are approximately 10 compounds on the market targeting EGFR. However, their effectiveness is limited in patient subpopulation with specific genetic characteristics and due to acquired resistance. Radioimmunotherapy (RIT) is based on radiolabeled proteins targeting cancer-associated structures. Besides antibodies, alternative protein formats with different pharmacokinetic characteristic such as nanobodies (VHH) have been explored for radionuclides diagnosis in the recent years. The aim of this project is to obtain a novel radiopharmaceuticals based in ^{177}Lu and VHH against EGFR receptor (VHH-EGFR) for theragnostic application. First, we labelled a monomeric VHH-EGFR with ^{177}Lu and characterized its performance in *kras* mutated and wild type cancer cells in colony assay. The results showed a specific and significative therapeutic effect in *kras* mutated respect wild type *kras* cancer cells. However, for in vivo application, it is necessary to slightly increase its size in order to avoid quick renal clearance and achieve a therapeutic effect. At this moment, we are working in different strategies to slightly increase nanobody size, for instance adding an albumin binder or perform VHH dimers to improve its pharmacokinetic and therapeutic effect in future in vivo experiments.

298. (440) **STUDY OF THE INTERACTION BETWEEN SILICA NANOPARTICLES AND CANCER CELLS MEMBRANE, USING SCANNING ELECTRON MICROSCOPY**

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Gattas *(INS UNSAM)*, Galo Soler Illia *(INS UNSAM)*, Sergio Moya *(CIC biomaGUNE)*, Marina Simian *(INS UNSAM)*

The aim of this work was to study the interaction between Mesoporous Silica Nanoparticles (MSN) as potential drug carries, with different cell lines. The MSN were synthetized and functionalized to use them on 2D and 3D cell cultures of B16F10 and U251.
Is still under discussion how cells interact with different types of nanoparticles, their cytotoxicity and selectivity. For this reason, we focused on studying in detail this MSN-cell membrane interaction through Scanning Electron Microscopy (SEM). To verify the presence of MSN, energy dispersion X-ray spectroscopy (EDS) was used as a complementary technique. Also, confocal microscopy experiments were carried out to compare the results between both microscopy techniques.
The main challenge was focused on preserving all the characteristics of the cell structure in the steps prior to its analysis by SEM. For this, a chemical dehydration and drying method was developed in order to avoid the use of expensive equipment, such as Critical Point Drying or Cryomicroscopy. We developed a cheap, fast, and reproducible method for cell fixation and dehydration, preserving the natural characteristics of the samples.

299. (449) **HISTAMINE H2 RECEPTOR AGONIST AMTHAMINE MODULATES CELL PROLIFERATION AND MIGRATION VIA ERK1/2 AND SRC PHOSPHORYLATION IN PANCREATIC CANCER CELLS**

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We have previously determined that histamine dose-dependently modulates cell proliferation, metalloproteases activity and migration in the human pancreatic cancer cells PANC-1 and BxPC3. In vivo, the histamine H2 receptor (H2R) antagonist ranitidine hindered the growth of PANC-1 and BxPC3 grafts and the development of PANC-1 lung metastasis in nude mice.
The aim of this work was to evaluate the effect of the H2R agonist amthamine on proliferation, gelatinolytic activity and migration in PANC-1 and BxPC3 cells.
Results from dose-response curves showed that 100 nM amthamine in PANC-1 and 10 μM in BxPC3 increased the number of colonies by the clonogenic assay ($p<0.05$ vs control) while the H2R antagonist ranitidine blocked these effects. Immunoblot studies revealed an enhancement in the expression of cyclin E2 ($p<0.05$ vs control) and a significant increase in phospho-ERK1/2 levels ($p<0.01$ vs control) in both cell lines. Interestingly, MEK inhibitor PD98059 counteracted both ERK activation and clonogenicity induced by amthamine ($p<0.01$ vs amthamine).
Besides, MMP2 in PANC-1 and MMP9 gelatinolytic activity in BxPC3 were increased when cells were treated with 100 nM ($p<0.05$) and 10 μM amthamine ($p<0.01$), respectively. Cell migration studies using transwell units showed that these amthamine concentrations increased the migratory capacity in both cell lines ($p<0.05$ vs control). These effects were associated with Src kinase activation/phosphorylation since amthamine augmented phospho-Src levels ($p<0.01$ vs control) and the selective Src inhibitor PP2 blocked the increase in the number of migrated cells ($p<0.05$ vs amthamine).
Collectively, results suggest that H2R might be at least partially involved in the stimulatory action exerted by histamine on cell proliferation, gelatinolytic activity and cell migration in pancreatic cancer cells and support the in vivo role of ranitidine in the growth of PANC-1 and BxPC3 xenotransplanted tumors and lung metastasis in nude mice.

300. (458) **BIRC6 AS A NOVEL THERAPEUTIC TARGET IN TRIPLE NEGATIVE BREAST CANCER**

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BIRC6 (baculoviral IAP repeat-containing protein 6) is a member of the inhibitors of apoptosis protein family thought to play an important role in the progression or chemoresistance of many cancers. The aim of this study was to investigate the role of BIRC6 in triple negative breast cancer (TNBC).
Firstly, we analyzed BIRC6 mRNA expression levels and Copy Number Variations (CNV) in three *Breast Cancer* databases (TCGA) using cBioPortal and USCSXena platforms comparing clinical and molecular attributes. In addition, we analyzed the BIRC6 expression in murine (F3II) and human (MDA-MB-231) TNBC cell lines by immunofluorescence. Moreover, we used a recombinant baculovirus able to silence BIRC6 previously constructed in our lab. Then, we determined apoptosis levels in F3II cells by TUNEL.
Our bioinformatics results showed that there was a differential expression of BIRC6 in cancer samples compared to normal samples (normal= -0,372±0,395, cancer= 0,113±1,055; *t-test*, $p<0,001$). TNBC tumors have higher proportions of *birc6* CNV amplifications and gains compared to those found in hormone dependent tumors (ER+: 2% gain, <1% amp; ER-: 15% gain, 2% amp; PR+: 1% gain, <1% amp; PR-: 9% gain, 1% amp; χ^2, $p<0,05$). Moreover, we found that there was a differential expression of apoptotic, PI3K/AKT/mTOR and Ras-MAPK pathways in conditions of high expression of BIRC6 (*t-test,* $p<0,05$), and also a strong correlation between the expression of proteins involved in these pathways and BIRC6 (*Pearson correlation*). Finally, we demonstrated that baculovirus efficiently transduced TNBC cell lines and elicited a reduction in the BIRC6 protein levels. Silencing baculovirus exerts a pro-apoptotic action (*t-test*, $p<0,05$).
These preclinical data suggest that BIRC6 would be proposed as a therapeutic target for gene therapy on the treatment of TNBC tumors.

301. (464) **PHOSPHORYLATED HSP90-ALPHA EXPRESSION IN PERIPHERAL BLOOD LEUKOCYTES FROM CISPLATIN-TREATED CANCER PATIENTS**

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The heat shock protein HSP90 plays important roles in protein homeostasis. The inducible form, HSP90α, may be an attractive target for new anticancer therapies, as many of its client proteins are involved in cancer hallmarks. The phosphorylated form in Thr-7 (p-HSP90α) accumulates at the DNA damage sites and has been proposed as a biomarker of genomic instability. Cisplatin (cisPt) is used for the treatment of solid tumors, but resistance limits its efficacy. Therefore, it is important to identify biomarkers to predict treatment response. Our aim was to evaluate the predictive/prognostic value of p-HSP90α in peripheral blood leukocytes (PBL) from cisPt-treated cancer patients. PBL from 7 healthy persons and 52 patients before chemotherapy were in vitro exposed to cisPt (200 μM, 1 h). The cells were harvested at: T0 (immediately after cisPt), at T24 and at T48 (after a recovery period of 24h and 48h, respectively). Nuclear and cytoplasmic expression of p-HSP90α was evaluated by immunocytochemistry. At basal conditions, cytoplasmic and nuclear p-HSP90α did not show statistically significant differences between healthy individuals and cancer patients. After cPt-treatment, the cytoplasmic levels of the protein did not significantly differ between experimental conditions, in both controls and patients. In contrast, the nuclear expression of p-HSP90α significantly increased in patients at T24 ($P<0.0001$) and at T48 ($P<0.001$). No statistically significant differences were found between patients with complete clinical response to chemotherapy and those with partial response, stable or progressive disease with respect to the cytoplasmic or nuclear p-HSP90α expression. In addition, no significant associations with

cancer patient?s survival outcomes were also found. Our preliminary data indicate that immunocytochemical detection of p-HSP90α in PBL from cancer patients does not appear to be a useful tool for either prognosis or response to cisPt-based chemotherapy.

302. (465) **DIFFERENTIAL RESPONSE TO FREE AND ENCAPSULATED CHEMOTHERAPEUTIC DRUGS IN GLIOMA AND MELANOMA CELLS**

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We aimed to study the effectiveness of MCM41 silica NPs as potential drug carriers for glioblastoma and melanoma tumor cells. Three drugs (Cis- platinum, Doxorubicin and Paclitaxel) of different chemical nature were encapsulated in MCM41 NPs with differential pore functionalization. In particular for Cis-Platinum, we funtionalized NPs with SATES. Secondly, for Doxorrubicin we funtionalized NPs with APTES and for the loading of Paclitaxel, we left the porus without funtionalizatio.

A chemical study of loading and release of the drugs was carried out under physiological conditions. MTT assays were performed on two human glioblastoma cell lines (U251 and U87) and a murine melanoma cell line, B16F10. Cytotoxicity was measured at 24, 48 and 72 hours of incubation.

Our experiments revealed that cell viability in response to the drugs was different for each cell line. B16F10 cells were susceptible to encapsulated and free Cis- platinum at 48 and 72 hours (n=3; $p< 0.001$) and the empty NP showed no toxicity. U251 cells responded to free and encapsulated Doxorubicin and Paclitaxel (n= 3; $p<0.001$) and were not affected by the empty NPs. U87 cells did not respond to any of the drugs either in their free form or encapsulated in NP.

Our results show that the effectiveness of nanoformulations is dependent on cell context and that results in one cell line should not be extrapolated to other systems.

303. (470) **MIFEPRISTONE IMPROVES THE EFFECT OF CHEMOTHERAPY ON EXPERIMENTAL BRAIN METASTASIS INHIBITING THE EXPRESSION OF P-GLYCOPROTEIN OF THE BLOOD BRAIN BARRIER**

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The presence of multidrug resistance efflux transporters, such as the P-glycoprotein (Pgp) on the blood brain barrier (BBB), limits the efficacy of chemotherapeutic agents on brain tumors or metastasis. The antiprogestin mifepristone (MFP) is known to inhibit Pgp activity on tumor cells. The murine mammary carcinoma C4-2-HI, whose growth is not inhibited by MFP treatment and the human triple negative MDA-231Br cells were injected into the brain as previously described. Both models are sensitive to Doxorubicin (doxo) treatment. However, when low doxo doses were used, a significant decrease in tumor size was only observed in mice treated with MFP and doxo ($p<0.05$). We hypothesized that MFP inhibits the activity of BBB Pgp allowing a high entry of doxo to the brain. Human Brain Microvascular Endothelial Cells (HBMEC) were treated with doxo (20 μM) with or without MFP (10 μM) for 60 or 120 min. An increase in the intracellular retention of doxo was observed in MFP-treated cells (fluorescence intensity; $p<0.05$). In addition, we evaluated the expression of Pgp by Western blots in HBMEC cells treated with MFP for 24 hours in the presence or absence of dexamethasone, which is known to induce Pgp overexpression. MFP showed an inhibitory effect in both experimental settings ($p=.05$) We conclude that MFP may be a promising agent to increase the effectiveness of chemotherapeutic agents on brain tumors, regardless of their progesterone receptor expression through its action on BBB Pgp.

304. (473) **INTRATUMORAL GENE SILENCING OF MUSCARINIC RECEPTORS BY RNA INTERFERENCE DECREASE HUMAN BREAST CANCER CELLS GROWTH.**

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The ability of interfering RNA (RNAi) to silence target genes with high efficiency and specificity has stimulated efforts to develop these molecules as therapeutic agents in oncology. It has been reported that human breast cancer MCF-7 cells express muscarinic receptors (MR) M_3 and M_4 subtypes and its activation promotes tumoral progression. The aim of this work was to evaluate if intratumoral delivery of RNAi against M_3 and M_4 affects the growth kinetics of MCF-7 cells, *in vivo*. For this, cells treated with or without the cholinergic agonist carbachol (Carb, -8M), were injected subcutaneously in female Balb/c NUDE mice ($1x10^7$cells/0.2mL) that had been implanted with a 17β-estradiol pellet (1mg) a week earlier. After 5 days, mice had small visible tumors and were divided into groups: MCF-7 cells (control) and MCF-7+Carb (RNAi once, RNAi repeated (once a week, four times) and without RNAi). Tumor diameter were measured three times per week and the tumoral volume was estimated. After 38 days, all mice were sacrificed, the tumors were photographed and measured. The data analysis showed that tumors of cells stimulated with Carb had higher growth kinetic and tumor volume compared to control group (day 38: 527.5±77.6 mm^3 vs. 740.5±72.8 mm^3;$p<0,001$). Additionally, repeated RNAi treatment against M_3M_4 subtypes decrease MCF-7+Carb cells capacity to growth (day 38: control: 527.5±77.6 mm^3 vs. MCF-7+Carb+RNAi repeated: 165.2±59.8 mm^3;$p<0,0001$). We also found that tumoral weight of RNAi-repeated group was significantly lower than MCF-7+Carb without RNAi and MCF-7+Carb+RNAi once (100mg±57.1 vs. 335mg±43.8 ($p<0,01$) and 261.5mg±69.8 ($p<0,05$), respectively). Our results show that intratumoral administration of naked RNAi is an effective systemic delivery and prevents its degradation. We observed that gene silencing of MRs, by weekly administration of specific RNAis, decrease both MCF-7 tumors weight and their growth kinetic; suggesting that MR are important therapeutic targets.

305. (479) **NIVELES DE CORTISOL SALIVAL EN PACIENTES CON DESÓRDENES POTENCIALMENTE MALIGNOS Y CÁNCER DE LA MUCOSA ORAL. (ESTUDIO PRELIMINAR)**

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Introducción: La desregulación del eje hipotálamo-hipófisis-suprarrenal por factores psiconeuroinmunológicos como el estrés, aumenta la función suprarrenal elevando los niveles de cortisol en sangre y en saliva. Dicha elevación podría influir tanto en el desarrollo de los desórdenes potencialmente malignos (DPM), como en la evolución del cáncer de mucosa oral (COCE). Un número creciente de estudios muestra que el cáncer la progresión no ocurre solo debido a factores locales relacionado con el tumor, pero que también está influenciado por factores psiconeuroinmunológicos inherentes a el paciente.

Objetivos: 1) Evaluar niveles de cortisol salival en pacientes con DPM y COCE. 2) Determinar presencia de estrés en la población analizada.

Metodología: Estudio de casos y controles aprobado por bioética de la FOR durante 2018-2019. Se incluyeron 15 casos (DPM y COCE) y 15 controles. Se recolectaron muestras de saliva para la determinación del nivel de cortisol. Se evaluó la presencia de estrés con la escala de Holmes y Rahe. **Resultados:** los promedios de cortisol salival fueron 6.980 ng/ml para los casos y 5.740 ng/ml para los controles ($p< 0,05$). Se determinó estrés en 12 casos y 5

controles.
Conclusiones: los niveles de cortisol salival están aumentados en los pacientes con DPM Y COCE comparados con los del grupo control.

306. (485) **ESTROGEN RECEPTOR-ID4 CROSS-TALK IN BREAST CANCER**
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Inhibitor of differentiation (ID) 4, a member of the ID family, has been shown to act as a tumor suppressor and as an oncogene in breast cancer. Our group has investigated this apparent discordant information and has found evidence that ID4 acts as a tumor suppressor only in estrogen receptor ER+ tumors and as an oncogene only in ER- tumors. Here we focus on ID4?s tumor suppressor role and further investigate why ID4 is aberrantly methylated exclusively in ER+ tumors.EZH2 is a histone methyltransferase involved in the tri-methylation of lysine 27 on histone 3 (H3K27me3) and also promotes DNA methylation via DNMT recruitment. In breast cancer EZH2 is overexpressed and downregulates the expression of tumor suppressor genes via increased promoter H3K27me3. Since ID4 is hyper-methylated in ER+ tumors and since EZH2 expression is induced by estradiol we hypothesize that estradiol induces ID4 methylation through EZH2. We performed siRNA (EZH2), immunofluorescence and chromatin immunoprecipitation (CHIP) experiments in MCF7 breast cancer cell lines. Our results show that EZH2 regulates ID4 expression as confirmed by siRNA experiments, that estrogen treatment increases EZH2 expression and ID4 methylation and CHIP experiments reveal that estrogen administration increases EZH2 and H3K27me3 marks on ID4 promoter. Taken together our results show for the first time that estradiol induces ID4 methylation trough EZH2 in breast cancer cell lines.

307. (509) **SOLITARY FIBROUS MENINGEAL TUMOR PRIMARY CULTURES DEVELOPMENT TO STUDY POTENTIAL THERAPEUTIC TARGET**
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Solitary fibrous tumors (SFT) are relatively uncommon spindle-cell neoplasms that may be distinguished from mesotheliomas. SFT are often pleura neoplasms, rarely occurring in the meninges. Most of these tumors harbor a NAB2–STAT6 gene fusions. The activation domain of STAT6 converts NAB2 from an EGR1 repressor to an activator. EGR1 has been shown to activate VEGF, involved in vascular development, and to enhance HIF1α, involved in tumor glycolytic phenotype. Here, we present a multidisciplinary work from the clinic to the basic research of a SFT. After being clinically evaluated, a 45 years old woman underwent a gross total resection of a highly vascularized right frontal parasagittal meningeal tumor. The immunohistochemistry (IHC) showed a strong nuclear positivity for STAT6 confirming SFT entity. Next, we were able to establish a primary culture from a fragment of this tumor both as monolayer and spheroids in DMEM/F12 supplemented with 10% SFB and ATBs. Preliminary results showed that SFT primary cultures viability (determined by Crystal Violet staining, 5 days after treatment) resulted particularly affected with the combination of metformin (an oral antidiabetic drug with potential antitumor activity) with 2DG (up to 80%), which is a glucose analog that inhibits Hexokinase (HK, the first and limiting enzyme of glycolysis) and with 6-AN (up 60 %), which is a Glucose-6 phosphate dehydrogenase (G6PD, the first and limiting enzyme of Pentose Phosphate Pathway) inhibitor. In addition, we found that SFT cells express iNOS (by immune staining), a potential druggable enzyme probably involved in the highly vascularized phenotype of these tumors. Then, we hypothesize that the biological features of SFT may provide therapeutic opportunities for its treatment. Our results denote the relevance of a multidisciplinary work and support further studies to uncover the remarkable mechanisms behind the nature of SFT.

308. (516) **NANOMEDICINE CO-DELIVERY FOR CHEMORESISTANT COLORECTAL CANCER TREATMENT**
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Despite the use of surgical resection and chemotherapy, nearly 50% of patients with colorectal cancer develop recurrent disease, highlighting the need for improved therapies. Administration of antineoplastic drugs in nanovehicles (NV) optimizes the localization of drugs in tumor tissue. This occurs manly due to the enhancement permeably retention effect that reduce peripheral toxicity and increase the local therapeutic effectiveness. Oxaliplatin (Oxp) is a high efficiency chemotherapeutic drug but with adverse effects, thus the encapsulation could significantly improve the effect. Moreover, the combination with sensitizing molecules, such as curcumin (Cur), could yet increase the possibilities of therapy success. This molecule is hydrophobic, so its encapsulation in NV also could improve its delivery. The aim of this work was to evaluate the effect of Cur combined with Oxp in a chemoresistant *in-vivo* model encapsulated in NV performed by microfluidic technology. We generate an *in-vivo* chemoresistant model by serial passaging of bulk T84 subcutaneous tumor xenografts in nude mice treated with Oxp and re-derived at least by four times, resulting in a solid tumor that no respond to Oxp treatment. Furthermore we developed a microfluidic chip for the encapsulation of Oxp in liposomes, obtaining NV of 125.9±1.5nm and 0.23mg/ml Oxp. Moreover, we performed Cur polymeric micelles of 21.3±1.4nm and 2.5mg/ml. In first term we test the Cur (20mM) and Oxp (40mM) effect in a T84ROxp cells administrated independly or in combination *in-vitro* assays (MTT assay), obtaining a cell survival of 70% vs 50% respectively. Next we evaluated these

treatments in the *in-vivo* model. We administrated both compounds free and encapsulated in NV independently or in combination (i.v) and obtained similar effects, but with toxicity signal in mice, such loss of weigh. However, the administration of Cur and Oxp in NV improved significantly the effectiveness in tumor and avoided the peripheral toxicity.

309. (524) **MICROBIOME RELEVANCE IN TUMOR TISSUE OF BREAST CANCER PATIENTS**

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Microbiome composition reflects the host's genetic and lifestyle variables, contributing it's dysbiosis to the risk of developing breast cancer. Patients from diverse countries show different microbial profiles, thus led us to analyze the microbiota in breast cancer patients in Argentina.

In order to study the prognostic relevance of the microbiota in the evolution of early breast cancer, a study was carried out including biopsies of frozen/fresh breast tumor tissues (Hospital Roffo, n=7 and Hospital Italiano, n=1, respectively). Inclusion criteria: women with invasive ductal breast carcinoma, clinicopathological stage I/II with a 5-year follow-up minimum. Exclusion criteria: patients with prior treatment, underlying immune disease or another prior tumor. Samples were processed and DNA was extracted using the QIAamp-DNA-Mini-Kit. The bacterial profile was identified studying V4-V4 hypervariable regions of the 16S rRNA gene. OTUs classification and Alpha and Beta diversity analysis were performed, in order to study possible associations with parameters such as age, tumor size, histological grade, Ki-67, Her2/neu, ER, PR, axillary lymph nodes and tumor evolution.

Taxonomic analysis of these samples indicated that Firmicutes (34.6%) and Proteobacteria (30.8%) are the most representative phyla. We observed that the sample from the H. Italiano presented greater diversity (165k) than the ones from the H. Roffo (43k-96k), therefore the first was not taken into account for the analysis. Alpha diversity analysis showed a significant difference between tumor dimensions, showing a decrease in diversity with the tumor size increase (p-value <0.0001, "t-test" two-tailed).

To conclude, H. Italiano's sample presents a higher OTUs diversity in comparison with H. Roffo samples. In addition, the analysis of different bacterial populations among patients with different tumor sizes opens up new paths on the development of alternative therapies to improve the patient's life-quality.

310. (543) **STUDY OF HV1 CHANNEL EXPRESSION IN CLINICAL SAMPLES OF BREAST CANCER THROUGH CLINICAL DATABASES ANALYSIS.**

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Metabolic reprogramming, an emerging hallmark of cancer, conduces to a large production of acidic substances requiring the over expression of H^+ extrusion cell structures. We have previously reported that proton channel (HVCN1) has a key role in this process in breast cancer cell lines and Jurkat T cells, and its inhibition induces apoptosis. The aim of this work was to analyze the HVCN1 channel expression (mRNA) in clinical samples of normal and neoplastic human mammary tissue and its correlation with key metabolic reprogramming-related gene increased in several types of cancers (Tanner et al., 2018, Cell Systems 7, 1?14). To this end, GEPIA and cBioPortal databases information was used. The analysis of GEPIA database showed that not significant differences of HVCN1 expression (Transcripts per Millon) appears between normal (N=291) and pathological (N=1085) mammary tissues. However, we observed different HVCN1 expression levels within tumor samples of the Breast Cancer METABRIC, Nature 2012 & Nat Commun 2016 study. Selecting samples in which HVCN1 mRNA level was major to one standard deviation of the median (high Hv1 group; N=250) and compared this group with the rest of the samples (control group; N=1653), we observed that the high Hv1 group contains a significantly increased number of triple negative and claudin-low samples respect to the control group (Chi-quadrate test, $p<10^{-10}$), and found that 4530 genes are significantly enriched (Student test) in the high Hv1 group, including metabolic reprograming related genes such as GLUT3 ($p=3.07e^{-19}$), GLUT5 ($p=4.66e^{-25}$), PFKP ($p=9.44\ 6e^{-9}$) and PFKL ($p=2.04e^{-3}$), which are indicated as key genes of glycolytic flux. Altogether, our results demonstrated that Hv1 expression could be associated invasive subtypes and glycolytic phenotype, pointing out the role of this channel in breast cancer.

311. (550) **HISTAMINE H4 RECEPTOR EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER: AN EXPLORATORY STUDY**

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Triple-negative breast cancer (TNBC) is an aggressive BC subtype. Unfortunately, there are neither universally accepted prognostic markers to predict outcomes, nor specific molecular targets related to TNBC. The histamine H4 receptor (H4R) has been characterized in TNBC experimental models, demonstrating its critical role in tumor development and progression. Limited information about the association of H4R expression with markers of prognosis is available. In this study, we investigated the H4R expression in samples of 26 TNBC patients and its correlation with clinicopathological features, and survival estimated by the Kaplan-Meier method. Positive H4R immunoreactivity was observed in about 65% and 81% of tumor specimens and peritumoral breast tissue, respectively. A moderate positive correlation was found between the expression of H4R in the tumoral and peritumoral tissue (Spearman r: 0.4412, P=0.0398). Elevated H4R expression in peritumoral tissue and tumors was observed in patients with negative lymph node metastasis and unifocal TNBC. Even more, a negative correlation between H4R expression and the number of lymph node involvement was observed in peritumoral tissue (Spearman r: -0.5429, P=0.0110), accompanied by a similar tendency in relation to the tumor size. However, no significant association was observed between the H4R expression and tumor grade, stage, Ki67 percentage and lymphovascular invasion in both tumor and histologically-normal samples. Negative H4R expression was associated with reduced survival.

In summary, these results suggest that H4R might represent a potential prognostic biomarker in TNBC patients. Further studies in larger cohorts are needed to better understand the significance of H4R in breast cancer biology.

312. (558) **MUTATIONS IN *RB1* GENE IN RETINOBLASTOMA PATIENTS**

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Retinoblastoma (RB) is the most frequent intraocular malignant tumor in childhood, caused by mutations in *RB1* tumor suppressor

gene. RB may be hereditary (bilateral tumors) or non-hereditary (mostly unilateral tumors). The first mutation can be germinal (RB hereditary) or somatic (RB non-hereditary), the second mutation is always somatic. RB is a potentially curable cancer depending on early diagnosis and treatment. Besides, patients with hereditary RB can transmit the predisposition to their offspring. The aim of our work is the identification of *RB1* mutations, for risk assessment in the offspring and siblings of bilateral patients and for discrimination between hereditary and non-hereditary RB in unilateral patients. Out of a total of 215 patients with RB studied in our lab, the results of 2 bilateral and 2 unilateral are presented here. Mutational detection was conducted by a combined approach of Exome analysis and MLPA. Variants in donor splicing sites were detected in the 2 bilateral patients: c.1389+1G>A and c.2106+1del. Moreover, a newly born sibling could be excluded from RB risk. One of the unilateral patients showed a homozygous deletion of *RB1* entire gene and surrounding genes (*ITM2B-5,RCBTB2-8,DLEU1-2*) in the tumor; this mutation was not present in blood DNA, denoting a non-hereditary RB. The other unilateral patient displayed a heterozygous germline deletion of whole *RB1* gene and neighboring genes (*ITM2B-5,RCBTB2-8,DLEU1-2,PCDH8-3,PCDH8-2*), denoting a hereditary RB and a likely contiguous deletion syndrome. The 3' end was not found, that is, the deletion continues beyond *PCDH8*.
Identification of *RB1* mutations allows detection of RB predisposition in patient´s offspring and siblings which provides access to early treatment to preserve survival and vision. Identification of germinal mutations in unilateral patients allows detecting the risk of tumor development in the other eye and its absence eliminates the need for frequent eye *exams under anesthesia*.

313. (561) **MELANOMA XENOGRAFTS GROWTH INHIBITION BY SOLANUM TUBEROSUM ASPARTIC PROTEASES 3 (*St*AP3) TREATMENT**

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The swaposin domain is present in plant aspartic proteases (APs) inserted into the C-terminal domain as an extra region known as plant-specific insert (PSI). The PSI domain interacts with the plasma membrane, causing cell permeabilization, thus killing plant and human pathogens. A typical Solanum tuberosum AP (*St*AP) named as *St*AP3 was demonstrated to have cytotoxic activity against cancer cells and no effect in normal cells in vitro. The toxicity of *St*AP3 was assessed in a mice model, showing no signs of systemic toxicity. Malignant melanoma (MM) is one of the most aggressive cancers, with high metastatic ability and resistant to therapies. Thus, the pursuing of novel agents against MM is still challenging. Herein, we aimed to evaluate the *in vivo* antitumor effect of *St*AP3 in MM. Subcutaneous A375 human melanoma xenografts in athymic nude mice were induced. Once tumors developed (mean larger dimension = 3.8 ± 0.09 mm), mice were treated with *St*AP3 (6 μg/mg body weight, subcutaneously under the tumor at a single dose) or physiologic solution (controls). Animal experiments complied with the ARRIVE guidelines and were performed with protocols approved by the Argentine National Atomic Energy Commission Animal Care in accordance with the EU Directive 2010/63/EU and NIH Publications No. 8023, revised 1978. A significant inhibition of MM tumors growth was observed in *St*AP3-treated mice ($p < 0.05$) *vs.* controls. This was detected immediately after treatment and was sustained until 15 days post-treatment, with a maximum inhibition of 76%, when control tumors reached 200 mm^3 and animals were sacrificed. As far as we know, this is the first report showing the *in vivo* effect of a plant AP whose action mechanism would be mediated by membrane destabilization, which may be explained by the plasma membrane composition with high levels of phosphatidylserine in the outer leaflet of cancer cells. These results suggest the potential of these plant proteases as anticancer agents.

314. (394) **HYALURONAN MODULATION BY 4-METHYLUMBELLIFERONE (4MU) CONFERS CHEMO SENSIBILITY TO LUNG CANCER STEM CELLS**

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Taxane-platin chemotherapy is widely used for non-small cell lung cancer (NSCLC). However, the majority of patients will progress or relapse. In the tumor microenvironment (TME) cancer cells co-exist with cellular and non-cellular components that drive tumor processes such as chemotherapy resistance. Cancer stem cells (CSCs) are tumor initiating cells identified by CD133, CD44 and ALDH1 among others markers, which form residual niches involved in tumor recurrence. It has been partly described how the TME hyaluronan (HA) regulates CSCs function. We have demonstrated that 4-Methylumbelliferone (4Mu), a modulator of HA synthesis, reduces CSCs properties in hepatocarcinoma. Here, we observed that HA was present on mice Lewis Lung Carcinoma (LLC) tumors. We found HA+ LLC cells; thus cancer cells produced, at least in part, the HA observed in tumors. We observed about 6.58 ± 0.83 % of CD133+ CSCs on *in vitro* cultured LLC cells. Isolated CD133+ cells showed higher expression of HA and CD44 in comparison with non-CSCs population ($p<0.05$). Analysis of HA synthases (HAS), hyaluronidases (HYAL) and the CSCs transcription factors KLF4 and SOX2 expression from NSCLC patients using The Cancer Genome Atlas data showed that while HAS3 positively correlates with levels of KLF4 and SOX2, HYAL2 inversely correlates with SOX2 expression. Also, HAS3 correlates with a shorter disease-free survival when it is highly expressed. Also, 4Mu treatment of whole, CD133+ or CD133- LLC cells showed an inhibitory concentration 50 (IC50) of 0.90mM, 0.60mM and 1.06mM respectively, while non tumor cells showed an IC50 of 4.22mM ($p<0.01$). When CD133+ cells were treated with 4Mu plus chemotherapy we observed a significant decrease in viability with an IC50 of 0.32nM pemetrexed (Pe) vs. 0.15nM Pe+4M, and 7.52nM paclitaxel (Pa) vs. 0.50nM Pa + 4Mu. Our results suggest that targeting HA could turn tumors, particularly CSCs, more susceptible to chemotherapy promoting an effective antitumoral response.

REPRODUCCIÓN

315. (22) **EFFECT OF ZN ON SPERM PHYSIOLOGY**

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Optimal preservation of boar sperm for artificial insemination (AI) is at 16°C. At this temperature, neither cellular metabolism nor microbiological growth conditions can be effectively reduced in seminal doses. Under the hypothesis that Zn's effect on sperm physiology could improve their preservation at lower temperatures, the ob-

jective of this work was to evaluate Zn supplementation on vitality (eosin-exclusion test), motility (Computer-Assisted Sperm Analysis, CASA) and reactive oxygen species concentration (ROS, luminol) in seminal doses stored at 4°C. Also, mitochondrial and DNA integrities were evaluated by developing a new technique based on sperm chromatin dispersion test and cytochrome c oxidase activity. Aliquots of seminal doses were supplemented with 0 (Control, C), 0.5, 1, 2 or 3 mM $ZnCl_2$ and kept at 4°C for three days. Vitality, motility and ROS concentration data were analysed by ANOVA or Kruskal-Wallis in the case the assumptions were not met by ANOVA. Mitochondrial and DNA integrity data were analysed by linear mixed models for longitudinal data. The percentage of motile sperm was higher in doses supplemented with 1 and 2 mM $ZnCl_2$ by day 2 (63.25^a C vs 68.5^a 0.5 mM vs 73.75^b 1 mM vs 71.5^b 2 mM vs 65.5^c 3 mM de $ZnCl_2$ p=0.082) while, by day 3 all doses supplemented with Zn showed higher percentage of motile sperm than the control (47.75^a C vs 61^b 0.5 mM vs 65.75^b 1 mM vs 60.50^b 2 mM vs 61.50^b 3 mM $ZnCl_2$, p=0.0184). CASA analysis demonstrated differences in the linear pattern of movement and percentage of rapid sperm. Mitochondrial integrity was affected only by the time of storage while vitality, ROS concentration and DNA integrity did not show changes neither by treatment nor time of the experiment. The results indicate that Zn preserves motility of sperm storage at low temperature allowing its use for AI.

316. (30) **REGULATION OF LIPID STORAGE BY LEPTIN IN SERTOLI CELLS**

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The process of spermatogenesis and consequently male fertility are dependent upon the somatic cells that are present in the testis. Sertoli cells (SCs) are necessary in order to provide the structural and nutritional support for germ cell development. SCs convert glucose to lactate, the main energy substrate for spermatogenic cells. Consequently, SCs cannot rely on glucose for their own energy requirements and it has been postulated that they utilize fatty acids (FA) as energy source. In addition, FA are stored as triglycerides (TAG) in lipid droplets (LD), being the latter essential to keep ATP levels in SCs. On the other hand, according to the World Health Organization, obesity prevalence has risen dramatically and has been shown to negatively affect male reproductive function through multiple mechanisms. Adipocytes, the main cell type within adipose tissue, secrete Leptin (Lep) and its secretion is increased in obesity. Therefore, the analysis of Lep effects on SC function might explain some mechanisms related to subfertility associated with obesity. 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 Lep on TAG storage in SCs. Cultures of SCs from 20-day old rats were maintained under basal conditions (B) or stimulated with concentrations of Lep observed in non-obese (10ng/ml) and obese (100 ng/ml) patients for 48 h. It was observed that both conditions augment the LD number (B: 0.67±0.17; Lep 10: 1.67 ± 0.41*; Lep 100: 2.9±1.0*. X±SD, LD/cell in one representative experiment out of three. *P < 0.05 vs B). This change was accompanied by an increase in TAG content and in mRNA levels of PLIN1, protein that coats LD. The results presented herein suggest that Lep regulates lipid storage in SCs. Considering that the FA are the main energy source of SCs, Lep would play an important role in the lipid homeostasis of this cell type (PICT2015-228; PICT2018-1291; PIP2015-0127).

317. (35) **ASSESSMENT OF POTENTIAL METFORMIN EFFECTS ON BLOOD-TESTIS BARRIER (BTB) FUNCTION IN JUVENILE RATS**

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Sertoli cells (SC) are the main cellular component of the BTB as intercellular junctions between neighboring SC set up the physical interaction of this structure. The BTB isolates germ cells (GC) providing an adequate microenvironment for GC development. Concordantly, several animal models have shown that defects in the BTB lead to detrimental effects on spermatogenesis. Metformin (Met) is the first-line therapy for children suffering type 2 diabetes. However, no studies have been conducted to assess if early life exposure to Met represents any potential risk to BTB function. The objective of this work was to analyze the *in vitro* and *in vivo* effects of Met on BTB integrity and expression of BTB-related proteins during BTB formation. SC isolated from 20-day-old Sprague-Dawley (SD) rats were incubated in basal conditions or with testosterone (T, 1 μM) (a known positive BTB regulator) in the absence or presence of Met 10 mM. P<0.05 was considered statistically significant. The BTB junctional integrity was measured as Transepithelial Electrical Resistance (TER). Met decreased T-stimulated TER (B:60.2±5.4, Met: 64.0±3.3; T:120.0±7.1*; T+Met: 92.4±6.5$^{\#}$ $\Omega.cm^2$, * vs B; $^{\#}$ vs T). This reduction was accompanied by a decrease in mRNA levels of claudin 11, occludin, ZO-1 and Cx-43. For *in vivo* assays, male SD rats received a daily oral dose of Met (200 mg/kg) while controls (C) received vehicle (water) from postnatal day (PND) 14 to PND 30, period in which BTB is being established. On PND 31, BTB integrity was evaluated using a biotinylated tracer. Gene expression and meiosis progression analyses were also performed. Met treatment led to a mild but significant increase in the percentage of permeated tubules (C:14.5±1.5%; Met:18.8±0.9%*) although no changes in BTB-related gene expression or meiosis progression were observed. Overall, Met disturbed *in vitro* BTB parameters and exerted a slight increase in BTB permeability in juvenile rats. (PICT2015-228)

318. (43) **VIP-DEFICIENT MICE PREGNANCIES EXHIBIT PLACENTAL GLUCOSE UPTAKE AND TRANSPLACENTAL TRANSPORT ALTERATIONS**

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Adequate glucose uptake by trophoblast cells (Tb) is crucial to allow placental and fetal development. Placental metabolic alterations were reported in pregnancy complications and impairment of mTOR activity was observed in placentas with fetal growth restriction. Vasoactive Intestinal Peptide (VIP) has embryotrophic effects. We have recently shown its modulatory effect on Tb metabolism and we described a murine pregnancy model with VIP-deficient Tb cells presenting impaired placentation and reduced fetal weight at gestational day (gd) 14.5/17.5. VIP treatment at gd6.5 restored fetal weight. Our aim was to evaluate *in vivo* placental glucose uptake and transfer to the fetus in this model and the effect of VIP treatment on placental metabolism. We mated VIP+/+ females with VIP+/+ or VIP-/- males. For VIP treatment 2 nmol VIP were injected at gd6.5. At gd17.5 females were intraperitoneally injected with 3mM glucose fluorescent analogue 2-NBDG for 1.5h. Fluorescence of fetal/placental tissue was quantified in a fluorimeter. Placental/fetal GLUT1/mTOR expression was measured by RT-qPCR. For *ex vivo* experiments, VIP+/+ placental explants were incubated with VIP antagonist prior to the *in vitro* addition of 2-NBDG. We found that VIP antagonist impaired glucose uptake in placental explants (p<0.05) from WT mice. VIP+/- placental weight at gd17.5 did not differ from VIP+/+ placentas. Surprisingly, *in vivo* assays showed an increase of glucose uptake by VIP+/- placentas (0.57±0.11 nmol/

gplac vs. 0.38±0.04 nmol/gplac; p<0.05) in line with an increase of GLUT1/mTOR expression in placental/fetal tissue, however transplacental transport remained constant. VIP treatment tended to restore mTOR/GLUT1 expression. These results suggest that while VIP regulates at the cellular level Tb glucose uptake, VIP deficiency *in vivo* triggers compensatory mechanisms at both the placental and fetal tissues that would contribute to placental metabolic adaptations in order to restore fetal growth.

319. (60) **PARTICIPATION OF SIRT1 IN THE REGULATION OF MATURE SERTOLI CELL (SC) ENERGY METABOLISM**

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SCs provide structural and nutritional support for germ cells (GC) development. SC metabolism has particular characteristics. It converts glucose to lactate, the main energy substrate for GC, and uses fatty acids (FA) as its own energy source. SC is also capable of synthesizing triglycerides and storing them in lipid droplets (LD). In this context, the simultaneous regulation of lipid metabolism and lactate production may be relevant to the seminiferous tubule physiology. Sirtuins (SIRT1-7) belong to a NAD^+-dependent enzymes family that act as cellular energy sensors. SIRT1, the most studied member, plays an important role in processes ranging from cell cycle regulation to energy homeostasis. The aim of this work was to evaluate the participation of SIRT1 in the regulation of lactate production and of FA metabolism in SCs. SC cultures obtained from 20-day-old rats were incubated in the absence (B) or presence of resveratrol 50 μM (RSV, SIRT1 activator). Results are expressed as X±SD of three independent experiments (*p<0.05). It was observed that RSV increases lactate production (B: 3.62±0.73; RSV: 5.24±0.76*μg/μg DNA) and glucose consumption (B: 21.34±5.21; RSV: 40.14±5.71*μg/μg DNA). Possible mechanisms involved in the increase of lactate production after SIRT1 activation were evaluated and it was observed that treatment with RSV augments GLUT1 mRNA levels (1.86±0.44* fold variation respect to B). Regarding FA metabolism, RSV treatment decreases LD content (B: 0.52±0.06; RSV: 0.13±0.02* LD/cell) and increases the expression of FA transporter FAT/CD36 (2.01±0.47* fold variation respect to B). In addition, RSV increases Acetyl CoA Carboxylase phosphorylation levels, which is related to active FA oxidation. Taken together, these results suggest that SIRT1 activation would play an important role in the regulation of SC glucose and lipid metabolism, essential for a normal spermatogenesis (PICT2014-0945; PIP2015-0127).

320. (77) **DISRUPTION IN THE SPERM QUALITY OF THE OFFSPRING CAUSED BY MATERNAL OVERNUTRITION IN RATS**

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Obesity has increased in recent years and is the most important noncommunicable chronic disease. Maternal overnutrition may induce multiple pathologies in both women and their offspring. Our previous studies showed that male offspring from high-fat-fed rats exhibited higher body and testis weight and altered puberty. Also, we found a lower number of germ cells, percentage of motile sperm and capacitation. Thus, the aim of the present study was to evaluate the effects of maternal overnutrition, induced by high-fat diet, on the quality and function of sperm in the offspring.To this end, maternal overnutrition were induced by a high-fat palatable (cafeteria) diet, which was supplied continuously until weaning of their offspring, including pregnancy and lactation. Male offspring from rats fed standard (OSD) or cafeteria diet (OCD) were fed with a standard diet, inspected periodically, and euthanized at 60 days of age. In the germ cells we examined the presence of the reactive oxygen species by flow cytometry using a fluorescent probe (2,7-dichlorofluorescein diacetate), DNA fragmentation by TUNEL kit, mitochondrial function using the probe 3.3-diaminobenzidine, the membrane functional status by hypoosmotic swelling test, and the presence of abnormal chromosomes by cytogenetic assay (Evan test). Compared with OSD rats, OCD group showed a lower percentage of the hypoosmotic-reacted sperm (15±1 vs 23±2, p<0.01) and an increase in the abnormal metaphases (6±1 vs 2.1±0.7, p<0.001). No differences were found in the TUNEL positive cells, but OCD exhibited higher fluorescein intensity expressed as relative units (577±74, p<0.01) compared with OSD (233±27). Finally, 50% of OCD rats displayed a lower mitochondrial function, expressed as relative units (94.8±4 vs 97.7±0.4 from OSD, p<0.01).These results indicate that diet-induced maternal overnutrition may contribute to disorders in the fetal programming, particularly in the germ cell quality.

321. (172) **HYPERTHYROIDISM INCREASES MILK IMMUNE CELLS AND IMPAIRS OFFSPRING DEVELOPMENT IN EARLY LACTATION**

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Hyperthyroidism (H) reduced milk ejection and quality, impairing maternal behavior and mammary gland development. However, it remains unclear if H impacts in milk immune cells numbers. Our aim is to assess the influence of H on i) pup maturation and development ii) prolactin secretion and iii) milk immune cells. For this purpose, 10-12 weeks old *Wistar* rats were injected daily with T_4 (0.25 mg/kg until day 18 of gestation, then 0.1 mg/kg until day 2 of lactation L2) to induce H or with vehicle in control group. Rats were mated 8 days after starting T_4 treatment and euthanized L2 (after ketamine/xylazine sedation and oxytocin stimulation for milking). Afterwards, milk and mammary gland samples, minced to reach single cell suspension, were dyed with fluorophore labeled mAbs ($CD45^+$, $CD3^+$, $CD11b/c^+$) and analyzed by flow cytometry. Offspring weights on L1 and 2, head circumference and body length (L2) were measured. Serum of dams and offspring was obtained to determine total T_4 and prolactin levels by RIA. Our results show that H mothers had more implantation sites and pup number (*p<0,05*) and higher pup mortality rate than controls (*p<0,001*). The H pups had lower weight on days 1 and 2 (*p<0,001*), less weight gain, and diminished length and head circumference (*p<0,001*). H group T_4 and prolactin levels were increased in dams (*p<0,01; p<0,001*) but T_4 reduced in the pups (*p<0,001*). The H group had increased % of $CD45^+$ cells (*p<0,05)* and % and absolute quantity of $CD3^+$ cells/μl compared with control while the number of CD11 b/c^+ cells was diminished (*p<0,05*). No changes were observed in mammary gland resident immune cells. These results suggest that T_4 impairs pup development on early lactation. Additionally, milk leukocytes are modulated by H with a cell-lineage specific response. These data suggest that then maternal immune protection transferred through milk to the offspring may be altered in H and highlight the need of evaluating thyroid status in pregnancy and lactation.
Área temática: Reproducción.

322. (186) **PRESENCE OF SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* IN ENDOCERVIX OF ASYMPTOMATIC PREGNANT WOMEN: NOVEL PATHOGEN RESPONSIBLE FOR ADVERSE PREGNANCY OUTCOMES?**

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E. coli can colonize the vagina, usually asymptomatically, although epidemiologic studies have showed that the presence of this bacterium in the endocervix microbiota could be a risk factor for pregnancy. We have previously reported that Shiga toxin (Stx) producing *E. coli* (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Stx2 in rats in early or late stage of gestation. **The goal** of this study was to detect STEC in the endocervix from asymptomatic pregnant women. Endocervical swabs from 103 asymptomatic pregnant women with gestational age of 12 to 30 weeks from the National Hospital Posadas were enrolled. Swab samples were enriched in Tryptic Soy Broth and then streaked on sorbitol-MacConkey (SMAC) agar. *E. coli* was confirmed by the presence of *uidA* gene detected by polymerase chain reaction PCR. The positive samples for *E. coli* were analyzed for STEC virulence factors genes such as: *stx1, stx2, eae,* rfb_{O157}, $lpfA_{O113}$ and *hcpA genes*. The *stx2* positive *E. coli* samples were grown in Luria-Bertani Broth and the filter-sterilized bacterial supernatants (SN) were used to evaluate Stx2 activity on Vero, Swan and Hela by cell viability assay. **Our results** showed that 14.6% (15/103) of the endocervical samples were positive for *uidA* gene. Additionally, we found that 8.7% (9/103) was positive for *stx2* and 5.8 % (6/103) for $lpfA_{O113}$ and *hcpA* genes. The SN of one of them expressing *stx2* gene had a high cytotoxic activity on Vero, Swan 71 and HeLa cells. Stx2 identity was checked using an anti-Stx2 antibody in order to neutralize the cytotoxic effects. **In conclusion**, we demonstrate that STEC can be asymptomatically present in the endocervix and that can potentially express Stx2. This study may open a new perspective to understand whether STEC can be a novel pathogen involved in adverse pregnancy outcomes.

323. (197) **OVARIAN PROTEOME OF VIZCACHAS (LAGOSTOMUS MAXIMUS, CAVIOMORPHA, RODENTIA) IS MODULATED BY ENVIRONMENTAL FACTORS**

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Proteome is the complete set of proteins expressed by an organism, and it actively changes in response to both internal and external conditions. Since estradiol levels of pregnant vizcachas strongly correlate with climate variables we aimed to study the effect of such variables on the ovarian proteome. For this, ovarian protein extracts from two groups of pregnant females (n=2 each group) with 105±6 days of gestation were used for proteomic analysis. The years of capture of each group, 2011 and 2015, were characterized by low temperature/high precipitation and high temperature/low precipitation levels respectively. Briefly, equal amounts of protein extracts were analyzed using MALDI-TOF/MS and then, LC-ESI/MS (Orbitrap). The resulting peptides were identified with Proteome Discoverer Software using the Rodentia UniProt Database, and functional enrichment analysis was performed using DAVID, STRING and FunRich softwares. Proteins differentially expressed in each year were plotted on a volcano plot (t-test, $p < 0.05$). Through functional enrichment analysis it was corroborated that apoptosis regulation and platelet degranulation processes prevailed in 2011-ovaries, while in the 2015-ovaries signal transduction was favored. In addition, the interactomes defined by the overexpressed proteins showed a very distinct topography in 2011 vs 2015, with different nodal peptides in each situation. This is the first large-scale data analysis of the vizcacha proteome. The present work showed an ovarian expression profile that significantly varies under different climatic conditions. Finally, this work provides new markers for future investigations on the modulation of ovarian function. Grants: Fundación CIentífica Felipe Fiorellino; PIP (CONICET)110/14

324. (223) **RESVERATROL IMPAIRS CELLULAR MECHANISMS ASSOCIATED WITH ENDOMETRIOSIS DEVELOPMENT**

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Endometriosis (EDT) is a benign gynecological disease with no available effective treatment due to its adverse side effects. Resveratrol (RES) is a natural polyphenol with well-known anticarcinogenic properties found abundantly in grapes, peanuts, and berries. We and others have shown its inhibitory effect on EDT development but its molecular mechanism is still unknown. The aim of this study was to evaluate the effect of RES (50-100μM) on the proliferation, migration and apoptosis of endometriotic epithelial cell line 12Z and endometrial stromal cell line St-T1b. We also studied its effect on gene expression related to cell migration and angiogenesis, and on the maintenance of stem cell pluripotency in both cell lines and in primary endometrial epithelial cell cultures (EEC). RES significantly decreased cell viability after 48h in both concentrations and both cell lines ($p<0.01$), and reduced wound healing size after 8h and 20h with 100μM and 50 μM RES respectively ($p<0.05$). The number of apoptotic cells, assessed by FITC Annexin V/PI, was increased after 24h ($p<0.01$) as well as cleaved caspase-3 levels, assessed by Western Blot ($p<0.05$). On the other hand, real time PCR showed that treatment with 100μM RES reduced MMP2 and increased Timp1 mRNA expression in both cell lines ($p<0.05$). Angiotensin1 mRNA levels decreased with both RES doses ($p<0.05$). Besides, 100μM RES decreased VEGF mRNA levels only in St-T1b ($p<0.05$). EEC treated with 100μM RES displayed an increase of Timp1 and a decrease of MMP2, VEGF and Angiotensin1 mRNA levels ($p<0.05$). Among the stem cell pluripotency markers, 100μM RES provoked an increase in Notch1, Snail1, KLF4, Sox2 and Tert mRNA levels in both cell lines and in EEC ($p<0.05$). Also, the expression of Oct4 mRNA increased in St-T1b and EEC ($p<0.05$) and Vimentin mRNA exerted a significant upregulation only in EEC ($p<0.01$). These findings revealed that RES treatment affects several signaling events implicated in EDT development and progression.

325. (280) **GLUCOCORTICOID RECEPTOR CHARACTERIZATION AND DEXAMETHASONE LEPTIN REGULATION IN PLACENTAL CELLS**

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Leptin is a key hormone in placental physiology. It regulates trophoblast survival and fetal maternal tolerance by the induction of HLA-G in placental cells. The expression of leptin in trophoblastic cells is regulated by different endogenous signals. Previous results from our lab demonstrated that estradiol (E2) regulates leptin expression. In this study we aimed to characterize glucocorticoid receptor (GR) and analyze the effect of the synthetic glucocorticoid dexamethasone (DEX) on leptin expression in human placental cells. BeWo cells cultured under standard conditions, and human placental explants were used as well. Western blot immunofluorescence and transient transfection analysis were carried out. We analyze (GR) expression in placental explants. Two isoforms of 67 and 56 KDa were characterized and the smaller one was increased after E2 treatment. Neither of them was induced by 100 nM DEX. Besides the incubation with 100 nM DEX significantly diminished leptin expression regardless the presence of E2. Endogenous GR was not able to be detected in BeWo cells. However, overexpression of a recombinant GR-GFP protein in BeWo cells localized in the nucleus and activated

MMTV promoter dependent luc transcription after DEX stimulation. These results demonstrated that GR pathway is active in these cells. In conclusion GR protein is expressed in placental cells as 67 and 56 KDa isoforms and probably mediates the regulation of leptin expression by DEX.

326. (283) **POLYCYSTIC OVARIAN SYNDROME ALTERS THE EXPRESSION OF MOLECULES INVOLVED IN THE UTERINE FUNCTIONAL DIFFERENTIATION OF PERIPUBERTAL RATS**

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The uterus functional differentiation is a process that involves changes in cell proliferation and differentiation at all stages of life. Polycystic ovarian syndrome (PCOS) is associated with higher risk of infertility and endometrial hyperplasia. The aim of this study was to evaluate whether PCOS alters the expression of molecules involved in functional differentiation of the rat uterus. To induce PCOS, Wistar rats were injected subcutaneously with dehydroepiandrosterone (6mg/100g body weight, PCOS group) from postnatal day 21 (PND21) to PND40, control group receive sesame oil. At PND41, the uterine horns were collected. Molecules involved in cell growth and proliferation [ie: Insulin-like growth factor 1 (IGF-1), IGF-1 receptor (IGF-1R), Homeobox gene A10 (Hoxa10), phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN)] and involved in uterine development and differentiation [ie: Wingless-related MMTV integration site member 5a and 7a (Wnt5a and Wn7a) and β-catenin] were evaluated by RT-PCR and/or immunohistochemistry. In PCOS rats, IGF-1 mRNA was increased whereas IGF-1R mRNA was similar to Control rats. Hoxa10 mRNA expression was similar between experimental groups whereas the expression of the tumor suppressor gene, PTEN, was decreased both in subepithelial stroma and in myometrium of PCOS rats. In these rats, Wnt7a mRNA was not modified but a decrease of Wnt5a (mRNA and protein) and an increase of β-catenin (mRNA and protein) was observed. Wnt5a was expressed in all uterine compartments whereas β-catenin was markedly expressed in the cell membrane of epithelial cells. The results suggest that increased IGF-1 and decreased PTEN could be responsible for dysregulation of uterine cell proliferation in PCOS rats. Furthermore, the decrease in Wnt5a and the increase in β-catenin in the cell membrane suggest a down-regulation of mechanism regulated by canonical Wnt signaling. These changes may help explain the uterine abnormalities observed in women with PCOS.

327. (350) **DEVELOPMENT OF A MURINE MODEL TO STUDY THE EFFECT OF METABOLIC SYNDROME ON ENDOMETRIAL FUNCTION AND FEMALE FERTILITY**

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The correct functioning of the reproductive axis depends on an adequate energy balance, therefore studying the influence of the physiological alterations associated with metabolic syndrome (MS) as a single entity is important to understand the associated female fertility problems. The aim of this study was to develop and characterize an optimal experimental in vivo model for MS with the future objective of studying the effect of MS on endometrial physiology and reproductive disorders. Twenty one-days-old female C57BL/6 mice were divided in two groups: one received a high fat diet (HF) and the other one, a control diet (CD) for 13 or 14 weeks. Animals were weighed weekly and a significant increase in weight of HF mice was observed at week 11 ($p<0,05$). On the last day of experiment, glycemia was measured using a glucometer with One Touch test strips at two fasting times: 4 h and 6 h, observing a significant increase in HF group after the longer fast ($p< 0,01$). Animals were sacrificed at estrus and the blood was collected by cardiac puncture for biochemical analyses of cholesterol, triglycerides, HDL and LDL. The uterine horns, the adipose tissue and the liver were removed and fixed. Significant changes in cholesterol, LDL and HDL were observed in HF mice ($p<0,05$), but no changes were found in triglycerides levels. Adipose tissues were weighed, seeing a significant increase in the weight of visceral and gonadal adipose tissue in HF mice ($p<0,05$). Steatosis was evaluated by hematoxylin eosin (H-E) staining in liver sections, observing a fatty liver in the HF group. As an approximation to study the reproductive profile, cell proliferation, was assessed by PCNA immunohistochemistry and endometrial glands and leukocytes were count in H-E stained uterine sections in a preliminary experiment, but no changes were observed between groups. These results are promising for the further efforts to evaluate the effect of MS on female reproduction.

328. (355) **PLACENTAL APOPTOSIS INDUCED BY CHEMICAL HYPOXIA IS COUNTERACTED BY LEPTIN**

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Leptin acts as a regulatory hormone in the maternal fetal interface. We demonstrated that leptin promotes proliferation and survival of trophoblastic cells. Moreover, leptin prevents cellular stress under hypoxic condition in trophoblastic cells. In this sense, Leptin is incremented in different pregnancy pathologies such as preeclampsia. In this work we aimed to elucidate the mechanisms involved in Leptin antiapoptotic effect on placental apoptosis induced by cobalt chloride ($CoCl_2$). This agent stabilizes HIF-1α transcription factor. We used Swan-71 cells, a cytotrophoblast human cell line and human term placental explants cultured under normoxia and hypoxia conditions. Swan-71 cells and placental explants were treated with $CoCl_2$ (50 or 100 μM) in presence or absence of leptin (100 ng/ml). The expression of HIF-1α, p53, Caspase-3, cPARP and Mdm2 was determined by Western blot. Apoptosis was determined by the visualization of apoptotic nuclei by IF. All procedures were approved by ethical review committee at the Alejandro Posadas National Hospital.

We observed that HIF-1α stabilization increased apoptosis (1.54 ±0.1) in Swan-71 cells. Treatment with $CoCl_2$ increased (1.8 ± 0.5) PARP-1 and (1.7 ± 0.2)

Caspase-3 levels indicating that apoptosis was induced. Leptin treatment diminished this effect ($p<0.01$). Leptin was capable to regulate p53 pathway, increasing Mdm2 (1.4 ± 0.4) expression, a p53 negative regulator in hypoxia condition. All these results suggest that HIF-1α stabilization enhances placental apoptosis and leptin is capable to protect these cells under hypoxic conditions.

Key words: Placenta, apoptosis, hypoxia, leptin

329. (371) **HIGH FAT DIET FEEDING IN FEMALE MICE AFFECTS OVARIAN FUNCTION**

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Female fertility is highly dependent on a correct energy balance. Metabolic disturbances are increasingly common in women of reproductive age, leading to menstrual dysfunction, subfertility and pregnancy complications.

However, it is still not clear how high fat diet (HFD) affects reproductive function.

Angiogenesis is a physiological process in the ovary that allows the correct follicular development. Alterations in the balance of pro and anti angiogenic factors may cause ovarian pathologies.

Hypothesis: HFD causes ovarian alterations that affect follicular development and ovulation.
Objective: To analyze the effects of a HFD on metabolism and ovarian function in female mice.
Methodology: 21 days-old female C57BL/6 mice were fed with a HFD (45Kcal% fat) or a control diet during 14 weeks. The animals were weighed once a week. Estrus cycle was evaluated by vaginal cytology and glycemia was measured. After the sacrifice, serum, gonadal and visceral fat and the ovaries were extracted to measure metabolic parameters and to perform histochemical and western blot analysis. Unpaired t-test was used.
Results: the animals fed with a HFD had higher body weight, glycemia, total cholesterol (Cho), HDL-Cho and LDL-Cho. Their adipocytes were hypertrophied and the animals showed increased gonadal and visceral adipose tissue. The estrus cycle was shorter in HFD-fed animals compared to controls. The number of cycles was increased in the HFD group. However, anovulatory stages were longer in HFD animals. In the ovary, periendothelial cell area and cellular proliferation were increased, while the percentage of corpora lutea was decreased. In addition, PDGFB was decreased in the ovaries of HFD-fed animals.
Conclusion: HFD feeding affects metabolism and ovarian function, leading to an alteration in the estrous cycle and a decrease in ovulation. Changes in cellular proliferation, periendothelial cell area and PDGFB may be some of the possible causes of the observed alterations.
KEY WORDS**:** High fat diet, ovary, angiogenesis, metabolism

330. (399) **INTERLEUKIN-6 CONCENTRATION IN SERUM AND PLACENTAL EXTRACTS DURING PORCINE GESTATION**
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Mammalian pregnancy requires coordinated interactions between the conceptus and gestating endometrium that involves the generation of an inflammatory immune response. In earlier stages after implantation, this local inflammatory immune response produces an anti-inflammatory response, that includes numerous cytokines that regulate the intrauterine and systemic environment. This study aimed to evaluate the profile expression of IL-6 during porcine gestation. Swine placentas and sera were collected at 32, 44, 50, 60, 70, 80, 90, 114 days of gestation (dg) from slaughterhouses near General Pico, La Pampa, Argentina. Animals were sacrificed according to the animal welfare manual of National Agrifood Health and Quality Service (SENASA). The placentas were separated in fetal and maternal portions to obtain homogenates of the fetal placenta (HoFP) and maternal placenta (HoMP). As a control, we prepared homogenates from non-pregnant uteri (HoU). IL-6 concentration was determined by a specific porcine ELISA kit (R&D Systems, USA). In this first approach of this study, it was observed that this cytokine is elevated at 32 dg in HoFP (878.29 pg/ml). Around 32 days of gestation begins the ossification of the embryos and the stage of the exponential growth of the placenta. After this peak, its concentration decreased reaching basal values (below 37 pg/ml), which are similar concentrations than that determined in HoU (34 pg/ml). On the other hand, analyzing the results in HoMP, we found a markedly increasing of IL-6 at 50-80 dg: 50 (48.4 pg/ml), 60 (126.74 pg/ml), 70 (66 pg/ml), and 80 dg (87 pg/ml). In this period occurs the end of placental development and the beginning of the exponential growth of fetuses. Finally, we found a pattern of two biphasic curves of IL-6 expression. The first corresponds between 44-50 dg and the second correspond to the 70-90 dg, reaching a maximum value at 90 (384.85 pg/ml). Interestingly, we observed a basal value in serum at 60 dg which is the same period in that we found the maximum value for IL-6 in HoMP. As a preliminary conclusion, the variability of the IL-6 expression observed, suggest that this cytokine could be necessary in the molecular events that occur at placental remodeling during gestation.

331. (410) **LPS FROM *P. GINGIVALIS* AFFECTS TROPHOBLAST-NEUTROPHIL INTERACTION THROUGH TLR4 AND FAVORS A PROINFLAMMATORY MILIEU**
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During placentation, trophoblast cells interact and secrete cytokine in order to regulate and maintain immune homeostasis. Changes or defects in this interaction may lead to pregnancy complications. In fact neutrophil activation is associated with poor placentation and severe pregnancy complications. *Porphyromonas gingivalis (Pg)* is an important pathogen of periodontal disease that has been implicated in adverse pregnancy outcome although the mechanisms involved are still unclear. Pg-LPS is the most important virulence factor of Pg and activates both TLR4 and TLR2.
The aim of this work was to evaluate the effect of conditioned media of trophoblast cells primed with Pg-LPS on neutrophil function.
Human trophoblastic cell line Swan-71 was treated with Pg-LPS (10ng/ml) or Pg-LPS ultrapure, variant that only activates TLR4. Cytokine expression was evaluated by RTqPCR, flow cytomery and ELISA. Peripheral blood neutrophils (Neu) and mononuclear cells (PBMCs) were purified from healthy donors and cultured with conditioned media from trophoblast cells (TbCM) treated or not with LPS (PgLPS-CM) or LPS ultrapure (PgLPSultra-CM). Apoptosis and reactive oxygen species (ROS) were evaluated by flow cytometry. Regulatory T cell induction was evaluated by flow cytometry.
Pg-LPS ultrapure increasde the expression of TNFa, IL6 and IL1b respect to basal while Pg-LPS had no effect. In line with this result, Pg-LPS-CM had no effect on neutrophil activation whereas PgLP-Sultra-CM increased neutrophil activation with higher release of ROS and decreased apoptosis rate ($p \leq 0.05$). Neutrophils exposed to TbCM induced FOXP3 expression on CD4+ T cell after 48h of co-culture with PBMCs. This induction was diminished when neutrophil were preconditioned with PgLPS-CM or PgLPSultra-CM ($P<0,05$).
The inflammatory effects of TLR4-dependent Pg-LPS on the trophoblast-neutrophil interaction could contribute to the pathogenic mechanisms of Pg infection in early pregnancy.

332. (421) **ENDOPLASMIC RETICULUM STRESS THROUGH ATF6A PATHWAY INDUCES AN INFLAMMATORY RESPONSE: POTENTIAL REGULATION BY MIRNAS**
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During decidualization, endometrial stromal cells undergo endoplasmic reticulum stress (ERS) and unfolded protein response (UPR), which will allow them to expand their endoplasmic reticulum with the corresponding machinery for protein folding. These processes are directed by miRNAs that regulate the expression or stability of their transcription factors. Here we focus on the role of ERS/UPR during decidualization to induce a physiological sterile inflammatory response and whether it might be regulate by miRNAs. We used an *in vitro* model of decidualization represented by human telomerase-immortalized endometrial stromal cell line St-T1b, treated with 8-Br-cAMP (0.5 mM) during 5 days, or Thapsigargin (Tg, a RS-inducer 1 μg/ml); and endometrial biopsies from patients with recurrent spontaneous abortions (RSA) and recurrent *in vitro* fertilization failures (RIF).
We evaluated the expression of the ERS-sensor ATF6 and the UPR marker, CHOP. Both markers increased in decidualized cells, and Tg induced even higher levels in comparison with non-decidualized cells ($p<0.05$, t-test). Then, we evaluated the modulation of TXNIP, a link between the ERS-pathway and inflammation. TXNIP increased

in decidualized cells, and also the inflammasome NLRP3 and IL-1β expression ($p<0.05$, t-test). Then, using an *in silico* analysis using miRTarBase v8.0 we selected two miRNAs able to regulate the ERS and UPR pathways: miR-193b-3p and miR-21-5p. Both miRNAs significantly decreased in non-decidualized cells in the presence of Tg ($p<0.05$, t-test). Finally, we studied the expression and localization of miRNAs through an *In Situ* Hybridization (ISH) technique in endometrial samples. Both miRNAs were expressed in stromal cells and endometrial glands in samples from RSA and RIF patients at similar levels. The present results suggest that decidualization in St-T1b cells is accompanied by an ERS and UPR associated with a sterile inflammatory response potentially regulated by miR-193b-3p and miR-21-5p.

333. (532) **EPIGENETIC DISRUPTION OF PLACENTAL GENES BY CHRONIC MATERNAL CAFETERIA DIET IN RATS**

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Cafeteria (CAF) diet is an experimental rodent model which reflects variety, palatability, and energy density food of western diet habits. Previous results indicated that CAF diet alters fertility and pregnancy performance, although the mechanism involved remains unknown. Our aim was to study the effects of a maternal CAF diet on feto-placental parameters on embryonic day 21 (E21) and analysed the implications of key placental systems: insulin growth factor (IGF) and vascular endothelial growth factor (VEGF). Female Wistar rats were fed with control (CON) diet (pellets) or CAF diet (i.e.: cheese snacks, sweet biscuits, and chocolate) from weaning. After 14th week of feeding, females were mated and half of the animals of each group were euthanized at E21. We determined fetal weight and length, and placental weight and index (placental weight/fetal weight ratio). The rest of the animals were maintained until delivery with the respective diet to assess the weight of pups at birth. Placentas were collected for mRNA quantification of IGF1, IGF1R, IGF2, IGF2R, VEGF and VEGFR and for DNA methylation analysis of their promoter regions. Feto-placental parameters were analyzed using Student´s T test; for mRNA expression and DNA methylation levels a Mann-Whitney U test was performed. CAF diet produced a decrease of placental weight and index on E21 and a low weight of pups at birth. In addition, we found an upregulation of IGF2 and down regulation of VEGF placental mRNA expression in CAF dams. Importantly, these changes were associated with modifications in DNA methylation levels of their respective promoter regions. These results indicate that maternal CAF diet impairment of placental growth and pups weight at birth could be explained, at least in part, with an epigenetic disruption of IGF and VEGF systems. Identifying how epigenetic targets are dysregulated by diet factors will allow the development of prevention strategies to improve human and animal reproductive health.

TOXICOLOGÍA

334. (12) **EFFICACY AND TOXICOLOGY OF SILVER NANOPARTICLES AS A COMPONENT OF WOUND HEALING DRESSING IN ZEBRAFISH EMBRYO AND LARVAE**

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Silver nanoparticles (AgNPs) have an important role in nanotechnology since they possess exceptional bactericidal activity. Increasing applications are the AgNP-based wound dressing, providing a large surface area with high bactericidal efficacy. We aimed to design a film of hydrogel, composed of autochthonous and low cost polymers. Within the film, oil-in-water emulsions will be used to encapsulate and delivery AgNPs, vitamins, anesthetic, and anti-inflammatories. This novel device for the treatment of wounds and burns will be enriched with supplements focused in the reduction of pain and in the acceleration of healing process. As a first step, we will carry out an analysis of efficacy of the AgNPs in opportunistic bacterias commonly found on the skin, and toxicology of the AgNPs in zebrafish embryo and larvae. Results will help to select a concentration to next use in the emulsion. To evaluate effectiveness of AgNPs, Staphylococcus aureus and Pseudomonas aeruginosa were incubated with 1.6-60 ppm AgNPs, and minimal inhibitory concentration (MIC) was determined. To evaluate toxicology of AgNPs, Zebrafish embryo and larvae were incubated with 0.1-100 ppm AgNPs, then studies of LC50, acute toxicity (mortality), teratogenicity (hatching rate and morphology), general developmental abnormalities and specific tissue toxicity (brain and heart function) were conducted. The bacterial inhibition indicated a high susceptibility of Staphylococcus aureus and Pseudomonas aeruginosa from 30 ppm. It was observed that AgNPs, in the highest concentrations, caused a delay in the development of embryos, morphological abnormalities in the larvae, decreased swimming and heart rate. In the larval lethality study, it was observed that the LC50 at 1 hs post-incubation was 683 ppm, and at 48 hs post-incubation, it was 108 ppm. The comparison of in vitro and in vivo results allows us to find a functional and harmless AgNPs dose.

335. (27) **EFFECTS OF DEVELOPMENTAL EXPOSURE TO GLYPHOSATE AND PROPICONAZOLE ON THE MALE RAT MAMMARY GLAND**

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Several agrochemicals have been shown to impaired mammary gland development, some of them acting as endocrine disrupting chemicals. Since the male rat mammary gland is susceptible to endocrine disruption, it is a useful model to study the effects of these compounds. Here, we evaluated whether developmental exposure to glyphosate (GLY), propiconazole (PRO) or both (PROGLY) alter the mammary gland morphology and its endocrine response in postpubertal male rats. Pregnant rats were exposed orally to vehicle (saline solution), 4 mg/kg/day of GLY, 4 mg/kg/day of PRO or both doses of GLY and PRO, from gestation day 9 until weaning. On postnatal day 60, the male offspring were sacrificed and mammary gland samples were collected. Total area, perimeter, longitudinal growth, number of terminal end buds and mammary development score were analyzed in mammary gland whole-mounts (WMs). Lobuloalveolar development and the presence of hyperplastic structures were evaluated in histological sections, as well as estrogen (ESR1) and androgen receptor (AR) protein expression. Among the parameters evaluated in WM, only mammary gland total area was reduced in GLY exposed males (Control: 492±16 mm^2, GLY: 419±21 mm^2, PRO: 436±17 mm^2, PROGLY: 473±19 mm^2; Control *vs* GLY $p<0.05$). Regarding mammary gland morphology, PRO animals presented an enhance lobuloalveolar development ($p<0.05$) and 27% of the animals developed lobular hyperplasias, whereas no differences were observed in the percentage of hyperplastic ducts between experimental groups ($p>0.05$). The protein expression of ESR1 was lower in GLY males (Control: 15.3±0.72%, GLY: 11.0±0.74%, PRO: 15.5±1.4, PROGLY: 12.6±1.0%; Control *vs* GLY $p<0.05$) and AR expression was similar between experimental groups ($p>0.05$). In conclusion, GLY and PRO have different effects in mammary gland development; however, these effects are not observed when the

males are exposed to both compounds.

336. (72) **EFFECTS OF *IN UTERO* EXPOSURE TO BENZOPHENONE-3 IN PUBERTAL MURINE MAMMARY GLAND**

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Benzophenone-3 (BP3), an ultraviolet radiation filter commonly used in sunscreens and other personal care products, has shown endocrine disrupting properties. Our aim was to evaluate whether *in utero* exposure to BP3 alters mammary gland (MG) development during puberty. Pregnant C57BL/6 mice were dermally exposed to vehicle (sesame oil; Control), 0.15 (0.15B) or 50mg BP3/kg/day (50B) from gestation day 8.5 to 18. Gestational parameters such as the length of gestation, the weight gain of dams and the number of pups per litter were recorded. Also, the offspring body weight (BW) gain during lactation was evaluated. After weaning, the onset of puberty in females was recorded as the day of vaginal opening. MG samples were obtained in diestrus on postnatal day 45. Total and epithelial area, perimeter, longitudinal growth, branching and number of terminal end buds (TEBs) were measured in MG wholemounts (WMs), whereas MG morphology was analyzed in histological sections. The expression of Ki-67, estrogen receptor and progesterone receptor (PR) was evaluated by immunohistochemistry. The gestational parameters in the dams, as well as the BW gain and the onset of puberty in the female offspring were similar between groups. Histo-morphological analysis showed similar MG growth between groups, with ducts extending beyond the lymph node, ductal-side branching and TEBs. The proliferation index (Ki-67) and the expression of steroid hormone receptors were similar in all groups. However, in animals exposed to BP3, PR-positive cells were observed in clusters in MG ducts, suggesting future branching points. Although *in utero* exposure to BP3 induces limited changes in the female mammary gland at puberty, these changes could be associated with mammary ductal branching and an acceleration of its development.

337. (173) **CHLORPYRIFOS PROMOTES THE MIGRATION OF BREAST CANCER CELLS ACTIVATING C-SRC, AKT, GSK-B AND P38 PATHWAYS**

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Chlorpyrifos (CPF) is a pesticide whose effects on breast cancer risk are not clear. Our aim was to analyze if CPF promotes migration and the pathways involved in MCF-7 and MDA-MB-231 cell lines. Migration was evaluated by 2D (wound healing and Boyden Chamber assays) and 3D assays (multicellular spheroids). c-Src, AKT, P38 and GSK3-β were studied by Western Blot. In MCF-7 cells, 0.05 μM CPF induced the migration after 48 h ($p<0.01$) that was reversed with IC182,780 (1 nM) and PP2 (1 μM) together ($p<0.001$). 50 μM CPF ($p<0.001$) promoted the migration in a c-Src-dependent way ($p<0.001$). 0.05 ($p<0.001$) and 50 μM ($p<0.01$) CPF induced migration on 3D model after 5 days. This increment induced by 0.05 μM and 50 μM CPF was retarded in presence of IC182,780 ($p<0.01$ and $p<0.05$, respectively). No changes were observed after adding PP2. Migration induced by 0.05 μM and 50 μM was retarded by adding IC182,780 and PP2 ($p< 0.001$). Healing assays showed that migration induced by 0.05 µM CPF ($p < 0.001$) and 50 µM CPF ($p< 0.001$) was reverted by adding PP2 ($p< 0.001$) after 24 h in MDA-MB-231 cells. c-Src-dependent-migration was also promoted by 0.05 µM and 50 µM CPF ($p< 0.05$) in Boyden chamber assays.

In MCF-7 cells 2D culture, 0.05 ($p<0.01$) and 50 µM CPF ($p<0.001$) increased p-c-Src after 15 min. p-AKT was induced by 0.05 μM CPF after 60 min in ERα-dependent way ($p<0.05$). p-AKT was also induced by 50 μM CPF depending on c-Src ($p<0.05$). p-GSK-3β was induced by 0.05 μM CPF after 60 min in ERα ($p<0.01$) and c-Src ($p<0.01$) dependent way. p-P38 induced by 0.05 ($p<0.01$) and 50 µM CPF ($p<0.05$) was dependent on c-Src. In MDA-MB-231 cells, CPF induced p-c-Src after 30 min ($p< 0.05$) and 60 min ($p<0.001$). p-AKT was increased by CPF in an independent way of c-Src activation. p-P38 and p-GSK-3β could be countered by PP2. We show that CPF promotes the migration of breast cancer cells activating c-Src, AKT, GSK-3β and P38 pathways pointing out importance to minimize the exposure to the pesticide.

338. (181) **LINE-1 RETROTRANSPOSON ACTIVATION IN NON-TUMORIGENIC MAMMARY EPITHELIAL CELLS EXPOSED TO PESTICIDES. A LINK TO WHY IS BREAST CANCER ON THE RISE?**

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Pesticides exposure is linked to the rise in breast cancer incidence. Long interspersed nuclear element-1 (LINE-1) is inhibited in somatic tissues but its reactivation was implicated in mammary tumorigenesis. The pesticides hexachlorobenzene (HCB) and chlorpyrifos (CPF), both ligands of the aryl hydrocarbon receptor (AhR), increase LINE-1 activation in breast cancer cells MDA-MB-231, through AhR/c-Src and TGF-β1/Smad3 signaling. Our aim was to evaluate if LINE-1 can be activated by pesticide exposure in non-tumorigenic mammary epithelial cells NMuMG. We used environmentally relevant doses of CPF (0.05-50 μM) and HCB (0.005-5 μM).

LINE-1 mRNA levels were examined by RT-qPCR, finding an increment after 48 h of exposure with CPF (0.05-5 μM, $p<0.001$) and HCB (0.005 μM, $p<0.05$). c-Src phosphorylation was enhanced after 15 min of CPF treatment at all assayed doses ($p<0.05$), but Smad3 was activated only at 5 and 50 μM ($p<0.001$), analyzed by Western blot (WB). Cells were pretreated with 5 μM 4,7-orthophenanthroline (PHE, AhR inhibitor) or 2 μM SB431542 (TGF-β1 pathway inhibitor), in order to evaluate the role of these pathways in the 5 μM CPF-induced LINE-1 expression. RT-qPCR data showed that both inhibitors prevented the CPF-induced LINE-1 mRNA levels ($p<0.01$). Previous results showed that 0.005 μM HCB activates the AhR/c-Src axis in NMuMG, without changes in Smad3. Here we observed that PHE blocked the HCB-induced LINE-1 mRNA levels ($p<0.05$). CPF (5-50 μM) and HCB (0.05-5 μM) raised the expression of ORF1p, a protein encoded by LINE-1 (WB, $p<0.01$). Given that ORF1p nuclear levels are associated with poor prognosis, ORF1p subcellular localization was examined. WB analysis shows that 0.05, 0.5 and 5 μM CPF induced ORF1p nuclear import ($p<0.01$). HCB enhanced nuclear and cytosolic ORF1p levels at 0.05, 0.5 and 5 μM ($p<0.001$). In conclusion, HCB and CPF increased LINE-1 expression and ORF1p nuclear import in NMuMG, which could contribute to breast cancer risk.

339. (191) **PESTICIDE EXPOSURE INDUCES MAMMOSPHERES FORMATION AND PROANGIOGENIC FAC-**

TORS EXPRESSION IN BREAST CANCER CELLS HER2 POSITIVE

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Exposure to environmental pollutants, such as pesticides, has gained importance as a risk factor for breast cancer, the leading cause of death by cancer in women. We have previously demonstrated that the pesticide Hexachlorobenzene (HCB) stimulates breast cancer progression, facilitating cell migration, invasion, tumor growth, angiogenesis and metastasis. HCB is a weak ligand for the Aryl Hydrocarbon Receptor (AhR), a ligand-activated transcription factor related to tumor and vascular development. Stem cells are of great interest due to their ability to originate, maintain and expand tumors, as well as to lead to metastasis and recurrences. Vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) are associated with tumor angiogenesis. The aim of this work is to examine the HCB action on the capacity to mammospheres formation, expression of VEGF and COX-2, production of interleukin-10 (IL-10), and to determine if the AhR pathway is involved in the effects of HCB on tumor cell proliferation and migration. To this end, we used a Human Epidermal Growth Factor Receptor 2 (HER2) positive murine breast cancer cell line, LM3, incubated with HCB in the presence or absence of AhR inhibitors. Environmentally relevant HCB doses were used (0.005-5 μM). HCB stimulated the development of mammospheres at all doses tested ($p<0.01$). In addition, HCB induced the protein expression of VEGF and COX-2 ($p<0.05$), favoring a proangiogenic environment. In a first approach to the immune compartment, the results showed that HCB decreased intracellular levels of IL-10 ($p<0.05$). In addition, the exposure to the pesticide increased cell viability ($p<0.05$), proliferation ($p<0.001$) and migration ($p<0.01$) in these cells through an AhR pathway-dependent mechanism ($p<0.01$). These results suggest that HCB promotes a dedifferentiated profile and the proangiogenic factors expression, contributing to tumor progression in a HER2 breast cancer model.

340. (263) **EFFECTS OF ANTHRACENE AND MAGNETITE NANOPARTICLES COATED WITH OLEIC ACID ON BREAST CANCER CELL PROLIFERATION.**

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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) on breast cancer cells proliferation. These NP were developed in the CITAAC for the remediation of water contaminated with PAH.

We have previously studied the effects of the water-soluble fraction of petroleum (WSF) simulating a possible oil spill. WSF exposure significantly decreased the viability and significantly increased catalase (CAT) activity of MCF-7 and MDA-MB-231 breast cancer cells compared to controls. As the WSF is a mixture of hydrocarbons we considered important to study the effects of PAHs individually to understand if the observed effects were induced by the individual action of its components or by the interaction between them.

MCF-7 and MDA-MB-231 cell lines were treated for 7d with either anthracene (0; 3,5; 7; 15 and 28 μM) or NP (0; 12,5; 25; 50; 100 and 200 mg/L) to perform clonogenic assays. No significant differences were observed in the clonogenicity of anthracene treated cells compared to control. However, the exposure to NP from 12,5 to 200 mg/L and from 25 to 200 mg/L significantly decreased the clonogenicity of MCF-7 and MDA-MB-231 cells respectively ($p<0,0001$). These results suggest that other PAHs, such as naphthalene, pyrene and others should be evaluated in order to understand the mechanism of action of the toxicity of the hydrocarbons present in the WSF. We also envisage the need of evaluating NP containing PAH after remediation to determine potential risks. The effects derived from NP alone alert for secure uses avoiding nanomaterial release to the environment during remediation processes.

341. (302) **ENDOCRINE DISRUPTING ENVIRONMENTAL POLLUTANS HEXACHLOROBENZENE AND CHLORPYRIFOS INDUCE ENDOMETRIOSIS ASSOCIATED ANGIOGENESIS**

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Endometriosis is a frequent and chronic illness which is defined by the existence of endometrial tissue outside the uterus. Angiogenesis is critical in endometriosis progression, is a complex process involving endothelial cell migration, proliferation, tube formation, and survival. Vascular endothelial growth factor (VEGF) is a powerful angiogenic factor, which activates downstream effectors such as ERK1/2. This signaling pathway is involved in endothelial cell proliferation and migration. Endocrine-disrupting environmental pollutants are thought to play a role in the development of this disease. Hexachlorobenzene (HCB) is an organochlorinated pesticide that increases microvessel density and VEGF levels in a rat endometriosis model. Chlorpyrifos (CPF) is an organophosphate insecticide that acts as an Endocrine Disruptor. The present study examined the effect of HCB and CPF on endometriosis angiogenesis *in vitro*. T-HESCs cells (stromal endometrial cell line) were exposed to HCB (0.005, 0.05, 0.5 and 5 μM) and CPF (0.05, 0.5, 5 and 50 μM) or vehicle for 48h, and the conditioned media were then used to stimulate EA.hy926 endothelial cells. The results showed that HCB (0.005, 0.05 μM) and CPF (0.5, 5, 50 μM) induced VEGF secretion ($p<0.05$) in T-HESCs. Moreover, the conditioned media of HCB treatment enhanced the EA.hy926 cells proliferation (PCNA expression and MTT assay) (0.005-5 μM) ($p<0.05$), migration (scratch motility assay) (0.005-0.5 μM) ($p<0.05$), ERK1/2 phosphorylation (Western blot) (0.005, 0.05 μM) and tube formation (tube-like structure formation in Matrigel assay); increasing total tube length (0.005 μM $p<0.05$; 0.5 μM $p<0.01$) and branching points (0.5 μM $p<0.05$). The results with CPF conditioned media showed an increase in cell migration rate (5 μM $p<0.005$). Our results demonstrated that HCB and CPF exposure induces VEGF secretion in human endometrial cells triggering angiogenesis in endothelial cells, a critical event for the endometriosis progression.

342. (324) **SOY PROTECTS AGAINST CADMIUM INTOXICATION IN LUNGS. EFFECTS ON ANTIOXIDANT RESPONSE AND HEAT SHOCK PROTEINS (HSP) EXPRESSION**

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Cadmium is a toxic metal and a widespread contaminant. The mechanisms of its toxicity are not yet fully understood, but it has been shown that cadmium increases lipid peroxidation and oxidative stress in many organs. On the other hand, it has been shown that soy consumption has many benefits for human health. Heat shock proteins are a superfamily of proteins that have an important role in folding/degradation of proteins. Their expression can be used as a sensitive biomarker when cells are under stress conditions. Antioxidant response includes enzymatic and non-enzymatic systems. These systems exert a protective effect against free radicals and oxidative damage. We decided to study the expression of Hsp and the antioxidant response in rat lungs under cadmium intoxication, analyzing the possible protective effects of a soy based diet. 4 lots of adult Wistar rats were used: 2 lots received casein and 2 lots, soy as protein source. Within each group, 1 lot received regular tap water and the other 15ppm of Cd (as $CdCl_2$) in drinking water for 60 days. Lungs were removed, fixed and paraffin embedded. Immunohistochemistry was realized using Hsp27 and Hsp70 antibodies. Total RNA was obtained using QuickZOL reagent, retrotranscribed and used for PCR amplification of GPx and SOD-2. CAT activity was measured in lungs homogenate. Results showed that Hsp27 expression increased in both Cd intoxicated groups ($p<0.05$), being higher in soy fed groups. Hsp70 expression showed no differences between casein groups, but it revealed a significant increase in Soy-Cd vs its control. GPx expression augmented in both intoxicated groups ($p<0.05$), being higher in those fed with soy. SOD-2 mRNA levels showed no differences among casein groups, but it increased in Soy-Cd group vs its control ($p<0,01$). CAT activity augmented in Cas-Cd group vs its control ($p<0.01$); however, activity was higher in soy fed groups ($p<0,05$). Cadmium intoxication generates oxidative stress in lungs. Lungs responded increasing the expression and/or activity of antioxidant enzymes, being both higher in soy groups. Soy diet also improves the expression of Hsps, that protects against protein damage caused by oxidative stress. We concluded then that a soy rich diet could be a good therapeutic strategy in cases of Cd exposure.

343. (333) **CHLORPYRIFOS INDUCES CYTOTOXICITY, B-ESTERASE INHIBITION AND OXIDATIVE STRESS IN ENDOTHELIAL CELLS**

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**contribución equitativa*

Chlorpyrifos (CPF) is an organophosphate insecticide widely used in Argentina and throughout the world. In animals, exposure to CPF produces inhibition of B-esterases (acetylcholinesterase, AChE, and carboxylesterases, CES) and multiple toxic effects. However, little is known about the effect of CPF on endothelium. The aim of this work was to explore in vitro effects of CPF on endothelial cholinesterase and non-cholinesterase targets. Mouse endothelial cell cultures (H5V) were treated with CPF for 24 h. The biochemical parameters analyzed were AChE, CES, antioxidant enzymes (catalase, CAT and superoxide dismutase, SOD) and the levels of reduced (GSH) and oxidized (GSSG) glutathione. Enzyme activities were measured spectrophotometrically and GSH and GSSG levels were determined by the recycling method using GSH reductase. ROS production was determined by flow cytometry with the fluorescent probe DCF-DA and the morphological analysis was performed with Image J using phase-contrast images. When analyzing cell morphology, a decrease in cell area was observed at CPF concentrations > 50 μM ($p<0.05$). After performing a viability curve, a working range of sublethal concentrations (0-50 μM) was established. In this range, AChE inhibition (up to 50%) and CES inhibition (up to 80%) was observed ($p<0.05$). CAT activity showed an increase at 10 μM and a decrease at 50 μM CPF ($p<0.05$). SOD was inhibited around 30% with respect to controls in all treatments ($p<0.05$). GSH/GSSG ratio decreased (≈ 60%) in all treatments ($p<0.05$) and the amount of DCF+ cells increased at 10 and 30 μM CPF. Our results show that endothelial cells respond to the presence of sublethal concentrations of CFP, showing B-esterase inhibition and redox state alteration. These *in vitro* studies represent a first approach to understanding the mechanism of toxic action of organophosphate pesticides on the endothelium.

344. (354) **UTERINE GLANDULAR DYSFUNCTION IN RATS EXPOSED TO GLYPHOSATE OR A GLYPHOSATE FORMULATION: A POSSIBLE MECHANISM OF IMPLANTATION FAILURE**

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Glyphosate (Gly) is the active ingredient of multiple herbicide formulations known as glyphosate-based herbicides (GBHs), which are the most globally used pesticides. We have shown that perinatal exposure to either Gly or GBH decreased the number of implanted embryos in female rats. Here, we investigated whether Gly or GBH exposure alter endometrial gland function as a possible mechanism responsible for implantation failures. 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 weaning. F1 females were pregnant and uteri were sampled on GD5 (pre-implantation period) for morphological, immunohistochemical and mRNA analysis. Uterine sections were stained with hematoxylin-eosin to analyze the following morphological features: luminal epithelial height, number of glands, and thickness of myometrium and subepithelial stroma. We also determined the expression of molecules that regulate uterine gland function and implantation such as, Forkhead box A2 (FOXA2) and b-catenin which were evaluated by immunohistochemistry, and leukemia inhibitory factor (*Lif*) that was assessed by qPCR. A lower number of uterine glands in Gly (**$p<0.01$) and GBH (***$p<0.001$) groups was detected regarding to the control. Both Gly and GBH exposure (*$p<0.05$ vs control) decreased the expression of *Lif*. Also, FOXA2 (Gly: **$p<0.01$; GBH: *$p<0.05$ vs control) and b-catenin (Gly and GBH: **$p<0.01$ vs control) expression levels were decreased in the glandular compartment in both groups. In conclusion, Gly and GBH exposure decreased the number of glands in the pre-implantation uterus, in association with a downregulation of key molecules for endometrial gland activity and implantation. These findings suggest that uterine gland dysfunction might be a mechanism of Gly- and GBH-induced implantation failures. Importantly, Gly and GBH induced similar changes suggesting that both compounds may act through similar pathways.

345. (383) **THIOREDOXIN OVEREXPRESSION SHOWS A CARDIOPROTECTION AGAINST THE REDOX METABOLISMS ALTERATIONS INITIATED BY AIR POLLUTION EXPOSURE**

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Epidemiological studies showed correlations between daily changes in particulate matter (PM) concentration with cardiovascular diseases such as heart failure. PM-initiated reactive O_2 species leading to oxidative damage initiates adverse mechanisms. Thioredoxin-1 (Trx1) through redox homeostasis regulation have shown an ischemia/reperfusion injury cadioprotection, reducing the infarct size. Therefore, the aim of the present project was to further assess the Trx1 protection against the cardiopulmonary toxicity initiated by

Residual Oil Fly Ashes (ROFA) exposure, used as a PM inhalation model. Wild type (WT) and overexpressing Trx-1 (TRX) transgenic mice were intranasally instilled with a ROFA suspension (1 mg/kg) or saline. Heart tissue redox metabolism and cardiac function were evaluated 3h after treatment. Trx-1 overexpression was able to revert the tissue O_2 consumption decreased observed after ROFA exposure (p<0.05). Regarding the antioxidant system, higher GSH levels found in TRX mice lead to no differences in GSH/GSSG ratio between groups, while SOD activity was decreased in TRX-ROFA mice (p<0.05). The increased phospholipids oxidation observed in ROFA treated WT mice (p<0.01) returned to control values in the TRX group even after ROFA exposure. Left ventricular contractile and lusitropic reserve was evaluated as LVDP and t_{50}, before and after a β-adrenergic stimulus with isoproterenol (ISO) (1 μM), revealing that the LVDP increment was significantly lower in WT mice exposed to ROFA compared to saline while no differences were observed among TRX groups (p<0.05). Similar trends were observed in T_{50}, showing an impaired relaxation response due to ROFA inhalation which was abolished by the Trx-1 overexpression. Here we are showing that Trx-1 overexpression restores the ventricular reserve after an acute exposure to ROFA. These findings contribute to the understanding of the adverse health effects triggered by PM-initiated redox metabolism alterations.

346. (417) **UNDERSTANDING THE MECHANISM OF SILVER NANOPARTICLE TOXICITY**

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Nanotoxicology has increasingly gained attention along with the AgNP applications development. As inhalation is one of the main routes of exposure, the aim of this study was to evaluate the AgNP harmful effects in the lung. Characterization of AgNP was performed by DLS, TEM and EPR, while biodistribution studies were done using radio labeled ^{99m}Tc-AgNP. *In vivo* studies in balb/c mice intranasally instilled with 0.1 mg AgNP/kg b.w were performed, where O_2 metabolism was analyzed 1h after AgNP inhalation. *In vitro* studies using A549 cells and a 3D lung tissue model, were done after 1h exposure to 2.5 μg AgNP/mL. AgNP showed a hydrodynamic diameter of 17 ± 6 nm and were able to initiate the hemolytic cleavage of H_2O_2 leading to $OH^{\cdot}$ production. *In vivo* results showed the lung as the main deposition organ, where AgNP initiate alveolar epithelium barrier injury, revealed by increased protein content and total cell count in BAL samples (46%;120%; p<0.05). AgNP triggered tissue O_2 consumption increase by 31% (p<0.05), due to increased 44% NOX activity (p<0.05) and a higher mitochondrial active respiration (55%; p<0.001), leading to increased H_2O_2 production rate as mitochondrial complex I activity increased (28%; p<0.01*;* 39%; p<0.05 respectively). Increased ROS release triggered the antioxidant system activation as increased SOD and catalase activities (p<0.01; p<0.01) and a 35% decreased GSH/GSSG ratio (p<0.05) were observed. In A549 cells, AgNP showed 49% increased NOX activity (p<0.05), 40% decreased mitochondrial ATP associated respiration and higher H_2O_2 production rate (72%; p<0.001) leading to oxidative damage (23%; p<0.01). Moreover, the lung 3D tissue model showed AgNP-initiated barrier alterations as TEER values decreased (20% p<0.05). Taken together, these results show that AgNP exposure alters O_2 metabolism leading to a redox imbalance. AgNP-triggered oxidative damage may be responsible for the impaired lung function observed due to alveolar epithelial injury.

347. (495) **THE UV FILTER BENZOPHENONE 3 ALTERS BLASTOCYSTS IMPLANTATION AND THE EARLY EMBRYONIC DEVELOPMENT**

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The aim of our study is to analyze the effect of the UV-filter benzophenone 3 (BP3) on early gestational processes by a complementary in vitro and in vivo approach. Using an in vitro assay of blastocyst implantation, we analyzed the effect of three different BP3 concentrations: a) BP3-2: the predicted non-effect concentration (PNEC: 2 μg/L), b) BP3-20: the concentration detected in the amniotic fluid (20 μg/L) in our previous studies and c) BP3-200: the plasma concentrations reported in humans (200 μg/L). Blastocysts from 3.5 days pregnant mice (C57BL/6J) were transferred to a monolayer of autologous uterine epithelial cells (UECs) and cultured in the presence of vehicle (0.01 % DMSO) or BP3 for 6 days. Blastocyst expansion, hatching and implantation in the monolayer as well as implantation area were analyzed microscopically and recorded every 12 h. To verify the in vivo relevance of the in vitro results, pregnant C57BL/6J mice were exposed via dermal route to BP3-50 (50-mg BP3 kg.day) or olive oil (vehicle) from gestation day (gd) 0 to gd10. The mice were sacrificed at gd10 and the size of the whole implantation sites (WIS) was measured.

In vitro exposure to BP3-2 and BP3-200 altered the blastocysts expansion. Moreover, the hatching and the implantation time were delayed and the implantation areas were significantly smaller than those from the control with all BP3 concentrations assayed. In vivo study reaffirms the in vitro results, since we found that BP3-50-exposed WIS was smaller than those exposed to the vehicle. Previously, we showed that in vivo dermal BP3-50 produced an intrauterine growth restriction (IUGR) phenotype and lower offspring weight of first progeny. Here, we could demonstrate that BP3 disrupts blastocyst implantation and early embryo development. Our results suggest that an underlying mechanism by which BP3 affects pregnancy are linked to disruption of the implantation stage, leading consequently to a reduction in embryo size.

348. (541) **ROLE OF H_2S AND TGF-β1 IN THE ANGIOGENIC PROCESS AND IN THE DEVELOPMENT OF HEPATOCARCINOMA**

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Hepatocarcinoma (HCC) represents 90% of primary liver tumours. Hexachlorobenzene (HCB) is a dioxin-type toxic and promoter of preneoplastic foci in rat liver. We have shown that it alters regulatory parameters of cell growth *in vivo* and *in vitro*. In the present work, our objective was to evaluate the mechanisms of action of HCB in the development of HCC. We will study the key molecules present in the tumor microenvironment that collaborate in the angiogenesis process and in the development of HCC. We used *in vivo*, nude mice treated with HCB i.p. (3mg/kg) inoculated with HepG2. We evaluate: a) *in vivo*, PCNA (Western Blot (W));b) morphology(H);c) tumours and d) angiogenesis (vessels/skin). *In vitro*, HepG2:a) PCNA (W);b) cell cycle (flow cytometry); c) p21,p27,TGF-B1(W);d)

cD1 (W) and e) H2S. In Ea-hy926: a) PCNA (W), b) tubulogenesis (length of tubules); d) H_2S and e) role of TGF-β1 in the effect of HCB(5μM) on PCNA (W) using specific inhibitor (SB431542); f) effect of the conditioned medium (CM) of HepG2 treated with HCB(5μM) on Cell Migration (Cmi). *In vivo* it increased in the HCB group: PCNA (30%,p<0.01); TGF-β1 (29%,p<0.01); mitotic nuclei; tumours, size 8 mmx9 mm/day 30 and vascularization, (35%,p<0.01). In HepG2, increase: PCNA (22, 31%, p<0.01) (0.5 and 5 μM HCB); S phase (21.26%,p<0.01) (0.5 and 5 μM HCB); TGF-β1 (20, 28, 40%,p<0.01) (HCB 0.05, 0.5 and 5μM) and cD1 (18, 25, 32%,p <0.01) (HCB 0.05 ; 0.5 and 5 μM). They decreased: p21 (14, 21, 28%,p<0.05); p27 (19, 27, 31%,p<0.01) (HCB 0.05, 0.5 and 5 μM) and H_2S (20, 27%,p<0.05) (HCB 0.5 and 5 μM) . In Ea-hy926 increased: a) PCNA (40%, p<0.01) and tubule length (56%,p<0.01) and decreased: H_2S (21%,p<0.05). PCNA did not vary when treating with SB431542/ HCB (5 μM). The CM of HepG2/HCB(5μM) increased (CMi) (32%, p<0.01). We conclude that in the *in vivo* model of HCC, HCB deregulates cell growth and favours the development of neo-angiogenesis, H_2S and

349. (101) **ERYTHROCYTE SENESCENCE MARKERS AS PARAMETER OF TOXICITY OF FUMONISIN**

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Fumonisin B_1 (FB_1) is a mycotoxin that occurs as a frequent contaminant of corn and corn-based foods in Argentina, has an inhibitory effect on ceramide synthetase, key enzyme in the biosynthesis of sphingolipids. The effects of FB_1 on the plasma membrane and on oxidative stress led us to propose a possible role of FB_1 on the aging of erythrocytes (EC). Our **objective** was to assay techniques to determine the aging of EC to apply to the exposure study by fumonisins. **Materials and Methods:** Male 1-month Wistar rats were separated into two groups of 1 animal. The control rat was given saline solution while the other rat received orally for 20 days 1 mg/kg/day of FB1 from a culture of Fusarium proliferatum. Erythrocyte malondialdehyde (MDA) was measured according to the method of Kumar et al. The binding of alcian blue (AB) to human or rat EC, to measure the effect of fumonisin on erythrocyte charge, was studied by the method of Ponce de Leon et al. *In vitro* experiments, EC 5% in PBS was added to three tubes (0, 50 and 100 ng of FB1), incubated at 37 º 24 hs. **Results:** In experiments *in vivo*, TBA results showed no significance differences between treated and control rat (p = 0.7117, p <0.05) neither in conditions *in vitro* between erythrocytes incubated with 50 ng or 100 ng of FB1 vs the control (p = 0.8706 and p = 0.6128, p <0.05 respectively). For AB, measured as percentage erythrocyte anionic charge (EAC%), no significant differences were found between the control vs the treated rats (p = 0.7044, p <0.005) nor in the results of EAC% of treated with 50 ng or 100 ng of FB1 vs control(p = 0.418 and p = 0.3488, p <0.05 respectively). **Conclusions:** Considering *in vitro* experiments, it was observed that concentration of FB1 used in our case, were much smaller than other studies, therefore we will continue the analysis under those conditions. In case of *in vivo* experiments, we need to continue these preliminary assays to study the effects of fumonisin exposure.

350. (29) **RELATIONSHIP OF ErbB-2 WITH EGFR AND ErbB-3 IN THE CLEAR CELL RENAL CELL CARCINOMA (ccRCC)**

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The most common subtype of renal cancer is clear cell renal cell carcinoma (ccRCC), which accounts for 80-90% of all renal cancer cases. Currently kinase inhibitor therapy is used in advanced ccRCC, because the ccRCC is resistant to chemo- and radiotherapy. And there is no biomarker to guide therapy. While among the EGF receptors in the ccRCC, EGFR and ErbB-3 are expressed, ErbB-2 presence is not clear. In this context, we explored the expression and localization of ErbB-2 and its relationship with EGFR and ErbB-3. The technique used was immunohistochemistry. We selected 72 paraffin- embedded tissue samples from a cohort of archived ccCCR from the files of the Histolopathology service in Vidal Hospital, Corrientes from 2005 to 2019. Our results show that the membrane expression of ErbB-2 (MErbB-2) is 40% (29/73). However, it is not related to the stage (Chi-square test ns, Stage I and II vs III and IV). We explored the nuclear expression of ErbB-2 (NErbB-2) and 67% resulted positive (1+ 33%, 2+ 28%, 3+ 8%). We show that nuclear ErbB2 (NErbB-2) is correlated with high Fuhrman Nuclear Grade (FNG) Chi-square test *P=0.017 FNG 1 vs 2, ***P=0.0009 FNG 1 vs 3/4. Nuclear EGFR and ErbB-3 expression and the correlation with nuclear ErbB-2 were also explored. Our results show that, nuclear expression of EGFR (NEGFR) is 1+ (11%), 2+ (38%), 3+ (30%) and nuclear expression of ErbB-3 is 1+ (9%), 2+ (14%), 3+ (40%). The correlation of Pearson was positive for both NEGFR (**P=0.02) and NErbB-3 (**P=0.07) vs NErbB-2. Our findings showed that ErbB-2 is implicated in the carcinogenesis renal. It is very important to locate ErbB-2 because in the clear cell renal cell carcinoma nuclear ErbB-2 is associated with high FNG. The molecular mechanism of ErbB-2 nuclear location seems to be related to ErbB-2/EGFR and ErbB-2/ErbB-3 in renal cell carcinoma.

351. (45) **MAGEB2 INVOLVEMENT IN NUCLEOLAR STRESS RESPONSE**

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Beside its role in ribosome biogenesis, the nucleolus senses stress and is a central hub for coordinating the stress response.

MageB2 is a tumor specific protein with nucleolar localization. Related with its localization, we have previously observed that MageB2 enhances pre-rRNA transcription and ribosome biogenesis.

Here we aim to study the involvement of MageB2 in the nucleolar responses to stress.

First, we screened MageB2 protein interactors by performing yeast two-hybrid and immunoprecipitation (IP) follow by mass spectrometry assays. Our results, together with reported ones, showed that, beyond MageB2 interactors involved ribosome biogenesis, 34 and 17 out of 213 interactors were related with the GO terms “Cellular response to stress” (GO:0033554) and “Regulation of apoptotic signaling pathway” (GO:2001233), respectively, supporting a role of MageB2 in these biological processes.

Actinomycin D (ActD) at low concentration inhibits RNA Pol I and induces nucleolar stress, with the consequent redistribution of some nucleolar proteins. Under this condition, we observed that MageB2 relocalized to the nucleoplasm. Besides, MageB2 IP followed by western blot showed that ActD treatment resulted in MageB2 phosphorylation in Tyrosine.

We also observed that MageB2 expression correlated with resistance to apoptosis induced by ActD treatment. However, luciferase reporter gene assay of a p53 responsive promoter demonstrated that MageB2 was not able to regulate p53 function. Moreover, IP assays showed no binding between MageB2 and p53. In line with this, siRNA mediated down-regulation of p53 in U24 cells did not affect the resistance effect of MageB2, suggesting that MageB2 confers resistance to ActD in a p53 independent manner.

Altogether, this result suggests that nucleolar stress induced by treatment with ActD changes the localization and phosphorylation status of MageB2. Under this condition, MageB2 is able to counteract apoptosis in a p53 independent manner.

352. (89) **REGULATION BY MIRNAS OF THE STABILITY OF RAC3 DURING ADIPOGENESIS**

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Adipogenesis is regulated by several adipocyte-selective microRNAs (miRNAs) and transcription factors. We have previously demonstrated that the expression of the transcriptional coactivator RAC3 decreases during adipocyte differentiation, favoring the process through an increase of autophagy and a decrease in the proliferative rate.

In order to determine the mechanism whereby RAC3 expression levels are regulated during adipogenesis, we performed public datasets analysis and evaluated RAC3 and miRNAs expression during adipocyte differentiation (GSE20696 and 20298). After 7 days of differentiation, in the murine preadipocyte 3T3-L1 cell line (GSE20696), we observed RAC3 expression was downregulated (43.6% +/- 0.9% respect to control, p<0.01), corroborating the results obtained by our group in the L929 cell line.

By ISMARA platform, we performed an analysis of computationally predicted regulatory sites for transcription factors and miRNAs involved in RAC3 regulation during differentiation and we observed that the predicted binding activity for transcription factors was diminished but for miRNAs was increased, at 2 and 7 days after adipocyte differentiation.

Using TargetScan and MirSystem platforms, we found that the members of miR-17-92 family have a high score of binding in the murine and human RAC3 3?UTR. However, only miR17-5p has been validated for regulating RAC3 in human cells. Therefore, we analysed miRNA expression in GSE20698 dataset and observed that the expression levels of four mir-17-92 family members, miR-17-5p, miR-20a-5p, miR-20b-5p and miR-93-5p, were increased at 2 day of differentiation (>1.5-fold change respect to control).

We could conclude that the regulation of RAC3 expression during adipocyte differentiation not only occurs at transcriptional level but also may involve post-transcriptional regulatory mechanisms th

353. (254) **20-HYDROXYEICOSATETRANOIC ACID (20-HETE) MODULATES THE TRANSCRIPTIONAL ACTIVITY OF THE ANDROGEN RECEPTOR ACTING THROUGH THE GPR75 RECEPTOR IN ANDROGEN DEPENDENT PROSTATE CANCER CELLS**

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We have shown the role of 20-HETE in sustaining the viability of androgen-dependent prostate cancer cells (LNCaP) by acting as a signaling molecule downstream the androgen receptor (AR). Recently, the GPR75 receptor has been identified as the target for 20-HETE. The aim of this study was to identify the role of the 20-HETE/GPR75 axis in the AR transcriptional activity (ARTA) in LNCaP cells. The following methods were used: immunofluorescence (intracellular localization of AR); reporter gene assay (ARTA in LNCaP cells transfected with PSA-Luc); chemiluminescence (prostate specific antigen (PSA) quantification). Stadistic: two tails t-student test, one-way ANOVA followed by Dunnet?s. Both, 20-HETE (0.1nM, 45min) and DHT (10nM, 30min) increased by 63%, the abundance of AR in the nuclei (p<0.0001 for both), which was sustained for 60min (20-HETE) or 120min (DHT). Inhibition of the synthesis of 20-HETE by HET0016 (10uM, 36h) reduced by 65.7% the spontaneous ARTA (p<0.001) and by 76.5% the induced by DHT (10nM, 36h, p<0.001). 20-HETE (0.1nM, 36h) increased the ARTA by 178% (p<0.0001) and potentiated the effect of DHT by 58% (p<0.0001 vs. DHT). This response was inhibited by the GPR75 antagonist 19-HEDE (p<0.01 vs. 20-HETE; p<0.0001 vs 20-HETE+DHT). The effect of 20-HETE on ARTA was decreased by the inhibition of PKA, PKC and PI3K by 47.5% (p≤0.01), 39.9% (p≤0.01) and 56.9% (p≤0.001) respectively. Instead, inhibition of tyrosine- protein kinases (genistein, 5uM, 36h) increased ARTA by 196.8% (p≤0.01) per se, and augmented by 113.6% 20-HETE- induced ARTA (p≤0.0001). In line, 20-HETE (0.1 nM, 48h) increased by 58% PSA protein secretion to the conditioned medium (p<0.05) and this was abrogated by coincubation with 19-HEDE (p<0.01 vs 20-HETE). Our results strongly suggest a role for 20-HETE/GPR75 axis in the regulation of the ARTA, which involves the activation of PKA, PKC and PI3K pathways. 20-HETE- induced translocation of AR to the nucleus may account for this effect.

354. (326) **ROLE OF P110δ CATALYTIC SUBUNIT OF THE CLASS IA ISOFORM OF PI3K IN THE ANTI-INFLAMMATORY EFFECT OF BENZNIDAZOLE**

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Benznidazole (Bz) is the drug-of-choice in many countries for the treatment of Chagas Disease. Although it has been used in clinical settings for a long time, its mechanisms of action have not been fully elucidated yet. Indeed, there is a general premise that the etiological treatment contributes to a reduction of the parasite load and to fit the host immune response, leading to a balanced inflammatory response which is crucial to control Chagas disease morbidity. In addition to its parasiticidal effect, we have previously reported that Bz inhibits the activation of the NF-κB pathway by increasing the expression of SOCS3 through the IL-10/STAT3/SOCS3 pathway. Furthermore, in preliminary results, we showed that PI3K participates in this effect in cardiac cells. In this work, we assessed this issue in a macrophage (Mϕ) cell line (RAW 264.7). Mϕ were pre-treated with 15uM Bz and stimulated with 10 μg/ml of LPS. The treatments were performed, in parallel, in the presence of LY294002, a specific inhibitor of PI3K activity. To deepen the knowledge of the mechanism of action, we also used CAL-101, a specific inhibitor of the p110δ catalytic subunit of the Class IA isoform of PI3K. Inhibition was confirmed by the absence of phosphorylation of P70S6K (p<0.05). Under these conditions, Bz was unable to inhibit the activation of NF-κB, evidenced by the expression of cytosolic IκBα, the expression of IL-6 and TNF-α mRNA and the release of nitrites to the culture supernatant (p<0.05). Furthermore, Bz couldn't increase SOCS3 expression in both conditions (p<0.0001). These preliminary results suggest that Bz exerts it's anti-inflammatory effect, not only in an IL-10/STAT3/SOCS3-dependent but also in a Class IA-PI3K-dependent manner.

355. (328) **REGULATION OF PH IN CYSTIC FIBROSIS CELLS BY EGFR**

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the CFTR (CF transmembrane conductance regulator) gene. The pulmonary damage observed in severe patients is the major cause of morbidity and mortality due to chronic infections and unresolved inflammation. The reduction in the airway surface liquid (ASL) pH is one of the hypotheses that tried to explain the high susceptibility to lung infections. Together with a reduced bicarbonate transport

through CFTR, an increase in lactic acid secretion could also explain the changes in extracellular pH. The aim of the present work was to determine if the EGFR pathway is involved in the pH regulation in CF cells. Two cellular models were used: IB3-1 cells (bronchial epithelial cells derived from a CF patient with a ΔF508/W1282X CFTR genotype) and C38 cells (IB3-1 "corrected" cells). The results reported a decrease in pH in the extracellular medium culture ($p<0.05$) in IB3-1 cells concomitantly with an increased in lactate secretion and both LDH expression and activity ($p<0.05$) compared with C38 cells. These results confirmed that the CFTR regulates significantly ($p<0.05$) the pH, lactate secretion and LDH expression and activity. As we have previously observed the role of the EGFR pathway in the CF phenotype, we studied if this signaling pathway is also involved in pH regulation. Here, we observed that EGFR modulates significantly ($p<0.05$) the pH in the extracellular medium, the lactic acid (lactate) secretion ($p<0.05$) and the LDH expression and activity ($p<0.05$) in CF cells. In conclusion, our results showed that CFTR channel activity (or expression) regulates not only the pH, but also the lactic acid secretion and LDH expression and activity and the EGFR pathway is partially involved in this regulation. The low pH microenvironment observed in CF cells could promote the impairment of immune function and consequently the establishment of infections in people with CF (PWCF). Acknowledgements: ANPCYT, UCA and CONICET.

356. (352) **INTERCELLULAR MITOCHONDRIAL TRANSFER THROUGH NANOTUBULES IS PROMOTED BY CYCLIC AMP (cAMP) IN RAT ASTROCYTES AND HUMAN GLIOBLASTOMA CELLS**

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Nanotubules (Tunneling nanotubules, TnTs) are cell membrane projections made of F-actin fibers of nanometric diameter (up to 1 μm), which enable cytoplasmatic connections between cells. It has been shown that mitochondria, other organelles, and cellular components are transferred by TnTs in several normal and tumor cell lines. TnTs establishment has been extensively described in nervous system cells, as neurons and astrocytes. Published evidence indicates the existence of mitochondrial transfer through TnTs between different cell types, such as normal and tumoral cells. Mitochondrial passage from normal to tumor cells restores oxidative metabolism, decreasing tumorigenic potential. A similar effect has been observed with cAMP, a very well-known astrocytes stellation promoter, which mediates mitochondrial biogenesis and tumor growth inhibition. Mitochondrial transfer within TnTs in nervous system cells have not been demonstrated so far. Then, our goal was to analyze mitochondrial trafficking through TnTs in normal and tumoral astrocytes and a possible effect of cAMP. We used normal rat astrocytes and human glioblastoma U87 cells. Mitochondria and actin were probed with a mito-targeted green fluorescent protein and phalloidin, respectively. Astrocytes and U87 were incubated with or without 8Br-cAMP (cAMP analogue). We analyzed images by confocal microscopy and measured the width of actin connections between cells. We analyzed each culture separately; astrocytes and U87 establish thick projections containing mitochondria but treatment with cAMP promotes an increase of TnTs-like connections with mitochondria inside (control vs cAMP: astrocytes: 2.07±0.71 vs 0.85±0.21 μm, ***$p<0.05$; U87: 2.69±1.40 vs.0.84±0.22 μm, *$p<0.05$, ± SD, ANOVA, Tukey test). Thus, cAMP promotes TnTs-like structures and mitochondrial passage through them in normal astrocytes and glioblastoma cells, suggesting a role for intercellular mitochondrial transfer through TnTs in stellation process.

357. (416) **TPR-PROTEINS INFLUENCE THE NUCLEOCYTOPLASMIC SHUTTLING OF GR**

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Glucocorticoid receptor (GR) exists as a heterocomplex with the chaperone Hsp90 and a co-chaperone carrying a tetratricopeptide-repeat (TPR) domain through which it interacts with Hsp90. Steroid binding promotes the exchange of TPR proteins on the GR-Hsp90 complex, such that FKBP51 is replaced by its homolog partner FKBP52, an immunophilin that interacts with dynein motors favouring the retrograde transport of GR. In this study, we hypothesized that TPR-domain proteins regulate the subcellular localization of GR. It is shown that GR nuclear accumulation is impaired by overexpression of the recombinant TPR peptide. This disrupts the association between GR-Hsp90 an FKBP52, and consequently, the association with dynein is lost. It also causes loss of association with the importin-β1 adapter transporter (KPNB1), the nuclear pore-associated glycoprotein Nup62 and structures associated with the nuclear matrix. The final GR distribution is the result of the combination of two processes: decreased nuclear import and improved nuclear export. Interestingly, leptomycin B (a CRM1/exportin-1 inhibitor) abolished the effects of TPR peptide overexpression despite not having inhibitory effect itself on the nuclear GR export. These results strongly suggest the existence of a TPR domain-dependent mechanism for nuclear protein export. In summary, our study demonstrates a strong relationship between TPR proteins and the nuclear import mechanism of the receptor, as well as their potential capability to favour the anchorage of the GR to nuclear structures. We propose that the balance of expression of the TPR domain proteins bound to the GR-Hsp90 complex can determine the subcellular localization and the nucleocytoplasmic properties of the receptor and, therefore, its pleiotropic biological properties in different tissues or cell types.
KEY WORDS: Glucocorticoid receptor, Tetratricopeptide repeats, Immunophilins, FKBP52, Hsp90, Nuclear matrix

358. (527) **ACTIVATION OF THE LIVER X RECEPTOR (LXR) INHIBITS INFLAMMATORY EFFECTS ASSOCIATED WITH INVOLUTION AND PROMOTES LACTOGENIC FEATURES IN MOUSE MAMMARY CELLS**

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It is well-known that lactation is driven by glucocorticoids and prolactin (Prl) that induce milk protein expression through STAT5 activation. On the other hand, post-lactational involution is characterized by the secretion of pro-inflammatory factors that trigger mammary cell apoptosis. LXR is an anti-inflammatory transcription factor present in all developmental stages of the mouse mammary gland.The goal of this study was to determine whether LXR plays a relevant role during the lactation/involution switch. Female C57/bl6 mice were treated with the LXR agonist GW3965 (10 mg/kg) or DMSO (control) by IP injection at weaning, 48h or 96h post weaning (n=3 per group). At those times, mice were euthanized and pieces of their inguinal mammary glands were either fixed in formalin or frozen at -800C. Fixed tissue was later processed for histological studies, while proteins and RNA were extracted from frozen samples. On the other hand, we analyzed the effects of GW3965 (10-6M) compared to Dexamethasone (Dex), Dex+Prl or DMSO for 72h on HC11 mouse mammary cells in culture. Our results show that LXR activation inhibited expression of inflammatory cytokines IL-6, TNFa and LIF mRNA at 48h and 96h after weaning (by RT-qPCR), together with an increase of the anti-inflammatory tristetraprolin (TTP) protein (by Western blot analysis) at 96 hs. In addition, by immunohisto-

chemistry, we have also found a decrease of S100A9 (a stimulator of neutrophils and their migration) expression at 96h post-weaning. In culture, we determined that GW3965 treatment induced, similarly to Dex+Prl, but differently from Dex alone, an increase of TTP and b-casein mRNA expression. Importantly, GW3965 treatment did not trigger STAT5 phosphorylation as the lactogenic hormones Dex+Prl. Taken together, these results show that activation of LXR inhibits involution associated events and promotes the lactogenic phenotype in the mammary epithelium by a Prl-Stat5 independent signaling pathway.

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359. (545) **ABERRANT GLYCOSYLATION DURING INFLAMMATORY BOWEL DISEASES: MECHANISTIC INSIGHTS INTO THE ROLE OF MIRNAS AND HYPOXIA.**

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Even though alteration of the intestinal glycome during IBD has been known for many years, factors modulating and triggering these changes are still unclear. Recently, heightened hypoxia has been described as an important environmental factor in IBD, and microRNAs (miRNAs) have emerged as critical mediators of gene expression; however, a connection between them in IBD has not been thoroughly explored. Our objective is to unveil molecular and environmental factors modulating the intestinal glycome during IBD. We started by analyzing data from several miRNAs databases, searching for those dysregulated in IBD, hypoxic conditions and that could modulate inflammation, angiogenesis and glycosylation. Cross data analysis showed candidate miRNAs mir-155, mir-146 and mir-124 as regulators of several glycosyltransferases (ST6GAL1, ST6GAL2, FUT8, FUT11, MGAT4, MGAT5) and the mannosidase MAN1A1. By using publicly available datasets from IBD patients (E-MTAB-2967) we found that ST6GAL1, STGAL2, FUT8 y FUT11 were upregulated ($p<0,05$), while MGAT4A, and MAN1A1 were downregulated ($p<0,05$). Finally, in order to understand if these altered transcriptomic profiles were mirrored by glycophenotypical changes *in vitro*, we explored the glycosylation profile of two colon cell lines (HCT116 and HT29) cultured under hypoxia with proinflammatory cytokines relevant to IBD. Cell cultures were carried out in normoxic and hypoxic ($1\%O_2$) conditions with or without IL1, IL6 and TNFa. Glycophenotyping of these cells by flow cytometry showed an increase in b(1-6) complex N-glycans ($p<0,05$), α(1-3) and α(1-6) fucosylation ($p<0,05$) and α(2-6) sialylation ($p<0,05$). Interestingly, the most prominent driver of these changes was hypoxia. Taken together, our experimental data confirms a glycome shifting that correlates with dysregulated glycosyltransferases in transcriptomic data from IBD patients. We are currently connecting these changes with candidate miRNAs and explore their role during the course of IBD.

360. (260) **THE AQUEOUS EXTRACT OF *SMILAX CAMPESTRIS* INHIBITED THE OSTEOBLAST SECRETION OF IL-6 AND DECREASED THE OSTEOBLAST-INDUCED OSTEOCLAST DIFFERENTIATION**

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Background: *Smilax campestris* is a medicinal plant that has been used traditionally to treat inflammatory diseases. During bone remodelling, osteoblast cells secrete pro-inflammatory mediators that activate osteoclast differentiation and activity. This process is exacerbated in the absence of oestrogen. Aims: to determine whether the aqueous extract of *S. campestris* (SME) inhibits the osteoclast differentiation of RAW 264.7 cells induced by conditioned medium from inflammatory MC3T3-E1 osteoblast precursors, and the mechanism underneath. Methods: MC3T3-E1 cells were incubated with SME (10, 100, 1000 ng/mL) or oestrogen (E2 10^{-8}, 10^{-7}, 10^{-6} M) for 24 h, and then treated with IL-1β (2.5 ng/mL) for 24 h. After treatments, the cells were cultured for 24 h without stimuli to obtain conditioned media (CM). IL-6 concentration in CM was measured by ELISA. RAW cells were incubated with CM for 7 days and then tartrate resistant acid phosphatase positive (TRAP+) cells and MMP-9 activity were determined by histochemistry and gel zymography, respectively. Results: SME decreased IL-6 secretion induced by IL-1β in MC3T3-E1 cells, being this effect dose-independent (% inh= 91.8±3.8 at 10 ng/mL, $p<0.001$ respect to IL-1β). E2 dose dependently inhibited the IL-6 secretion induced by IL-1β (% inh= 69.7±6.4 at 10^{-6} M, $p<0.001$ respect to IL-1β). RAW cells treated with CM from MC3T3-E1 cells that had been cultured with IL-1β plus 10 ng/ml SME (IL-1β+SME CM), showed a reduction in the TRAP+ cell numbers and the MMP-9 activity compared to cells treated with IL-1β CM ($p<0.05$). Again, E2 exerted an inhibitory effect in a dose-dependent manner ($p<0.05$ respect to IL-1β CM). Our results suggest that SME is capable to diminish the osteoclast differentiation induced by inflammatory osteoblastic cells by a mechanism that could involve the inhibition of IL-6 secretion.

361. (534) **INFLUENCE OF DIETARY TRYPTOPHAN CONTENT ON THE ACTIVITY OF INFLAMMATORY BOWEL DISEASE.**

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Dietary tryptophan is catabolized by the intestinal microbiota into metabolites such as indoles and their derivatives. Our hypothesis proposes that a reduced availability of tryptophan metabolites at the interface of the intestinal mucosa causes a dysregulation of the epithelial barrier that results in Inflammatory Bowel Disease (IBD). To test this hypothesis, we evaluated the content of dietary tryptophan, the circulating tryptophan in plasma and the presence of urinary tryptophan metabolites in 11 control volunteers, 15 patients with Ulcerative Colitis (UC) and 10 patients with Crohn's Disease (CD). Daily tryptophan intake was calculated based on a food survey by a nutrition health professional, plasma tryptophan levels were measured using high performance liquid chromatography (HPLC) and tryptophan metabolites were determined in urine using mass detector coupled gas chromatography (GC-MS). IBD activity in patients was clinically estimated using the Mayo index (UC) or the Harvey-Bradshaw index (CD). Dietary and plasmatic tryptophan levels were comparable in all the study groups. The amount of tryptophan

consumed was directly correlated with the excretion values detected for the tryptophan metabolite 1H-indole-2,3-dione (r = 0.7631, p = 0.0168). In addition, non-tryptophan derived metabolites such as citric acid or 2-hydroxybutyric acid were able to discriminate patients with IBD from the control group. Although the amount of tryptophan consumed was not related to the activity of IBD in patients with UC, we found that the amount of tryptophan consumed was inversely correlated with the stage of IBD in patients with CD (r = -0.6971, p = 0.0251). Our findings suggest that increasing the amount of tryptophan in the diet may decrease IBD activity in patients with CD and that metabolomic profile of patients with IBD can provide a new precision medicine tool to improve the management and the quality of life of patients with IBD.

362. (581) **MEMORY T-CELLS IN AUTOIMMUNE LIVER DISEASE: A PRELIMINARY STUDY**

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Autoimmune hepatitis (AIH) is a progressive inflammatory disease involving many T lymphocyte subsets in its development. For instance, T cell subsets engaged in B cell activation and differentiation, as well as memory T cells. Based on their surface markers, memory T $CD4^+$ cells are classified into two subsets: the central memory T (TCM) cells expressing $CD44^+$ and $CD62L^{high}$ which facilitate their homing to the secondary lymphoid organs; and the effector memory T (TEM) cells which are $CD44^+/CD62L^{low}$ and exit into circulation toward peripheral tissues. The aim of this cross-sectional study was to characterize the immunophenotype markers of memory T lymphocytes in the peripheral blood from five patients with type 1 AIH, attending at the Gastroenterology Service of Hospital Provincial del Centenario de Rosario. Five healthy subjects were included as controls (Co). All patients were treated with prednisone and azathioprine at the time of blood collection. Peripheral blood mononuclear cells (PBMCs) were obtained and processed by flow cytometric analysis. AIH patients were positive for anti-ANA or anti-SMA antibodies showing higher levels of serum IgG, IgM, and IgA if compared to Co. The AIH group presented significantly increased and decreased levels of TCM and TEM cells, respectively in comparison from values in the control group ($P \leq 0.05$). Such increase in TCM cells may be reflecting a pathogenetic role in AIH. Specifically, the higher percentage of TCM cells at the peripheral level, may be associated with a Th2 response profile, and hence responsible for elevated gamma globulin fraction (mostly IgG and IgA) as well as the presence of autoantibody levels.

363. (560) E**VALUATION OF HIGH DIMENTIONAL REDUCTION AND CLUSTERING IN THE PHENOTYPIC DICRIMINATION OF $CD5^+$ B-CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES.**

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Introduction: Mature (peripheral) B-cell malignancies represent the malignant counterpart of normal mature B-cells that have differentiated into naive B cells or their progeny. Integration of a complex set of immunophenotypic, morphological, clinical, and cytogenetic information is essential for the subclassification of B-cell chronic lymphoproliferative diseases (B-CLPD).
Phenotyping is essential for the diagnostic classification of many B-CLPD cases, but the current immunophenotyping strategies also face several difficulties. In view of these issues, new methods to facilitate the identification of abnormal B-cell populations in routine clinical flow cytometric data would be desirable.
Methods: We used both 12 and 8 colour staining panels and we applied high dimensional reduction (viSNE) and clustering tools to discriminate between $CD5^+$ B-CLPD cases. Samples from already diagnosed CLL and MCL were analyzed (n=6) and healthy patients' cells were used as controls.
Results: High dimensional reduction (viSNE) revealed at least 6 individual clusters that corresponded to each $CD5^+$ B-CLPD sample. In addition, the two dimensions spatial distribution of the populations showed segregation by disease type (n=6, $p<0.05$). Conversely, healthy control samples were separated in two clusters, but all the samples showed overlapping (p=NS). Clustering suggested the heterogeneity in markers expression in each disease sample. For instance, we detected clusters with higher expression of Ig-k and CD20, that corresponded to MCL samples, $p<0.05$ and $p<0.01$, respectively. Deeper analysis of these samples is currently under investigation.
Conclusions: These preliminary results suggest that combination of high dimensional reduction and clustering might be an additional tool that can be used, at least, to distinguish between $CD5^+$ B-CLPD. Further research is required to confirm these results and to evaluate the power of these tools in the classification of atypical forms of the B-CLPD.

364. (13) **HYDATID FLUID FROM ECHINOCOCCUS GRANULOSUS INDUCE THE AUTOPHAGY PROCESS IN DENDRITIC CELLS AND PROMOTE ANTIGEN PRESENTATION AND T- CELL PROLIFERATION**

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Background: Autophagy is an important process for the presentation of endogenous and exogenous proteins on MHC I and II molecules, promoting activation of CD8+ and CD4+ T cells respectively. The aim of this work is to analyze if hydatid fluid (HF) from Echinococcus, constituted by a wide range of parasite proteins could trigger autophagy improving antigen presentation and T cell proliferation. Methods: BMDCs were cultured in complete RPMI. Hydatid fluid (HF) was punctured from the hydatid cysts collected of infected cattle slaughtered. Antigen uptake was measured with (FITC-OVA) in BMDCs using a standard method. HF-stimulated BMDCs, were evaluated in autophagy induction and MHC II expression. For it, fixed cells were immunostained with LC3-b (clone H50) and analyzed them by immunofluorescence confocal microscopy. CFSE-stained splenocytes were co-incubated with BMDCs using a DC: splenocyte ratio of 1:4 Cellular proliferation was assayed after 4 days of culture by flow cytometry. Results: First, we evaluated if stimulation of HF during 18 h in BMDCs, induce different rates of antigen uptake. Effectively, the presence of Echinococcus antigens induces a markedly decreased OVA-uptake compared to control (**p <0.01, n=3). Next, we studied if stimulation with Eg antigens induces changes in the basal level of autophagy. HF-stimulated BMDCs significantly enhanced the mean fluorescence intensity of MHC II and LC3 and showed a trend in the increment of number and the average size of LC3-positive structures in comparison with unstimulated cells (*p<0.05, **p<0.01, ***p<0.001 HF-stimulated cells vs controls). Finally, we observed that culture splenocytes in the presence of stimulated DC induce their proliferation % CFSE+ cells CTRL:99%, HF:55% (***p<0.001, n=3). Conclusions: These data suggest that HF of Echinococcus induces an increase in autophagy processes promoting the presentation of exogenous antigens presented in MHC II molecules to improve T cell proliferation.

365. (14) **MUCINS AND POLYSACCHARIDES FROM ECHINOCOCCUS GRANULOSUS LAMINAR LAYER INDUCE A MILD MATURATION PHENOTYPE IN DENDRITIC CELLS AND PROMOTE GENE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES**

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Background: The laminar layer is an acellular structure rich in glycans that surrounds the metacestode, creating a mechanical and immunological protective barrier, crucial in the Echinococcus?host interface. The aim of this work is to analyze if purified laminar layer (pLL) could trigger maturation and cytokine production in dendritic cells by activating mTOR pathway, a central regulator of cell metabolism and environmental signals. Methods: BMDCs were cultured in complete RPMI supplemented with 100 ng/ml Flt3-L.
The germinal layer was removed from pLL by washing with 2M NaCl. Flow cytometry: FITC or PE-conjugated mAbs directed to CD11c, CD40, CD80, CD86, MHC I and MHC II were used. Immunoblotting and confocal microscopy: mouse anti-puromycin and rabbit anti-Phospho-mTOR antibodies were used. Gene expression analysis of IL-10, IL-12p35, IL-6, TNF-a, IL-23p19, TGF-b was performed on a Real Time PCR System Results: The pLL of Echinococcus induce a non-statistically significant, but a trend in the up-regulation of CD86 and MHC II and that expression change was diminished by the use of rapamycin (n=5). Conversely, no changes in the expression of CD40, CD80 or MHC I were registered. Further studies were done to analyze whether pLL was also able to stimulate the production of cytokines by BMDCs. When BMDCs were stimulated with pLL induce the expression of IL-6 and TNF-a (n=3 *p<0.05, **p<0.01), but not differences compared to control were observed in IL-12, IL-23, IL-10 and TGF-b. Finally, we evaluated global protein synthesis and phosphorylation of mTOR using confocal microscopy or western blot. pLL (MFI:_979) showed an increase in translation levels compared to its counterpart without stimulation (MFI:_686, *p<0.05 n=3) and also induce an increase in mTOR phosphorylation levels compared to untreated BMCDs (n=3, p<0.01) Conclusions: These data suggest that Echinococcus pLL are recognized by DCs and induce activation of mTOR pathway favoring cell activation.å

366. (88) **HUMAN RARE SEROTYPE ADENOVIRUS AS VACCINE PLATFORM AGAINST *T. cruzi* INFECTION**

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Trypanosoma cruzi is an obligate intracellular parasite and stealth invader that causes a chronic infection affecting millions of people worldwide. Benznidazole, the first line antiparasitic drug, has been used for 50 years and is effective during the acute phase of the infection. However, its use in chronic phase where most cases are diagnosed, is still controversial. Considering these issues developing prophylactic and/or therapeutic vaccines might contribute to public health.
In this context, our laboratory has developed Traspain, a novel chimeric antigen that displays key domains for humoral and cytotoxic anti-parasite immune response. In order to improve antigen-specific cell-mediated immunity, we designed a low-seroprevalence recombinant adenoviral vector (Ad48) carrying Traspain gene.
METHODS
Traspain gene was fused to mouse immunoglobulin *Kappa* light chain signal peptide DNA sequence (SPTrasp) to facilitate extracellular antigen export upon vaccination. Adenovirus 48 carrying SPTrasp gene (Ad48-SPTrasp) was generated using traditional cloning and homologous recombination in HEK293 cells. Antigen expression was assessed *in-vitro* by RT-PCR and Western blot. Exploratory immunization experiments were carried out in C57/BL6 mice, administering two subcutaneous doses of Ad48-SPTrasp every 15 days.
RESULTS
We were able to rescue the replication deficient virus and detect antigen expression upon 48 hours of *in-vitro* infection. Vaccinated animals developed anti-Traspain antibodies (immunized vs control animals ELISA titers: 1782 and 235 respectively) and isotype determination indicated an IgG1/IgG2a ratio of 3.4. Robust antigen-specific CTL response was detected using tetramer staining (CD8+ VNHRLTVL+ cells, immunized: 3.5 % vs control: 0.6 %, p<0.01).
CONCLUSIONS
Considering these results, Ad48-SPTrasp appears as a high potential approach for improving the current strategies of vaccine-mediated control of *T. cruzi*.

367. (112) **POLYMORPHISM -174 G/C OF THE INTERLEUKIN 6 GENE, AND SUSCEPTIBILITY TO Toxocara canis IN HUMANS**

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Polymorphisms in the IL6 gene promoter region can give rise to interindividual variation in the transcription of IL6. Low levels of IL6 expression have been associated with susceptibility to some parasitic diseases. Human infection by Toxocara canis is a zoonosis transmitted by dogs, with a high prevalence in NE Argentina. OBJECTIVE: To investigate the -174 G/C single nucleotide polymorphism (SNP) in the promoter region of the IL-6 gene, related to the susceptibility to Toxocara canis human infection. MATERIALS AND METHODS: The study included 79 patients from Chaco, Argentina (without distinction of age or sex) with epidemiological history of exposure to Toxocara canis. The serological analysis was performed using an indirect ELISA test employing locally obtained secretory/excretory T. canis antigens and peroxidase-labeled anti-human IgG serum. The IL6 -174 G/C polimorphism was studied by a nested polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. RESULTS: Of the 79 patients studied, 14 resulted positive and 65 were negative for T. canis antibodies. The study of the polymorphism genotypes yielded three possible results with the following frequencies: GG: 58.2% (46/79), GC: 38.0% (30/79), and CC: 3.8% (3/79). For this sample, no differences between expected and observed genotype distributions were found. Therefore, Hardy-Weinberg equilibrium is assumed. There was a significant difference in the distribution of the CC genotype between the seropositive and seronegative groups (14.3% vs 1.5%, respectively; OR= 5.12; p=0.02), statistical significance level (p <0.05). CONCLUSION: This study suggests a possible association between the IL6 –174 G/C polymorphism and susceptibility to toxokaryosis in humans. Although the number of patients studied is small, the trend indicates that the presence of the CC genotype could be associated with increased susceptibility to infection.

368. (145) **VIRULENCE FACTORS DELIVERED THROUGH OUTER MEMBRANE VESICLES SHAPE THE OUTCOME OF *B. PERTUSSIS*-MACROPHAGE INTERACTION**

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The outer membrane vesicles (OMVs) are bi-layer structures naturally shed by Gram-negative bacteria that usually contain bacterial virulence factors. In previous studies we found that the OMVs of Bordetella pertussis (Bp), the etiological agent of whopping cough, might influence the outcome of Bp interaction with human MΦ. In this study we tried to dissect the bacterial factors involved in this process. As first approach we determined the proteome of spontaneously released OMVs. By means of LC-MS/MS we identified a total of 350 proteins belonging to a wide range of cellular processes. Importantly, most of the Bp virulence factors were found present in these OMVs. Among them, the main bacterial adhesin,

FHA, and the two main toxins, CyaA and PT. In agreement with the presence of FHA, electron and fluorescence microscopy showed that OMVs attached to and are internalized by MΦ. As determined by flow cytometry, OMVs internalized by MΦ induced a significant decrease in the surface expression of CR3, an effect attributable to CyaA according to recently published studies. CR3 is the main Bp docking molecule on MΦ. These results explain the observed decrease of MΦ Bp uptake in the presence of OMVs. We previously demonstrated that CyaA and PT are involved in the modulation of bactericidal MΦ response. In this study we observed a similar effect on MΦ treated with OMVs prior infection, as determined by RT-PCR ($p<0,05$). Accordingly, confocal microscopy studies showed that bacterial colocalization with the late endosomal/lysosomal marker LAMP-1 was significantly lower in cells preincubated with OMVs, as compared with MΦ untreated or treated with OMVs produced by Bp mutants defective in any of these toxins. Accordingly, bacterial survival inside MΦ preincubated with OMVs was significantly higher than that observed in untreated MΦ. Altogether, these results show that virulence factors delivered through OMVs shape the Bp- MΦ interaction, eventually promoting bacterial persistence.

369. (201) ***Bordetella parapertussis* ADENYLATE CYCLASE TOXIN MODULATES THE HOST DEFENSE AND INFLAMMATORY RESPONSE ULTIMATELY PROMOTING BACTERIAL INTRACELLULAR SURVIVAL**

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B. pertussis (Bp) and *B. parapertussis* (Bpp) are the etiologic agents of whooping cough in humans, which is a re-emerging infectious disease. We have previously found that both species survive and even replicate inside human macrophages, indicating that these cells might serve as a niche for persistence. We further found that pertussis toxin and adenylate cyclase (CyaA) are involved in the intracellular survival of Bp. Bpp expresses solely CyaA, which is the main toxin of this species. Although the genes encoding this toxin in Bp and Bpp display limited polymorphism, there are studies showing different post-translational modifications that eventually lead to less citotoxic Bpp-CyaA, as observed in certain host cells. The aim of this work was to investigate the effect of Bpp-CyaA in macrophage defense response and its eventual role in the non-bactericidal interaction of Bpp with macrophages. To this end, we constructed a Bpp deletion mutant defective in CyaA. The human monocyte cell line (THP-1) was differentiated into macrophages and further infected with the mutant or the parental strain. By means of specific markers and confocal microscopy for bacterial intracellular trafficking studies and antibiotic protection assays, we found that Bpp-CyaA is involved in the early stages of macrophage infection, affecting phagocytosis and lysosomal maturation promoting intracellular survival. Accordingly, by qRT-PCR assays performed at 3 h p.i., we demonstrated that Bpp-CyaA down-regulates the transcription of genes encoding antimicrobial peptides, proteins involved in enzymatic or oxidative defense mechanisms, and the pro-inflammatory cytokine TNF-α, while it up-regulates the transcription of anti-inflammatory cytokine IL-10. All the data were statistically significant with $p<0.05$ analyzed by one-way ANOVA. Taking together, the results suggest that Bpp-CyaA is active in macrophages and plays a relevant role in the bacterial intracellular survival by modulating host defense response.

370. (230) **HUMAN AIRWAY EPITHELIUM AS AN INTRACELLULAR SURVIVAL NICHE FOR *Bordetella pertussis***

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Whooping cough is a reemerging disease caused by Bordetella pertussis (Bp). Current vaccines are protective against symptoms but fail to prevent colonization and transmission, which resulted in pathogen persistence. We have previously found that Bp survives inside phagocytic and non-phagocytic cells, suggesting that an intracellular stage might be important for persistence. The aim of this study was to evaluate whether Bp compromises the epithelial barrier and uses it as an intracellular niche. To this end, human 16HBE14o- cells were used as polarized *in vitro* model. After 7 days of differentiation, functional tight junctions were formed, as verified by means of specific antibodies and fluorescence microscopy. We developed a subconfluent model in which cells exposed apical and basolateral membranes, allowing the study of Bp interaction with both of them. Bacterial attachment and internalization were quantified by fluorescence microscopy at 5h post-infection. The results showed that Bp has a tropism for the basolateral membrane, in which most of the attachment and internalization was observed. Confocal microscopy analysis showed that a high percentage of internalized bacteria avoided phagolysosomal fusion. Antibiotic protection assays confirmed a high survival inside these cells at late time points (48h) post-infection, suggesting that epithelial cells might constitute a niche of persistence for this pathogen. Adenylate cyclase toxin (CyaA), a virulence factor released by Bp both in its soluble form and inside outer membrane vesicles (OMV), was found involved in tight junction disruption. We recently found that Bp OMVs preferentially attach to polarized respiratory cells near tight junctions. This might facilitate CyaA delivery to these sites, eventually granting Bp access to the basolateral membrane during infection. Furthermore, these results also suggest that the respiratory epithelium might have been underestimated as a persistence intracellular niche.

371. (337) **BP1092, A *BORDETELLA PERTUSSIS* VIRULENCE REGULATORY PROTEIN INVOLVED IN ADAPTATION TO INTRACELLULAR ENVIRONMENT.**

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Bordetella pertussis (Bp), which causes whooping cough, survives within macrophages, and adapts its bacterial proteome during intracellular infection. Virulence factors play a key role in bacterial persistence inside the host cell. Transcription of *Bp* virulence factors are controlled by the interrelated two-component systems BvgAS and RisAS. The participation of additional regulatory systems in this process is not clear. The aim of this work was to characterize the contribution of the BP1092 protein, a two-component system histidine kinase overexpressed intracellularly, in virulence factor regulation and intracellular survival.

To this end, we constructed a *Bp* deletion mutant defective in BP1092 (BPΔ1092) and analysed the differential expression of virulence factors representative of the virulent (*fhaB*), intermediate (*bipA*), and avirulent (*vraA, kpsE*) phases by qRT-PCR under standard (SS medium) and modulating conditions (SS medium + 50 mM). We showed that BpΔ1092, unlike the parental strain (wt), was unable to downregulate fha and bipA expression in bacteria growing under modulating conditions, suggesting that this protein is involved in the transition to the avirulent phase. Accordingly, by mean of microtiter crystal violet assay, we showed that under modulating conditions BpΔ1092, but not the WT strain, was able to form a biofilm, a way of lifestyle mainly dependent of *fhaB* expression. Interestingly, microscopy and antibiotic protection assays, showed that the lack of expression of BP1092 resulted in a decreased survival of Bp inside macrophages. This result suggests that early events of the intracellular survival depend on BP1092 expression. All the data presented here were statistically significant with $p<0.05$ analysed by one-way ANOVA.

This work reveals a new level of complexity of the virulence regulations of *Bp*. Elucidating the role of BP1092 in the regulation of *Bp* virulence might shed some light on the poorly understood pathogenesis of these bacteria.

372. (342) **DEVELOPMENT AND ANALYTICAL VALIDATION OF COLORIMETRIC DUPLEX RT-LAMP ASSAYS FOR RAPID DETECTION OF SARS-CoV-2 RNA IN CLINICAL SAMPLES**

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AIMS To evaluate duplex RT-LAMP for detection of SARS-CoV-2 RNA.
METHODS i) 2 Sets of primers (6 primers/set) for each ORF1ab, Nucleocapsid(N) and Spike(S) viral genes plus 2 sets for human RNASeP gene as control were designed and tested in silico for inclusivity against sequences from strains circulating in Argentina and abroad and exclusivity against those from related viruses. We used NEB RT-LAMP kit in simplex or duplex formats, plus a RT-LAMP for sample integrity control, using RNAs from RT-PCR tested nasopharyngeal swabs and synthetic viral RNAs. ii) LOD95 was calculated for duplex RT-LAMP tests following FDA and WHO COVID-19 guidelines, using a quantified SARS-CoV-2 positive RNA control (GISAID EPI_ISL_420600) isolated from a local viral strain cultured in Vero cells, and quantified against a WHO SARS-CoV-2 standard. iii) RT-LAMP visualization was done by naked eye and by a mobile app. Strips were photographed using a simple capture device with visual references to standardize strip-tubes positions, focus and light intensity, and colour analysis was implemented by computer vision techniques. iv) a blind panel of RNAs from 20 qPCR positive and 21 qPCR negative samples was tested. Positivity was visualized by colour change between 30-40 min. of incubation at 65°C. Kappa agreement between RT-LAMP and RT-PCR was estimated.
RESULTS LOD95 of selected duplex RT-LAMPS (ORF+N, N+S and S+ORF) ranged between 12.5-15 RNA copies/reaction. The mobile phone app reported correctly photographs of tested strips in accordance with naked-eye visualization and reporting. All 41 swabs were RNASeP-RT-LAMP positive. Maximum Kappa indexes between RT-LAMP and RT-PCR were excellent: 0.902 for ORF+S at 30 min (95% Se&Sp) and 0.804 for S+N at 30 min and ORF+N at 40 min (80%Se,100%Sp).
CONCLUSIONS Optimization of rapid RNA extraction methods from clinical samples and prospective blind-based evaluation is a next step towards validating these tools for point-of-care COVID-19 diagnosis.

373. (41) **TH22 RESPONSE INDUCED BY MYCOBACTERIUM TUBERCULOSIS STRAINS IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS**

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Tuberculosis (TB) remains a major global health problem and is the second leading cause of death from infectious diseases worldwide. Regarding TB immunity, the most important response has been attributed to T cells, with CD4+ T cells playing a crucial role both for the control of infection and the tissue damage. And, although TH1 lymphocytes are essential to control TB infection, other lymphocytes, such as TH22, would participate in this response. IL-22 acts on the mucous membrane promoting tissue repair or inflammatory processes. During TB, CD4+ T cells can evolve to effector cells with membrane-bound IL-22 (Mb-IL-22) inhibiting M. tuberculosis (Mtb) replication into macrophages. Besides, the genetic variability of Mtb could influence immune response. The aim of the study was to explore the TH22 response induced by different Mtb strains during active pulmonary TB. Peripheral blood samples were obtained from patients with multidrug-resistant TB (MDR-TB, n: 59), drug-susceptible TB (S-TB, n: 27) and healthy donors (HD, n: 20). Peripheral blood mononuclear cells (PBMCs) were isolated and cultured alone or with γ-irradiated Mtb strains. IL-22 secretion was measured from plasma and culture supernatants by ELISA while intracellular IL-22 (ic-IL-22) and Mb-IL-22 levels as well as senescent T cells markers, CD57 and PD-1, were determined by flow cytometry. IL-22 T cells expression was higher in S-TB than in MDR-TB and HD despite the strain employed as antigen. Plasma IL-22 levels in TB patients were lower than in HD. Besides, in MDR-TB patients, the greater the bacillary load and the severity of the lesion, the lower IL-22 expression in T cells and IL-22 secretion by stimulated PBMC. In addition, CD57+ and PD1+ T cells were markedly increased in MDR-TB patients and inversely correlated with IL-22 expression, reflecting that T cell exhaustion could lead to a deficient of the host to mount adequate TH22 responses, decrease bacterial load and mount tissue repair mechanisms.

374. (124) **B CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS SHOW CONSERVED CD39-MEDIATED REGULATORY FUNCTION AND INCREASED CD39 EXPRESSION AFTER POSITIVE RESPONSE TO THERAPY.**

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**These two authors contributed equally to this work*

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive joint destruction associated with increased pro-inflammatory mediators. In inflammatory microenvironments, exogenous ATP (eATP) is hydrolyzed to adenosine, which exerts immunosuppressive effects, by the consecutive action of the ectonucleotidases CD39 and CD73. Mature B cells constitutively express both ectonucleotidases, converting these cells to potential suppressors. Here, we assessed CD39 and CD73 expression on B cells from treated or untreated patients with RA. Neither the frequency of $CD73^+CD39^+$ and $CD73^-CD39^+$ B cell subsets nor the levels of CD73 and CD39 expression on B cells from untreated or treated RA patients showed significant changes in comparison to healthy controls (HC). CpG+IL-2-stimulated B cells from HC or untreated RA patients increased their CD39 expression, and suppressed $CD4^+$ and $CD8^+$ T cell proliferation and intracellular TNF-production. A CD39 inhibitor significantly restored proliferation and TNF-producing capacity in $CD4^+$ T cells, but not in $CD8^+$ T cells, from HC and untreated RA patients, indicating that B cells from untreated RA patients conserved CD39-mediated regulatory function. Good responder patients to therapy (R-RA) exhibited an increased CD39 but not CD73 expression on B cells after treatment, while most of the non-responder (NR) patients showed a reduction in ectoenzyme expression. The positive changes of CD39 expression on B cells exhibited a negative correlation with disease activity and rheumatoid factor levels. Our results suggest modulating ectoenzymes/ADO pathway as a potential therapy target for improving the course of RA.

375. (161) **IMMUNOMODULATORY EFFECTS OF DIFFERENT INTRAVENOUS IMMUNOGLOBULIN PREPARATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA.**

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Hypogammaglobulinemia is the most frequent immune defect in chronic lymphocytic leukemia (CLL). Although CLL patients usually have low serum levels of IgG, IgM and IgA, most standard immunoglobulin (Ig) preparations contain > 95% of IgG. Pentaglobin is an Ig preparation of intravenous application (IVIg) enriched with IgM and IgA, with the potential benefit to restore Ig levels of all isotypes. Because standard IVIg preparations have well-documented immunomodulatory effects, we aimed to evaluate the effect of Pentaglobin (IVIgGMA) and a standard IVIg preparation (IVIgG) on leukemic and T cells from CLL patients.

Peripheral blood mononuclear cells from CLL patients were used. T and B cell activation, induced by immobilized anti-CD3 mAb or anti-IgM mAb respectively, was evaluated by flow cytometry (FC). T cell proliferation in response to anti-CD3 mAb or IL-15 was determined by FC using the CFSE dilution assay. We also evaluated by FC the effect of IVIg preparations on T and B cell apoptosis induced by the BCL-2 inhibitor used for CLL treatment, venetoclax. Experiments were done in presence of IVIgG, IVIgGMA or HSA (ct).

We found that IVIgG impaired CD25, CD69 and PD-1 expression (n=9, $p<0.05$) on activated T cells, while IVIgGMA did not. Both preparations decreased T cell proliferation in response to TCR/CD3 stimulation (n=11, $p<0.05$), whereas only IVIgG, impaired proliferation induced by IL-15 (n=15, $p<0.05$). Regarding leukemic B cell activation, CD69 and CD86 (n=15, $p<0.05$) expression on activated B cells was similarly decreased by both preparations. Finally, IVIgGMA but not IVIgG, decreased venetoclax-induced T cell apoptosis (n=9, $p<0.05$) without impairing venetoclax-induced leukemic cells apoptosis.

Our results add new data on the effects of different preparations of IVIg in CLL and show that the IVIgGMA preparation not only impairs leukemic cell activation, but also has a particular profile of immunomodulatory effects on T cells that deserves further investigation.

376. (188) **IMPACT OF THE AZITHROMYCIN ADMINISTRATION ON INTESTINE IMMUNE CELLS: FOCUS ON SEX AND MOUSE STRAIN**

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Azithromycin (AZM) is a widely used antibiotic, with additional antiviral and anti-inflammatory properties not well understood yet. It belongs to the family of macrolide antibiotics and is approved worldwide to treat community-acquired infections. AZM is widely distributed in tissues and body fluids. Although short-term treatment results in decrease of richness, diversity and taxonomic composition of the gut microbiota, long-term effects are unknown. The impact of acute administration of AZM in drinking water (50 mg/kg/day/5 days) was evaluated in lymphocyte (L) subsets and cytokine microenvironment of mesenteric lymph nodes draining small intestine (SI) and colon (C) of 3 mouse strains: C57BL76, Per2KO (clock gene KO) and Foxp3-GFP. Using flow cytometry, we determined frequency (%) and absolute number (#) of CD3+L and CD19+L in adult male and female hosts. In C56BL6 males, no differences were observed between control and treated groups. In Per2KO males the % of CD3+L decreased in SI and C ($p<0.001$) and the % and # of CD19+L increased in SI and C ($p<0.05$) without differences in females. In C of Foxp3-GFP females, decrease of %CD3+L ($p <0.05$) and increase of %CD19+L occurred ($p<0.001$), while males showed increase in # CD19+L in SI ($p<0.05$). We also evaluated the % of Foxp3+, CD49b+ and FOXp3+ CD49b+ regulatory L in Foxp3-GFP mice. Both male and female animals showed a reduction in regulatory subsets that was more pronounced in C of female and SI of male mice ($p<0.05$). In terms of microenvironment, we assessed IL-6 and IL-10 by ELISA. We found that the concentration of cytokines depended on the strain ($p<0.05$), although no differences were observed between control and treated animals. Our results show interesting differences in AZM effects related to anatomical location, strain, and sex. These differences suggest that sex is an important factor to consider for future evaluations of intestinal activity not only for AZM but also for other drugs administered orally.

377. (202) **FUNCTIONAL EVALUATION OF NOVEL HETEROZYGOUS CARD11 MUTATIONS ASSOCIATED WITH DIVERSE IMMUNOLOGIC PHENOTYPES IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES (PID)**

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CARD11 encodes a lymphocyte-specific scaffold protein necessary inter alia for proper NF-κB activation in B- and T- cells. Germline *CARD11* mutations have been described in PID patients with diverse clinical phenotypes including SCID (biallelic null mutations), BENTA (heterozygous, gain-of-function mutations, GOF) and severe atopic disease (heterozygous, loss-of-function, dominant-interfering mutations LOF/DN). Evaluation of novel and rare *CARD11* variants in patients with PID is needed to characterize their functional impact.

Our aim is to determine the functional relevance of heterozygous *CARD11* variants identified in pediatric PID patients treated in Hospital Garrahan. In this work we further analyse three novel variants in CARD11 p.T117P, p.E96K and p.R818Q, and two previously reported pathogenic mutations p.R30W (LOF/DN) and p.G123S (GOF) carried by five unrelated Argentinean patients.

To evaluate signalling and function of CARD11, transfection of the different mutant *CARD11* constructs into HEK293T, Jurkat and JPM50.6 was performed. Our results showed that p.T117P drive to a GOF activity of the protein, p.E96K resulted in LOF/DN activity, while p.R818Q behaved similar to CARD11 wild type (WT) in a heterozygous transfection assay upon antigen receptor-induced activation of NF-κB. The results of immunofluorescence assays showed that both the GOF mutation p.G123S and the novel variant p.T117P caused multimeric aggregation of CARD11 in cytoplasmic complexes in absence of stimuli which were previously described as indicative of active signaling. This unique aggregation from GOF *CARD11* mutants may collaborate with future clinical management.

The identification of novel *CARD11* mutations in PID patients along with the broad range of clinical manifestations point out the necessity for available functional studies validating their potential pathogenic outcome and contributing to further understanding the mechanism that underlies the development of the disease.

378. (243) **CHARACTERIZATION OF EXTRAFOLLICULAR PLASMABLAST RESPONSE IN *TRYPANOSOMA CRUZI* INFECTION**

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B cells are the unique population able to differentiate into antibody-secreting-cells (ASC). The Germinal Center reaction-GC- and the extrafollicular-EF- response generate different types of ASC which provide long- and short-term humoral immunity against infections, respectively. The participation of follicular T helper cells -Tfh-, is key in these processes. In our lab, we identified a population of EF-ASC ($B220^{int}CD138+Blimp\text{-}1+$, Plasmablasts/PB) characterized by high expression of PD-L1 and CD39, present in murine infection models but not in autoimmunity or immunizations. These PB have the capability to modulate T-cell response, since chimera models lacking $PD\text{-}L1^{hi}PB$ have an increase in TNF-IFNγ-producing PD-

1+CD4+ Tcells. The aim of our work is to characterize the phenotype of regulatory PB and the mediators involved in the PB-Tcell interactions. Therefore, B6 mice were infected with *T. cruzi* and splenic, lymph node and bone marrow (BM) lymphocytes were evaluated by FACS at different days post infection (dpi). The 99% of splenic PB expressed high levels of PD-L1 and CD39 until day 23pi and this frequency was significantly reduced from day 28pi ($p<0.005$). Only the 15% of BM-ASC from mice in the chronic phase of infection (130dpi) expressed high levels of PD-L1 and CD39. Splenic PD-L1^{hi}CD-39^{hi} PB also expressed high levels of CXCR4, MHCII, CD80, CD86 and Ki-67 and produced IL-21, IL-6 and IL-10, probably conditioning T-helper response. In addition, we found CXCR5+ and CXCR5^{neg}P-D1+ICOS+Bcl-6+CD4+Tcell populations in spleen of infected mice, which produce TNF, IFNγ, IL-4, IL-6, IL-21, probably sustaining PB response since infected Bcl-6$^{flo/flo}$CD23^{Cre} mice did not exhibit this ASC population. The CXCR5^{neg}Tfh-like population could be located in the EF-area and could be interacting with PB, either collaborating with ASC or being regulated by them.

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379. (248) **B CELLS ACQUIRE A UNIQUE AND DIFFERENTIAL TRANSCRIPTOMIC PROFILE DURING PREGNANCY IN MICE**

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Pregnancy is an intriguing state of double compromise where maternal immune system must undergo certain adaptations to tolerate the semiallogeneic fetus and fight pathogens. B cells are key components of the adaptive immunity, responsible for humoral immune response. There is solid evidence that pregnancy induces strong modifications in B cell development and function. Hence, our aim was to characterize the transcriptomic profile of B cells during pregnancy in mice. We performed a genome-wide transcriptome profiling on isolated splenic B cells from pregnant (P) and non-pregnant (NP) mice. We identified 1136 genes with differential expression in B cells from P mice compared to NP animals (≈1.5-2fold changes, Limma eBayes test, $p<0.0001$, n=4). For biological interpretation we performed a functional enrichment analysis with InnateDB source (http://www.innatedb.com/). Significantly over-represented (ORA) gene ontology (GO) terms and biological pathways showed up-regulation on DNA replication and cell cycle nodes, while down-regulated genes were grouped into an immune response node (hypergeometric algorithm: FC>1.5; BH multiple test correction:$p<0.05$,FDR<0.05). Within the immune response node, B cell activation, antigen processing and presentation, B cell differentiation, cytokine and TLR mediated signaling pathways were downregulated in B cells from P compared to NP mice ($p<0.05$). Diminished mRNA expression levels of key genes implicated on these processes were confirmed by qPCR (Unpaired-t-test, one-tailed,$p<0.05$,n=8). Differential mRNA expression pattern could traduce in a diminished capacity of B cells to differentiate, proliferate, and to produce cytokines and antibodies in response to antigens. Our results strongly suggest that B cells acquire a state of hyporesponsiveness during pregnancy, most probably to tolerate the semi-allogeneic fetus. However, this could also break new paths for understanding why pregnant women are at higher risk for certain infections.

380. (250) **INFLAMMATORY RESPONSES AT THE BOUNDARY BETWEEN THE HOST AND THE WORLD BEYOND: THE DILEMMA OF INFECTION VERSUS COLONIZATION FROM A TONSILLAR PERSPECTIVE**

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Obstructive sleep apnoea (OSAS) is a syndrome suffered by children with hypertrophied tonsils. We have previously demonstrated that these tonsils present a defective Breg compartment. Here, we extend those findings by evidencing the inflammatory cytokine pattern of tonsillar mononuclear cells (TMC) and investigating the grounds of such profile. OSAS TMC were the only cells used for this work. We showed the ability of Bcs to promote the loss of immune homeostatic control by promptly producing TNFα. Using FACS, we determined TNFα production by stimulated TMC in culture. Upon 24 hours, Bcs represented the majority of TNFα$^+$ cells (52,4% ± SEM 4,2% CD20$^{+/down}$ cells vs 41,7% ± SEM 4,0% CD3$^+$ cells, $p < 0.05$). Conversely, at the same time point, IL17 was produced primarily by CD4$^+$ T cells (Th17) which comprised 90% of the IL17$^+$ population. Also at 24 hs post stimulation, two thirds of the Th17 population (59% ± SEM 4%) co-expressed TNFα. Despite the pro-inflammatory profile displayed by TMC in culture, OSAS has long been considered of non-infectious etiology. We cultured the core tonsillar tissue of 31 children and identified 89 bacterial species by MALDI-TOF MS. The species identified had been previously found either causing ENT pathology or as harmless local flora, both situations in competent hosts. Pathogens differ from commensals in being able to penetrate the epithelial barriers. Hence, we performed fluorescence *in situ* hybridization (FISH) with a universal eubacterial (EUB338) probe followed by immune-fluorescence staining, on cryo-sections from excised tonsils. By confocal microscopy, we confirmed bacterial presence within the lymphoid compartment from OSAS biopsies. To conclude, while we cannot ascertain that the microorganisms detected *in situ* as well as through culture are the initiators of the ongoing inflammatory response characteristic of OSAS, the chronification of the process must be related to the evidenced bacterial spreading beyond the normal boundaries.

381. (282) **A NANOSTRUCTURE OF ASCORBYL PALMITATE USED AS VACCINE PLATFORM IMPROVE ANTIGEN-SPECIFIC MEMORY RESPONSE AND RETAINS THE ANTIGEN AT THE INJECTION SITE**

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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 immune response superior to those induced by the soluble counterpart. Here, we studied the effects of various formulations of vaccine components on antigen-specific memory response and on antigen persistence at injection site. Mice were subcutaneously immunized with a single-dose of OVA and CpG-ODN nanoformulated with Coa-ASC16 (OCC), with OVA and CpG-ODN in solution (OC) prepared to room temperature (RT), with OVA and CpG-ODN solution heated and then cooled down to RT (OCø), with OVA solution heated and then cooled

down to RT plus CpG-ODN solution at RT (Oø/C), with OVA solution at RT plus CpG-ODN solution heated and then cooled down to RT (O/Cø). Heating and cooling processes recreated the conditions of the nanoformulation preparation. On the 160th day of post-immunization (pi), mice were intraperitoneally challenged with OVA/CpG-ODN and sacrificed 7 days later. OVA-anti IgG titers (ELISA) and splenocytes by flow cytometry were evaluated. When antigens at the injection site were measured, the formulations were prepared using fluorescent-dye labeled OVA and in vivo scanning of mice was performed on Odyssey®CLx. OCC induced IgG titers higher than OCø and Oø/C at 145 day pi ($p<0.001$). Post challenged (day 167), OCC IgG titers were comparable to OCø group and higher to Oø/C group ($p<0.01$). O/Cø failed to generate OVA-specific IgG. At 167 days pi, OCC generated higher OVA-specific ($CD3^-F480^-CD19^+IgM^-IgD^-IgG^+GL7^-CD38^+OVA^+$) memory and germinal center ($CD3^-F480^-CD19^+IgM^-IgD^-IgG^+GL7^+CD38^-OVA^+$) B cells ($p<0.05$) than the other formulations. In OCC group, the OVA fluorescence signal in the injection site was higher than in the other groups since 0.3 ($p<0.0001$) until 12 days pi ($p<0.01$). Our results show that the nanostructure improve the memory response and induce antigen depot at the injection site.

382. (300) **EXPRESSION AND MODULATION OF PROLACTIN RECEPTOR IN IMMUNE CELLS: INDUCTION OF mRNA AND PROTEINS BY DEXAMETHASONE**

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It is known that there is a relationship between hyperprolactinemia and autoimmunity. However, the role of prolactin receptor (PRL-R) in autoimmune development is still controversial. The aim of this work is to analyze the expression of PRL-R in murine immune cells, and determine whether PRL-R are modulated during activation with Concanavalin A (conA) and suppression with Dexamethasone (dexa) in healthy mice and three mice models for Lupus. For this end, splenocytes from C57BL/6 (C57), and lupic FcgRIIb KO (RIIb), NZM, and MRL/MpJ-Faslpr (FAS) mice were stimulated with 1 μg/ml of conA or 1 μg/ml dexa and kept in culture for 24 hours. RNA from splenocytes was isolated using Trizol and retrotranscribed to cDNA to detect PRL-RL by qPCR. Protein determination was carried out by flow cytometry using Anti-CD4, Anti-CD19, and Anti-PRL-R. We found that splenocytes from RIIb mice have higher mRNA of PRL-RL expression than other strains. In CD4+ T cells from C57 mice PRL-R increased by suppression with dexa and did no changes when stimulated with conA. In contrast, in CD4+ T cells from FAS mice, PRL-R increased after conA and decreased with dexa, while the CD4+ T cells from RIIb and NZM mice displayed no changes. In CD19+ B cells from C57 and FAS mice, we observed an increase of total PRL-R when we stimulated with dexa and no changes with conA, while in CD19+ from RIIb and NZM mice, did not display changes. These results show a differential behavior of PRL-R expression in C57 mice compared with the lupic mice after stimulation with conA and dexa. The fact that C57 displayed low levels of PRL-R during activation but high in suppression gives us the notion that PRL-R may contribute to maintaining a cellular immune balance by limiting PRL trophic effects. By contrast, the high levels of PRL-R during activation in FAS mice suggest an exacerbated trophic effect of PRL. Our data show that differential PRL-R expression in immune cells may contribute to regulate the immune response.

383. (313) **ORAL ADMINISTRATION OF MINTHOSTACHYS VERTICILLATA ESSENTIAL OIL ACTIVATES CD4+ AND CD8+ T CELLS IN MICE**

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Minthostachys verticillata essential oil (EO) has demonstrated antimicrobial, antioxidant and immunomodulatory activity. The aim of this study was to evaluate the immunomodulatory effect of oral administration of *M. verticillata* EO in a murine model. Male Balb/c mice (n=9) were divided in three groups and administered orally by gavage once a day for 10 consecutive days as follows: Control group 100 μl of saline solution (ClNa 0.9%), Treatment 1 (T1) group 100 μl of EO (5 mg/kg/day) and Treatment 2 (T2) group 100 μl of EO (10 mg/kg/day). Body weight, fecal consistency and fecal occult blood were evaluated as important indicators of intestinal health. The blood parameters were evaluated trough total and differential leukocyte count in peripheral blood. At the end of the assay mice were sacrificed by cervical dislocation and spleens were collected for $CD4^+$ and $CD8^+$ T cells quantification by flow cytometry. An increase in the body weight gain was observed in T1 group compared to the control and T2 groups ($p<0.05$). No difference was observed in diarrhea scores among treatments neither damage in the digestive system evidenced by the absence of fecal occult blood in all groups. T1 and T2 groups showed an increase in *total leukocyte count* compared to the control group ($p<0.05$). The leukocyte formula revealed that lymphocytes percentage were significantly higher in T2 group compared to control group ($p<0.05$). The number of $CD4^+$ T cells in splenic tissue were significantly higher in T1 and T2 groups compared to control group ($p<0.05$). The number of $CD8^+$ T cells was not altered in mice treated with EO. These results indicated that oral administration of *M. verticillata* EO was safe and modulates the systemic adaptive cellular immunity in mice *through positive regulation of T cells subsets.* Further studies will need to be carried out to elucidate whether this natural product could be used as an immunomodulatory food additive.

384. (319) **MINTHOSTACHYS VERTICILLATA ESSENTIAL OIL ALONE OR MICROENCAPSULATED ACTIVATES TH1 CELLS AND INDUCES THE PRODUCTION OF OPSONIZING ANTIBODIES**

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In a previous study, we demonstrated the adjuvant capacity of the Minthostachys verticillata essential oil (EO) (2.5 mg/ml) or microencapsulated essential oil (EOMC) (5 mg/ml) on the humoral and cellular immune response against Enterococcus faecium in Balb/c mice. The aim of this study was to evaluate the functionality of the specific antibodies produced and to quantify the IFN-γ and IL-4 cytokines. Balb/c mice (n=4 per group) were inoculated by subcutaneous injection (days 0, 14 and 28) as follows: Group 1: 100 μl saline solution; Group 2: 100 μl bacterin+EO (2.5 mg/ml); Group 3: 100 μl bacterin+ microcapsule wall material (2.5 mg/ml); Group 4: 100 μl bacterin+EOMC (5 mg/ml). In all groups, bacterin was of E. faecium. Seven days after the last booster, blood was obtained and serum was separated. Mice were sacrificed by cervical dislocation and spleens were removed to obtain splenic lymphocytes for cell proliferation assay. To evaluate the functionality of the antibodies, the Opsonophagocytic Killing Assay was carried out using different dilutions of the sera (1/100, 1/200, 1/500, 1/1000, 1/2000), E. faecium (1x107 CFU/ml), bovine PMNs (1x107cells/ml) suspensions and a source of bovine complement. Sera antibodies of mice from Group 4 mediated the opsonization of E. faecium and enhance the

phagocytosis by PMNs up to the 1/500 dilution compared to Group 2, which was up to the 1/200 dilution (p<0.001). IFN-γ and IL-4 were quantified in the culture supernatants by sandwich ELISA. Higher levels of IFN-γ and IL-4 were detected in lymphocyte culture supernatants of Group 2 compared to other groups (p<0.001). In Group 2, IFN-γ levels were higher than IL-4 (p<0.001), indicating that the activation of CD4+ T cells by the vaccine formulation containing alone EO may result in a Th1 profile. The results obtained confirm that EO and EOMC activate humoral and cellular immunity and could be candidates for vaccine adjuvants.

385. (336) **CHARACTERIZATION OF SPECIFIC CAMELID SINGLE DOMAIN ANTIBODIES AGAINST MURINE IgG1 TO BE USED AS LIGAND IN CONVENTIONAL MONOCLONAL ANTIBODY PURIFICATION**

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In addition to classical antibodies (Abs), the adaptive immune system of camelids comprises heavy-chain only antibodies (HCAb), where antigen-binding is mediated exclusively by one variable domain. These single domains are the smallest functional Abs, known as VHH or nanobodies (Nbs). Due to their inherent favorable attributes, such as high affinity and specificity for their antigen, easy cloning and small size, VHH have great potential for diagnostic, therapeutic and biotechnological applications. Thus, Nbs are used as protein purification ligands because of their high stability, monomeric nature, and easy directional immobilization to solid supports. For this reason, our aim was to characterize VHH against murine IgG1 (mIgG1) to be used as ligands in a new affinity chromatography method to purify conventional monoclonal Abs (mAbs). To achieve our goal, two immune VHH libraries were previously constructed starting from llama blood, and specific Nbs against mIgG1 were selected by Phage Display methodology. Moreover, 9 out of the 24 most reactive selected VHH were confirmed as unique by sequencing. In this work, these Nbs were expressed as soluble protein in *E. coli* WK6 strain. Later, 4 of these VHH were purified from periplasmic extract by IMAC. Reactivity of these soluble-expressed Nbs against mIgG1 was confirmed by ELISA and Western Blot (WB). Also, the reactivity against IgGs from other species was evaluated by Dot Blot and WB. Moreover, all these VHH were able to successfully bind and immunoprecipitate IgG from mouse sera and ascites. In conclusion, we have characterized four specific VHH expressed as soluble protein, which showed high reactivity against mIgG1 in ELISA and WB as expected. All of them were able to recognize their target in a biological protein mixture. Even more, two of them were highly specific for only murine IgG. Further characterization of these Nbs will allow us to develop a new VHH-based method of mAb purification with potential advantages.

386. (338) **CHRONIC POLIOVIRUS INFECTION IN A FEMALE PATIENT WITH UNCHARACTERIZED IMMUNODEFICIENCY: CASE REPORT**

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Introduction: Patients with B-cell immune deficiency have increased risk of complications associated with enterovirus infections. Long-term excretors could reintroduce mutated polioviruses with increased neurovirulence into the general circulation. Treatments such as pocapavir can reduce environmental spread.
Objective: To present clinical behavior of a patient with uncharacterized immunodeficiency and chronic poliovirus infection.
Clinical case: A two-year-old female patient, term newborn, daughter of healthy non-consanguineous parents, without relevant family history is hospitalized at 9 months old due to a left brachiocrural hemiparesis. She is evaluated by Neurology who assumes acute disseminated encephalomyelitis and prescribes methylprednisolone and EV gammaglobulin. The patient responds favorably so she is discharged.
After 3 months, she is referred to Immunology for evaluation. Inmunological screening shows undosable IgA and IgM, IgG 228mg/dl. Lymphocyte subpopulations CD3 77% CD4 48% CD8 27% CD19 15% NK 8%. Subpopulations B Naive 98.9%, memory without change of isotype 1.04%. Absent plasma cells. Blood group 0+. Isohaemaglutinins anti A and anti B absent. Ac HBs non-reactive. Uncharacterized humoral immunodeficiency is assumed so EV gamma globulin is initiated. The patient had received sabin at 6 months old so samples are sent to Malbran Institute to rule out vaccine´s secondary infection, obtaining positive result for sabin 3 postvaccinal serotype. Actually the patient continues fecal viral shedding wich presents 30 mutations. Compassionate use of pocapavir is attempted but cannot be imported. Samples are sent to NIH in the United States for mutation testing.
Conclusions: Our case explain chronic poliovirus infection in a patient with humoral immunodeficiency. We consider important to provide an antiviral treatment wich stops the environmental spread of the virus, because it has suffered numerous mutations that can constitute a serious epidemiological risk.

387. (340) **QUANTITATIVE AND INTEGRATIVE ANALYSIS WITH HISTORICAL PERSPECTIVE OF INFLAMMATORY BOWEL DISEASE RESEARCH: UNDERSTANDING ETIOLOGY AND PATHOGENESIS AS A RESULT OF AN INTERACTOME OR "NETWORK EFFECT"**

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Inflammatory bowel diseases (IBD) present a complex etiology associated with multiple factors interacting at different functional levels (i.e., risk genes, molecules, immune cells, biological processes and environment). The wide diversity of factors and associated variables studied over the last 3 decades in the context of IBD has challenged our ability to integrate the empirical information available in humans. Thus, our goal was to quantitatively analyze how representativeness of components of different functional levels and their interactions have changed over the last 30 years of IBD research in humans. A bibliographic search in Pubmed was performed aimed at extracting experimental studies on IBD in humans published between July 1990 and June 2020. This resulted in 25971 valid abstracts. A search for 1380 specific variables in these abstracts was conducted automatically using customized code in Matlab. The selection of these variables was based on recent reviews and, genetic and molecular catalogues. Publications showed a 5-fold annual rate increase from 1990 to the present. Representativeness of molecular and cellular components remained relatively constant over time, while genes showed a peak increase in publications between 1990 and 2005, with an increasing diversity of genes studied. Microbiota-sensing related variables showed a constant increase in publications. Specifically, components such as IL-1, IL-6, IL-10, NOD2, IFNγ, NF-κB, ATG16L1, TNF, T helper, Treg, macrophages and monocytes emerged as the most frequently studied elements. These components appear as integrative nodes in the network of variables involved in the manifestation of IBD. Our quantitative analysis with historical perspective supports the need to comprehend IBD as the resultant of an interactome or 'network effect'. It also highlights the importance of elucidating the dynamic relations between nodes to comprise etiology and pathophysiology.

388. (344) **ANALYSIS OF THE T AND B CELL COMPARTMENTS IN TONSILS OF CHILDREN WITH TRISOMY 21**

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Trisomy 21(T21) is the most frequent chromosomal disorder in humans with approximately 1/700 live births, caused by an inherited extra copy of human chromosome 21. Children with T21 have a unique disease spectrum with increased incidence of obstructive sleep apnea, tonsil hypertrophy, susceptibility to autoimmunity, a severe reduction in switched memory B cells and poor response to vaccination (Farroni et al., 2018; www.ndss.org). Since follicular helper T cells (Tfh) facilitate B-cell activation and differentiation, we studied Tfh cells in tonsils obtained from two children with T21 whose tonsils were extracted due to hypertrophy and two age-matched controls, one of them with recurrent tonsillitis and the other with hypertrophy. We performed immunohistochemistry and multiparametric flow cytometry to characterize the different subsets. Our preliminary data indicate that the diameters of follicles in T21 tonsils were reduced compared to controls (~500 vs 700 μm). The frequencies of CD19+ and CD3+ cells among the total tonsil CD45+ cell population were not modified in T21 samples. The frequency of the $CD3^{+}$ $PD1^{high}$ population, associated with germinal center Tfh, was reduced compared to age matched controls (27% vs 36%). Also, the frequency of $CD3^{+}$ $PD1^{int}$ was increased in T21 tonsils (38% vs 28%). The expression of the activation marker CD38, measured as mean fluorescence intensity (MFI), is reduced in both CD3+ population ($MFI\text{-}CD38_{PD1int}$ = 2066 vs 3037; $MFI\text{-}CD38_{PD1hi}$ = 5393 vs 8454). We also analyzed the frequencies of the different B cell populations determined by the expression of CD19, CD27, CD24 and CD38 markers and we observed an increase in the $CD19^{+}$ $CD38^{high}CD24^{high}$ cell population, associated with a transitional phenotype (3,1% vs 1,6%). Our results open new avenues to keep on investigating the B and Tfh response in children with Down Syndrome.

389. (351) **THE USE OF SPECIFIC HISTAMINE RECEPTOR 4 ANTAGONIST REDUCES INFLAMMATORY RESPONSE IN ALLERGY**

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Non classical CD8 T lymphocytes are poorly studied in allergic processes. However, it has been shown that they play an important role in diseases such as asthma. CD8 T cells are resistant to steroids (the preferred therapy for asthma), and a subset of these cells can produce type 2 cytokines (IL-5, IL-13) that perpetuate the immune response. Our group has previously shown in a mouse model of asthma that the instillation of histamine treated DCs exacerbates Th2/Tc2 type immune response, and that this can be partially reverted with the use of the H3R/H4R antagonist Thioperamide. In the present work we focus on the role of H4R in this effect, using a highly specific H4R antagonist, JNJ7777120 (JNJ). We generated the murine OVA allergy model using two intramuscular doses of OVA/AlOH (concentración) in a sensitization phase, and 5 consecutive daily intranasal OVA 3% instillation as a challenge. On day 1 of the challenge, mice were also intranasally inoculated with bone marrow derived DCs (BMDCs) treated or not with HIS (10^{-6}M) and JNJ7777120 (10^{-5}M). Two weeks after day 1 of challenge, mice were sacrificed and the lungs obtained, processed and analyzed. We found that, whereas no significant differences were found in IL-13 production, another Th2/Tc2 type cytokine, such as IL-5, was significantly reduced, especially in CD8+ T cells. We also found an increase in IL10 production in mononuclear CD11c+ cells. This could indicate that JNJ generates an anti inflammatory milieu that helps reduce cytokine production. We have observed a similar effect on a murine model of allergic dermatitis, where JNJ increases IL-10 levels and decreases Th2 cytokines and T cell proliferation. These results suggest that JNJ effect on type 2 immunity is conserved across allergic pahologies.

390. (389) **IMMUNOMODULATORY EFFECT OF U-OMP19 IN A PAPAIN-INDUCED ALLERGY MODEL**

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Allergies are T helper 2 immune responses mediated by IgE, induced by an antigen called allergen. Many allergens are proteases; thus, allergy models were developed using cysteine proteases like papain.

At our laboratory we had demonstrated the potential of U-Omp19 as an adjuvant in vaccine formulations against infectious diseases inducing a Th1 response. Therefore, the main objective of this work was to evaluate its potential to modulate allergic responses.

To this end an allergy model induced by papain was performed at our laboratory. Mice (n=5/group) were subcutaneously immunized with OVA, OVA+papain or OVA+papain+U-Omp19. The antibody response was assessed by analyzing anti-OVA IgE, IgG1 and IgG2a in serum by indirect ELISA. The specific cellular immune response was also measured, analyzing the levels of cytokines associated with a Th2 response (IL-5 and IL-13) in OVA-stimulated splenocyte supernatants by ELISA.

Our results indicate that the allergic response was induced when mice were immunized with OVA plus papain. In this group, we have found a significant increase in anti-OVA IgE levels ($p < 0.05$, ANOVA + Bonferroni Test), and a specific OVA Th2 response, given the significant increases in IL-5 and IL-13 levels ($p < 0.01$ for IL-5 and $p < 0.001$ for IL-13, ANOVA + Bonferroni Test), compared to the administration of OVA alone. In contrast, when U-Omp19 was added to the formulation, anti-OVA IgE levels were modulated or not elicited and were similar to levels induced by OVA delivered alone group. Besides, the T helper 2 cytokines production were not induced.

Together our results using a model of allergic disease constitute a proof of concept that indicates that U-Omp19 is capable of preventing/modulating the papain induced allergic response. This work paves the way of future research on the immunomodulatory potential of U-Omp19 in allergic responses.

391. (395) **DECIDUALIZATION PROGRAM CONDITIONS MATERNAL MONOCYTES TO A DC-10 PROFILE INDUCING REGULATORY T CELL RESPONSE.**

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The decidualization program allows the secretion of immunoregulatory factors by endometrial stromal cells which may condition maternal leukocytes to a regulatory profile. In this context, we previously showed that decidualized cells conditions maternal monocytes to a tolerogenic profile with characteristics of Myeloid Regulatory Cells (MRC), particularly DC-10. This novel and distinctive subset of dendritic cell is characterized to be a potent IL-10 producer and type 1 regulatory T (Tr1) cells-inducer through HLA-G pathway. Considering that DC-10 was found increased in decidua, here we focus on the impact of decidualization in conditioning monocytes to this subset and its ability to induce $Foxp3^{neg}$ regulatory T cells. Thus, conditioned media (CM) of human endometrial stromal cell line decidualized with MPA+dbcAMP for 8 days (Dec-CM) or not (Non-dec CM) were collected. Then, isolated monocytes from peripheral blood mononuclear cells of healthy women were cultured with rhGM-CS-

F+rhIL-4 for 5 days in the absence/presence of CM.
Whereas both Non-dec and Dec-CM-cultures showed increased IL-10 secretion by monocyte-derived cells, only Dec was able to induce a higher expression of the characteristic DC-10 tolerogenic markers HLA-G and ILT2/CD85j ($p<0.05$, Friedman test). Then, Dec and Non-dec-CM-treated cells were co-cultured with allogeneic lymphocytes for 5 days. On the last day, we observed an anti-inflammatory microenvironment in Dec-CM-cultures, characterized by a higher IL-10:IFN-γ ratio production and a decreased $CD4^+CD25^+$ subset ($p<0.05$, Friedman test). Interestingly, while both co-cultures showed a higher frequency of $CD4^{neg}HLA\text{-}G^+$ cells, only Dec-CM-treated cells were able to induce $CD4^+HLA\text{-}G^+$ regulatory T cell subset ($p<0.05$, Friedman test). The present results suggest that decidualization process promotes the differentiation of DC-10 subset able to induce $HLA\text{-}G^+$ T cells that might play an immunoregulatory role in embryo implantation.

392. (442) **ANTIGEN PRESENTING CELLS PULSED WITH INACTIVATED FMDV REALEASE EXTRACELLULAR VESICLES WITH THE ABILITY TO ACTIVATE BOTH SPECIFIC T AND B-CELLS IN VITRO.**

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Foot-and-mouth disease (FMD) is a highly contagious disease of livestock worldwide and is economically important. The main strategy for the control is vaccination with FMD-Virus (FMDV) chemically inactivated with binary ethylenimide (FMDVi). In FMDV infection and in vaccination, the B cell response plays a major role by providing neutralizing/protective antibody in both animal models and natural hosts. Extracellular vesicles (EVs) are nanovesicles involved in cell–cell communication. EVs secreted by antigen-presenting cells (APC) participate in the activation of B and T cells through the presentation of native antigen membrane associated (to B cells) or by transferring MHC-peptide complexes (to T cells) and even complete antigen from DCs. In our previous work we demonstrated that murine APC cells can internalize FMDVi and release EVs expressing APC markers and high level of viral proteins during the first 24 h. In the present work we aimed to evaluate the immune properties of these EVs in the generation of B and T cell response against FMDV. We demonstrated that EVs-FMDVi induced specific in vitro proliferation in vivo sensitized splenocytes with FMDVi, EVs-FMDVi induced specific B cell ($16.05\% \pm 0.61$ $p<0.001$) and T cell proliferation ($8.5\% \pm 0.81$ $p<0.01$) when compared to unstimulated sensitized splenocytes ($9.66\% \pm 0.17$ and $5.70\% \pm 0.15$, respectively) detected by CFSE dilution.
Our results revealed that EVs FMDVi could present part of FMDV proteins in native conformation or partially processed. These peptides can be recognized by the BCR and stimulate specific B cells response against viral infection. In addition, EVs FMDVi activate direct or indirectly a T cell response that could collaborate in B cell activation.
The knowledge derived from this work will serve to deepen the knowledge of the interrelation between the FMDV and the immune system that will serve for the rational design of vaccines.

393. (500) **CLUSTERIN EXPRESSION PROMOTES T CELL PRIMING BY DENDRITIC CELLS**

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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 analyzed. Here we show that clusterin expression by DCs plays a role in their ability to initiate the adaptive immune response.
As we did before using monocyte derived DCs, we analyzed the expression of clusterin by BDCA+ blood DCs. We found that clusterin production was induced after LPS stimulation (mean 1,61 ng/ml unstimulated vs 6,86 ng/ml stimulated, n=4, $p<0,05$).To look into the role of clusterin on DC function we performed a knockdown (KD) strategy using clusterin shRNA carrying lentiviruses and a scramble (SCR) construction as a control. In a previous report we showed that silencing of clusterin expression (CLU-KD) resulted in an increased cell death of DCs upon LPS stimulation. We now analyzed the function of CLU-KD-DCs in response to LPS stimulation. Control and CLU-KD DCs were stimulated with LPS for 24hs, cells were collected and analyzed by FACS and supernatants were harvested and cytokine secretion was measured by ELISA. We found that phenotype 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 clusterin silencing (not shown). We next studied the role of clusterin in antigen presentation culturing control and CLU-KD DCs with allogenic CD4+ T cells. Interestingly, CLU-KD dendritic cell ability to expand CD4 was found to be strongly diminished (mean 33,87 %CFSE- CLU-KD vs 19,15 CLU-KD LPS, n=6, Paired T test $p<0.01$).
These observations suggest that clusterin might play a role in the control of the adaptive immune response.

394. (504) **HUMORAL AND CIRCULATING FOLLICULAR HELPER T CELLS RESPONSES IN HOSPITALIZED INFANTS WITH COVID-19**

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Background: Marked progress is achieved in understanding the physiopathology of COVID-19 pandemic. However, CD4+ TFH cell subset, which is critical for eliciting neutralizing antibodies, is poorly understood during pediatric COVID-19.
Aims: To characterize the circulating TFH (cTFH) cell subset and to determine SARS-CoV-2 IgM and IgG antibody response in children hospitalized with acute mild and severe (pediatric multisystem inflammatory syndrome, PIMS) COVID-19, compared to pre-pandemic controls.
*Methods:*Sera (n=337) and PBMCs (n=41) from hospitalized COVID-19 children (age 1-14yr.) during different phases after disease onset and healthy donors (n=15, age 1-10 yr., HD) were collected to evaluate:
1.Memory cTFH subset (CD3+CD4+CD45RA-CXCR5+) and cTFH profiles (cTFH1 –non-efficient helper associated with a suboptimal antibody production-, cTFH2, and cTFH17, –efficient helpers) by flow cytometry during 1st week of COVID-19 diagnosis.
2.Anti-SARS-CoV-2 Spike RBD IgM and IgG serum levels during the 1st week and/or 21 days after symptoms onset by ELISA.
Results: Mild COVID (n=30) showed a cTFH profile similar to HD

(n=15). However, we detected a decrease of %cTFH1 (*) but an increase of %cTFH17 (**) in peripheral blood of PIMS infants (n=11) compared to both mild COVID-19 and HD. Indeed, the ratio (cTFH2 and cTFH17)/cTFH1 was increase in PIMS (**). Seropositivity rates were 28% for IgM (n=109) and 25% for IgG (n=337) among children during 1st week of diagnosis, and 63% for IgM (n=51) and 64% for IgG (n=55) after 21 days of symptoms onset. All children with PIMS reached the maximum detectable IgG OD. Interestingly, %cTFH17 positively correlated with anti-SARS-CoV-2 IgG OD (*).
Conclusion: Our results showed that COVID-19 infected children displayed multiple hallmarks of effective humoral response, although the neutralizing activity of antibodies remains to be elucidated. Moreover, the elevated levels of antibodies in PIMS infants point towards their role in severity of disease.

395. (520) **NOVEL FLOW CYTOMETRY IMMUNOASSAY TO STUDY THE PREVALENCE OF ANTI-PROINSULIN AUTOANTIBODIES IN ARGENTINE CHILD-ADOLESCENT PATIENTS WITH TYPE 1 DIABETES MELLITUS**

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Anti-proinsulin autoantibodies (PAA) are often the first markers that appear in patients with type 1 Diabetes Mellitus (T1DM) and its prevalence varies from 10-60% in child-adolescent patients. The gold standard method for PAA detection is the Radioligand Binding Assay (RBA), a highly specific and sensitive technique, but expensive and polluting; thus, it is imperative to develop an alternative method.
The **aim** of this work was studying the prevalence of PAA in Argentine pediatric patients with T1DM using a novel flow cytometric microsphere-based immunoassay (FloCMIA).
Materials and methods: Human proinsulin (PI) was expressed as Thioredoxin fusion protein (TrxPI) in *E. coli* and a fraction was biotinylated. Sera from 100 normal human controls and 51 T1DM patients -all PAA positive by RBA- were used to optimize FloCMIA. A double paratope model was used in which samples were incubated with TrxPI-biotin and microspheres adsorbed with TrxPI. The immune complexes were revealed using streptavidin-Phycoerythrin. The geometric mean of the signals was analyzed, and the results were expressed in Standard Deviation scores. With the optimized FloCMIA, the prevalence of PAA was evaluated in 60 samples of patients with T1DM (age range 0.1-18 years).
Results: The study of ROC curves allowed choosing a cut-off value of 3.7 SDs and the AUC was 0.884, indicating that the method has good ability to distinguish between samples from each group. The specificity of FloCMIA was 97% and the analytical sensitivity 69%, calculated as the percentage of patients RBA positive that were also positive by FloCMIA. There was a substantial agreement between methods (kappa statistic=0.700).
A prevalence of 30% for PAA was obtained in the population of T1DM patients studied.
Conclusions: An alternative method to RBA was developed with good performance and less operational complexity and environmental impact. The novel assay was implemented in Argentine patients with T1DM to study the prevalence of PAA.

396. (529) **PRODUCTION OF RECOMBINANT GAD65 BY INSECT LARVAE AND ITS EVALUATION AS ANTIGEN-DIABETOGENIC SPLENOCYTES PROLIFERATION INDUCTOR**

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The 65kDa isoform of glutamate decarboxylase (GAD65) is one of the main autoantigens in Autoimmune Diabetes Mellitus.
The aim of this work was to express recombinant GAD65 (rGAD65) in insect larvae and assay its capacity as antigen-driven proliferation of NOD mice-derived splenocytes.
GAD65 was expressed in *Spodoptera frugiperda* larvae using the baculovirus expression system with 97% purity yielding 5.7 mg/g of larvae. rGAD65 immunoreactivity was corroborated by radiometric assay using sera from diabetic patients with antibodies against GAD65. Proliferation assays were performed to evaluate the capability of splenocytes to recognize rGAD65. Splenocytes from pre-diabetic and diabetic NOD mice were cultured in triplicates in 96-well U-bottom plates with RPMI (basal proliferation) or with different concentration of the following diabetogenic antigens: 0.01ug/mL to 1 ug/mL of rGAD65, 0.1 ug/mL to 4 ug/mL of insulin and pancreatic islet lysate and 10 ug/mL of ovoalbumin as negative control. A positive unspecific control was carried out with ConA 10 ug/ml. The cells were cultured for 5 days, [^{3}H]TdR was added in the last 18h of the assay. Cells were harvested and the radioactivity incorporated was determined by liquid scintillation counter. Cell proliferation was expressed as Stimulation Index (SI = antigen-proliferation/basal proliferation). SI obtained for the different doses of each treatment were not significant. Besides, all antigen tested induced proliferation of NOD splenocytes compared to de basal condition ($p<0.01$). SI of pre-diabetic NOD splenocytes ranged from 0.28±0.06 to 2.45±0.25 for rGAD65 at 1 ug/mL to 0.01ug/mL, from 5.34±1.38 to 4.06±0.44 for insulin at 4 ug/mL to 0.1ug/mL and from 5.15±0.03 to 3.58±0.48 for islet lysates at 4 ug/mL to 0.1ug/mL. SI of overt-diabetic NOD splenocytes ranged from 2.06±0.32 to 2.35±0.11, from 3.07±0.19 to 2.95±0.42 and from 2.83±0.28 to 2.01±0.44, respectively. SI for OVA was 0.44±0.6 and 0.57±0.02, and ConA 56.32±5.84 and 15.16±2.52 for pre-diabetic and diabetic, respectively.
In sum, rGAD65 was successfully produced in *S. frugiperda.* Moreover, rGAD65 stimulated diabetogenic splenocytes proliferation obtained from NOD mice, fortunately, similarly to insulin and islets lysate. The dose of 1μg/ml of rGAD65 seems to be toxic for cells. Our preliminary results suggested that rGAD65 can be a potential candidate to generate immunotolerance to prevent experimental autoimmune diabetes.

397. (293) **BONE MARROW TRANSPLANTATION MODIFIES THE RELAPSE TO COCAINE: A POSSIBLE ROLE FOR PERIPHERAL IL-17A SIGNALING**

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Introduction: One of the main challenges to understand drug addiction is defining the biological mechanisms that underlie individual differences in recidivism. Studies of these mechanisms have mainly focused on the brain, yet we demonstrate here a significant influence of the peripheral immune system on this phenomenon. Lewis (LEW)

and Fischer 344 (F344) rats have different immunological profiles and they display a distinct vulnerability to the reinforcing effects of cocaine, with F344 more resistant to reinstate cocaine-seeking behavior. Methods: Bone marrow from male LEW and F344 rats was transferred to male F344 rats (F344/LEW-BM and F344/F344-BM), and these rats were trained to self-administer cocaine over 21 days. Following extinction, these animals received a sub-threshold primer dose of cocaine to evaluate reinstatement of drug-seeking behavior. Results: F344/LEW-BM but not F344/F344-BM rats reinstated cocaine-seeking behavior, in conjunction with changes in their peripheral immune cell populations to a profile that corresponded to that of the LEW donors. Cocaine treatment increased the CD4+ T-cells in F344/LEW-BM rats, and the splenic expression of Il-17A, Tgf-b, Tlr-2, Tlr-4 and Il-1b was altered in both groups. Discussion: We propose that peripheral T-cells respond to cocaine, with CD4+ T-cells in particular undergoing Th17 polarization and generating long-term memory, these cells releasing mediators that trigger central mechanisms to induce reinstatement after a second encounter. Conclusion: This immune response may explain the high rates of recidivism observed despite long periods of detoxification, shedding light on the mechanisms underlying the vulnerability and resilience of specific individuals, and opening new perspectives for personalized medicine in the treatment of relapse.

398. (36) **DETECTION OF SERUM ANTI-LIPOPOLYSACCHARIDES (LPS) ANTIBODIES FROM ENTEROHEMORRHAGIC *E. COLI* (EHEC) IN ASYMPTOMATIC KINDERGARTEN TEACHERS OF BUENOS AIRES PROVINCE.**

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EHEC is responsible for developing typical Hemolytic Uremic Syndrome (HUS) and Argentina has the highest incidence in the world. The endemo-epidemic behavior that HUS presents in our country was associated with person-to-person transmission. Since children below 5 years old are the most affected, our objective was to determine the frequency of kindergarten teachers with anti-LPS-EHEC antibodies in serum.

One hundred and fifty teachers from J.C. Paz district from Buenos Aires Province participated in an informative talk given by LuSUH, a non-governmental association, about good practices to reduce HUS transmission. Then, 63 of them voluntarily gave, under a signed written informed consent, a sample of blood obtained by finger puncture. A commercial ELISA (Chemtest, Arg) was used to detect specific IgM and IgG isotypes against 4 types of LPS-EHEC: O157, O145, O121 and O103. These variants represent approximately 87% of the EHEC serotypes associated with HUS in Argentina. The IgM finding was considered as indirect evidence of current infection with the EHEC specific serotype.

Forty-two samples were evaluated, 60% (25/42) of them were positive for at least one type of LPS. Fifty-six percent of them (14/25) were IgM positive. Fifty percent of the positive samples were reactive for LPS O157, 26% for LPS O121, 12% for LPS O145 and 2% for LPS O103. Among the samples positive for LPS O157, 5% belonged to IgM isotype, 31% to IgG and 14% had both. The IgG isotype was the unique detected for LPS O145 and O103 (12 and 2%, respectively). For LPS O121, the IgM isotype was detected in 2%, IgG in 12% and both isotypes in 12% of the samples.

The elevated frequency of LPS-EHEC reactive samples (60%) reflects the high circulation of EHEC strains in our country. This agrees with the prevalence of anti-Stx2 antibodies reported in our population previously. Very strikingly, nearly half of these individuals carry IgM, suggesting that they would be in the active phase of infection.

399. (47) **INFLUENCE OF THE INTESTINAL ENVIRONMENT ON *ESCHERICHIA COLI* O157:H7 PATHOGENICITY AND INFECTIVITY**

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We previously demonstrated a different outcome post *E. coli* O157:H7 (O157) infection between weaned C57BL and BALB mice. Although bacterial burden is similar, C57BL show increased tissue damage and mortality. The aim of this work is to investigate how intestinal environment affects pathogen virulence and horizontal transmission in both strains.

3 days post infection (pi), we analyzed the Stx production of recovered O157 from C57BL and BALB feces. Results showed similar Stx production by VERO and ELISA assays. Besides, we determined the inflammatory activity of feces through a LPS-dependent Hek-Blue-hTLR4 reporter line assay. Feces from C57BL showed inflammatory activity at day 1, 2 and 3 pi ($p<0.0001$, $p<0.001$ and $p<0.01$ respectively vs control). The maximal activity was statistically different from BALB at day 1 ($p<0.0001$). Feces from BALB showed inflammatory activity only at day 2 pi ($p<0.01$) [2way-ANOVA].

We tested infectivity of O157 in C57BL and BALB through co-housing experiments (2 infected + 5 non infected mice of the same strain were co-housed). The number of infected naive mice was recorded by O157 shedding. Despite all O157 inoculated C57BL died, 60% and 100% of naive mates were infected at day 3 and 7 pi respectively (one of them died). All inoculated BALB survived and only 1 out of 5 naive mates was infected at day 3 pi; this mouse recovered and negativized. At day 5 pi, a second mouse resulted infected (none of them died).

The local anti-O157 IgA response was assessed in feces from both strains. A significant population of IgA-coated bacteria was detected in pellets from infected BALB at day 3 pi by cytometry (infected vs control $p<0.05$; 2way-ANOVA). Besides, anti-O157 IgA was detected in all surviving BALB at day 7 pi by ELISA (infected vs control $p<0.05$; Student's t test).

BALB mice showed reduced horizontal transmission, lower and delayed inflammatory response and a specific and early local humoral response in feces compared to C57BL.

400. (91) ***TRYPANOSOMA CRUZI* PROMOTES MATURATION OF DENDRITIC CELLS AND THE RECRUITMENT OF PROTEINS INVOLVED IN ANTIGEN CROSS-PRESENTATION TO THE PARASITOPHOROUS VACUOLE**

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$CD8^+$ T cells are crucial in the defense against *Trypanosoma cruzi* infection. Efficient priming of $CD8^+$ T cell responses requires not only the processing and presentation of antigens, but the expression of costimulatory molecules by activated dendritic cells (DCs).

To analyze whether the interaction of *T. cruzi* with DCs promotes cell maturation, primary cultures of DCs were generated from mouse bone marrow (BMDCs) and incubated with trypomastigotes (Tp) of the Tulahuén strain of *T. cruzi*. DCs activation was analyzed at different time points by measuring the expression of the costimulatory molecule CD86 by flow cytometry. The activation of BMDCs

was only evident after 18 hours of co-culture (CD86 expression increased by 40%; statistical significance analyzed by Bonferroni Two-way ANOVA).
Then, we analyzed whether DC activation upon interaction with *T. cruzi* increased the cell capacity to cross-present exogenous antigens. To do so, matured BMDCs (previously stimulated with Tp from *T. cruzi* for 18 hours) were incubated with soluble ovalbumin (OVA) and co-cultured with the B3Z hybridoma cell line. OVA cross-presentation promote B3Z activation and the generation of a colorimetric reaction measured by OD. Our preliminary results show that BMDCs incubated with Tp increases OVA cross-presentation.
We are also interested to understand whether the parasitophorous vacuole (PV) formed upon *T. cruzi* infection of DCs can serve as an organelle involved in the presentation of parasitic antigens. Hence, by using confocal microscopy, we evidenced the recruitment of Tapasin and Calreticulin (components of the cross-presentation machinery) to the PV after 2 hours of infection of BMDCs suggesting that this organelle can act as a cross-presenting organelle.
Summarizing, we consider that antigen cross-presentation after *T. cruzi* internalization by DCs is a likely efficient mechanism that could actively participates in the orchestration of $CD8^+$ T cell responses against the infection.

401. (113) **EVALUATION OF THE ROLE OF TISSUE REPAIR REGULATORY T CELLS DURING ACUTE AND CHRONIC *TRYPANOSOMA CRUZI* INFECTION**

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Tissue repair $CD4^+Foxp3^+$ regulatory T cells (tisTregs) are a specialized Treg subset that exhibit tissue-specific phenotypic, functional and transcriptional profiles. tisTregs maintain tissue homeostasis and also display conventional immunoregulatory properties. *T. cruzi* (Tc) triggers a strong effector response that controls parasite spreading but promotes pathological tissue damage. We previously determined that during the acute phase of Tc infection, there is a reduction in tisTregs frequency and numbers in Spleen, Liver and Skeletal Muscle (SM) that correlates with decreased systemic levels of their growth factor IL-33. We also found altered values of biochemical markers of tissue damage (LDH, AST, ALT, CK-MB, glucose) in plasma.
In the current work we aimed to evaluate the behavior of this cell population and its correlation with biomarkers of damage as well as systemic IL-33 levels during the chronic phase of this infection. To this end, Foxp3-GFP C57BL/6 mice were infected (INF) with 5000 Tc parasites (Tulahuen). At 170 days post infection, ($ST2^+KLRG\text{-}1^+$) tisTreg cell numbers were quantified at different tissues by flow cytometry. The frequency and numbers of tisTregs were increased in target tissues like skeletal muscle and visceral adipose tissue but not in spleen and liver from INF mice in comparison to non-infected controls. INF mice also showed higher plasma levels of IL-33, as determined by ELISA. Lastly, only an elevated level of LDH among other biochemical markers remained in INF versus NI mice as evidence of damage.
This results together with our reported data support the speculation that during the acute phase of Tc infection, the decrease of tisTregs may be necessary to allow the immune control of parasite replication; while their expansion in target tissues during the chronic phase might be necessary to avoid excessive damage over time. Modulation of this cell population would allow us to better understand its role in this disease.

402. (168) **EARLY TREG CELL DEPLETION DURING *TRYPANOSOMA CRUZI* INFECTION PROMOTES CD8+ T CELL IMMUNITY AND PARASITE CONTROL IN THE ACUTE AND CHRONIC PHASES**

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We reported that after *Trypanosoma cruzi* (Tc) infection, Tregs undergo a marked and sustained reduction in frequency. This natural contraction of the Treg response was critical to allow the emergence of protective anti-parasite CD8+ T cell immunity in the acute phase. Accordingly, we previously demonstrated that Treg depletion at day (d) 5 post-infection (pi) but not at d11pi impacted on the magnitude of anti-parasite CD8+ T cell response and the ability to control parasite replication in the acute phase. Considering this, we hypothesized that Tregs may exert a role during early events of T cell priming. To test this, DEREG mice were infected with Tc and injected with diphtheria toxin (DT) or PBS at d5 and 6pi. The next day, DT-treated animals showed increased numbers of B cells, monocytes and neutrophils in the spleen compared to controls ($p<0.05$). Furthermore, CD86 expression was upregulated on splenic dendritic cells and macrophages of DT-injected mice in contrast to controls ($p<0.05$). Evaluation of the expression of different suppression markers on Tregs from infected mice did not evidence significant phenotypic activation of splenic Tregs at 5 dpi. This suggests that an undetermined mediator and/or the basal expression of multiple regulatory markers might be involved in the suppression. Finally, we looked if Treg depletion at early time points had an effect over the effector response and parasite control at the chronic phase. We found that mice treated with DT as above showed higher frequency of total and activated CD8+ T cells infiltrating liver, skeletal muscle and heart at d97pi than control mice, which coincided with reduced parasite burden in spleen and liver ($p<0.05$). Altogether, our results suggest that during Tc infection Tregs suppress the CD8+ T cell response at the priming stage through indirect mechanisms. These events at the acute phase would have late effects over the effector, likely pathogenic, response and parasite control at the chronic phase.

403. (178) **ETIOLOGICAL DIAGNOSIS OF TYPICAL HEMOLYTIC UREMIC SYNDROME (HUS): HUMORAL RESPONSE CONTRIBUTION**

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The hemolytic uremic syndrome (HUS) is of a great concern in Argentina. The present study describes the diagnosis of 27 HUS patients attended at Hospital Municipal del Niño of San Justo, Province of Buenos Aires, during 2017-2020 period. The aim was to perform the etiological diagnosis of HUS cases based on the identification of Shiga toxin-producing *E. coli* (STEC) strains and to determine the contribution of anti-Lipopolysaccharide (a-LPS) antibodies in serum to confirm the cases as typical HUS.
Screening of stx_1, stx_2 and rfb_{O157} genes by a validated multiplex PCR was performed in fecal cultures and in isolated colonies using sorbitol agar culture (SMAC). Serological tests to detect a-LPS O157, O121, O145 IgM/IgG antibodies were carried out using Glyco iELISA (CHEMLIS®).
STEC infections were diagnosed in 10 out of the 27 HUS patients studied: stx_2/rfb_{O157}/a-O157LPS+ (n=5); stx_2/a-O145LPS+ (n=2); stx_2 (n=2); stx_2/a-O157LPS+ (n=1) In 17 HUS PCR negatives (PCR-), 12 a-O157LPS+, 4 a-O145LPS+ and 1 a-LPS negative were identified. A significant association was found between PCR and a-LPS results, Chi-square P = 0.0460. In brief, 40.7% of the patients were PCR+ and 88.9% were a-LPS+; 94.1% of the HUS PCR- were a-LPS+; 66.7% of the patients resulted a-O157LPS+.
Additionally, the excretion time was evaluated in 10 cases of HUS PCR+: 6 HUS stx_2 (range: 7-17 days, median: 8.5 days) and 4 HUS stx_2/rfb_{O157} (range: 2-8 days, median: 6 days). The excretion time

was significantly lower for the stx_2/rfb_{O157} genotype, Mann-Whitney P = 0.0333.
The detection of antibodies a-LPS (O157, O145, O121) is an effective method to complement the etiological diagnosis of typical HUS in Argentina. Mainly, because the difficulties to isolate the pathogens, probably due to either the low concentration at HUS stage and/or its short excretion time.

404. (218) **IMMUNE VARIATIONS THROUGHOUT THE COURSE OF TUBERCULOSIS TREATMENT AND ITS RELATIONSHIP WITH ADRENAL HORMONE CHANGES IN HIV-1 PATIENTS CO-INFECTED WITH *MYCOBACTERIUM TUBERCULOSIS***

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Purpose: Risk of developing active TB among HIV-coinfected (HIV-TB) patients is 19 times higher than in HIV-negative individuals. Host's immune response determines resolution or dissemination of TB infection. We aimed to identify immuno-endocrine responses over a six-month follow-up of anti-tuberculous (anti-TB) treatment in HIV+ individuals that were associated with control of TB.
Methods: Plasma levels of cortisol, DHEA and DHEA-S, percentages of CD4+ regulatory T cell subsets and number of IFN-γ-secreting cells were determined. Several cytokines, chemokines and C-reactive protein (CRP) levels were measured. Results were analyzed, correlated with clinical data and compared to similar data from HIV-1-monoinfected individuals, HIV infected individuals with latent TB infection and healthy donors.
Results: Throughout the course of anti-TB/HIV treatment, reduction of viral load ($p<0.001$) and increase of absolute CD4+ count ($p<0.05$) and CD4/CD8 ratio ($p<0.05$) were observed. DHEA ($p<0.05$) and DHEA-S ($p<0.01$) plasma levels raised while cortisol ($p<0.05$) diminished, which correlated to parameters underlying control of infections (CD4+ counts, CD4/CD8 ratio and lung-restricted TB infection). Furthermore, the balance between cortisol and DHEA may be used as predictor of anti-TB treatment efficacy in HIV+ individuals ($p<0.005$). Clinical improvement was associated with reduced frequency of unconventional Tregs ($p<0.05$), increment in *Mycobacterium tuberculosis*-specific IFN-γ-secreting cells ($p<0.05$) and diminution of systemic inflammation (CRP and IL-6, $p<0.05$). Finally, we found significant changes of circulating cytokines and chemokines.
Conclusion: This study suggests that the combined anti-HIV/TB therapies results in a recovery of the adrenal axis and immune responses toward similar values to HIV-chronically infected individuals and may expand the knowledge about specific treatment response in HIV-TB patients.

405. (268) **IMMUNO-ENDOCRINE ASPECTS AFFECTING THE VACCINE RESPONSE TO TRYPANOSOMA CRUZI ANTIGENS**

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T. cruzi infection induces the activation of hypothalamus–pituitary–adrenal axis with the consequent release of corticosterone (CT) as a counter-regulatory mechanism. Previously, our group showed that CT is the main responsible of thymus atrophy, characterized by a decrease in the number of immature double positive (DP) thymocytes and the accumulation of regulatory T cells within the CD4 simple positive compartment. Here, we evaluated if the deleterious immune-endocrine response caused by *T. cruzi* infection upon the thymus is modified by the administration of our promissory experimental vaccine candidate. Therefore, BALB/c female (5-6/group) were immunized intranasally (three doses, one every two weeks) with vaccine formulations based in trans-sialidase (TS) antigen plus c-di-AMP as adjuvant. As controls, mice were treated with saline solution (SS) or with TS or c-di-AMP alone. Fifteen days after the last immunization mice were orally challenged with 3000 parasites of Tulahuen strain. Parasitemia and clinical score were evaluated several times after infection. To assess the impact of immunization over the immune-endocrine response mice were sacrificed after 100 days of infection. The thymus was weighted and thymocyte subpopulations were evaluated by flow cytometry. Also, adrenal response was evaluated indirectly by determination of gland weight and StAR gene expression by qPCR (enzyme involved in CT synthesis).
The vaccinated group (TS+c-di-AMP) showed a diminished parasitaemia ($p<0,05$ vs infected SS). Thymic atrophy (weight and cellularity) was very prominent in infected SS mice compared to TS+c-di-AMP. Despite protective effects TS+c-di-AMP, only tended to prevent DP and Tregs loss, while at adrenal level, induce a minor level of StAR expression and a tendency to develop a minor adrenal hyperplasia. In conclusion, TS+c-di-AMP vaccination protects against oral challenge with T. cruzi but provokes minor effects upon chronic thymic atrophy and adrenal response.

406. (291) **A SINGLE NUCLEOTIDE POLYMORPHISM OF *FCGR2A* IS ASSOCIATED TO HIGHER SEVERITY IN RSV INFECTED INFANTS**

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Background
Respiratory syncytial virus (RSV) infection is the commonest cause for hospitalization in infants and it is not clear yet why some children with no apparent risk factors develop severe bronchiolitis. Genetic variation in the receptor for the Fc portion of IgG (FcγR) determines their affinity for IgG subclasses and has been linked to susceptibility and/or progression of several infectious diseases. Here we study the association between a polymorphism for FcγRIIa (H131R) and RSV disease.
Methods
Blood samples were collected from 182 infants ≤24-month-old (50 uninfected, 114 moderate RSV-infected with moderate progression and 18 with severe disease). FcγRIIa-H131R SNP genotypic frequencies (HH, HR, RR) and anti-RSV IgG1, IgG2 and IgG3 levels were studied.
Results
We found no differences among genotypic frequencies for FcγRIIa-131H/R SNP between uninfected and RSV-infected infants. However, we observed a significant higher frequency of HH genotype in severe RSV-infected children versus moderate patients. HH RSV-infants from severe group presented more factors associated to severity than HR or RR patients. Furthermore, compared to moderate RSV-infected infants, severe patients showed higher levels of anti-RSV IgG1 and IgG3.
Conclusions
We found that FcγRIIa SNP is not related with a higher susceptibility for RSV infection. However, we did find an association between an FcγRIIa-H131 variant and progression to severe RSV disease once infected, which might support the involvement of IgG immune complexes in RSV pathogenesis. This genetic factor could also help to predict the worse outcome and identify healthy infants at risk at time of hospitalization.

407. (301) **RECOMBINANT TS-BASED NASAL VACCINE PROTECTS AGAINST ORAL INFECTION WITH *T. CRUZI***

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Chagas disease, caused by the *Trypanosoma cruzi* (*Tc*) parasite, is an important public health problem in Latin America. Although there are drugs for its treatment, currently there are no prophylactic vaccines to combat the disease. Here, we evaluated the immunogenicity and prophylactic efficacy generated by a recombinant Trans-sialidase (TSr) expressed in *E. coli.* This fragment was selected by bioinformatics and contains different B and T epitopes. Thus, female BALB/c mice (n=5/6/group) were immunized intranasally (three doses, one every two weeks) with different formulations that combine the TSr with different adjuvants (c-di-AMP or ISPA). As control groups we used mice not immunized (NI) or only treated with TSr. Fifteen days after the last immunization, *in vivo* cell-mediated (delayed hypersensitivity test -DHT-), *in vitro* specific splenocyte proliferation (Ki67 by flow cytometry) and specific humoral (ELISA) response were assayed. Then, animals were orally challenged with 2500 *Tc*/mice (Tulahuen strain). During acute phase, parasitemia, clinical affectation (score), muscle damage (plasma CK) was evaluated. In terms of immunogenicity,TSr+c-di-AMP and TSr+ISPA groups developed an enhanced DHT after 24-48 h, compared to control groups (in all cases, $p<0.05$). Specific proliferation of CD4 lymphocytes was also enhanced in splenocytes from TSr+c-di-AMP and TSr+ISPA groups ($p<0.5$ vs. NI and TSr). Moreover, the same animals showed enhanced levels of IgG2a and IgG1 (in all cases, $p<0,5$). Early parasitemia are less notorious in TSr+c-di-AMP and TSr+ISPA, but only TSr+c-di-AMP animals control more effectively the infection along the acute phase, being their clinical affectation less evident. Coincidentally, CK levels were 9-times lower in this group than NI ($p<0.05$). Taken together, these results suggest that TSr+c-di-AMP formulation may be a good vaccine candidate for the development of a prophylactic mucosal vaccine against *T. cruzi* infection.

408. (306) ***CHLAMYDIA TRACHOMATIS* DISTURBS ANTIGEN CROSS-PRESENTATION IN INFECTED DENDRITIC CELLS.**

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Chlamydia trachomatis (CT) is an obligate intracellular pathogen and the leading bacterial sexually transmitted infection worldwide. Inside the cell, CT lives into a parasitophorous vacuole (inclusion). Recently DC has begun to be studied like a CT host. Dendritic cells (DCs) can cross-present exogenous antigens to T CD8+ lymphocytes, a process that requires several intracellular transport pathways. Knowing that CT perturbs the intracellular transport, we hypothesized that chlamydia may alter antigen cross-presentation by disturbing key intracellular transport events. By using the DC line JAWS-II and the CT serovar L2, we observed that CT evades most of the interaction with the endocytic pathway since CT does not localize to specific markers of early endosomes, lysosomes or multivesicular bodies. However, CT did showed a strong interaction with the recycling pathway marker TfR and with Rab proteins that control endocytic recycling. Also by confocal microscopy we evidenced a striking redistribution of MHC-I molecules in CT infected DCs. These cells lost their typical MHC-I location in both, the perinuclear recycling center and the plasma membrane. By flow cytometry and WB analysis, we confirmed that MHC-I molecules do not transport properly to the cell surface in infected DCs, as compared to uninfected cells. Although the total amounts of MHC-I molecules are similar in both conditions. By using the model antigen ovalbumin (OVA) and the specific CD8+ T lymphocytes (B3Z) to measure cross-presentation, we found a significant decrease in the cross-presentation ability of infected DCs with both, soluble and latex beads-associated OVA. Finally, we discarded that this effect is caused by loss of endocytic capacity in the infected DC. Altogether these results indicate that CT infection alters the normal MHC-I intracellular distribution and impairs antigen cross-presentation by DCs.

409. (367) **NOVEL RESPIRABLE RIFAMPICIN-CURCUMIN LOADED NANOPARTICLES AGAINST *MYCOBACTERIUM TUBERCULOSIS* INFECTION.**

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Rifampicin (RIF) is one of the most powerful and effective first line drug employed in the treatment of *Mycobacterium tuberculosis* (Mtb) infection. With the worldwide emergence of highly drug-resistant tuberculosis (TB), novel agents that have direct antimycobacterial effects or that enhance host immunity are urgently needed. It was described the immunomodulatory anti-TB effects of Curcumin (CUR), a potent anti-oxidant and apoptosis inducer compound. We develop novel RIF-CUR nanoparticles (RIF-CUR NP) with improved drug aqueous solubility and stability for inhalator administration. Then, we analyzed by confocal microscopy the *in vitro* uptake of CUR-NP (20 μg/ml) in human macrophages (derived from PBMCs) at different time points (1h, 18h, 24h and 48h). We found a higher drug cellular uptake levels (intensity/ area) for Mtb antigen-stimulated cells (0.25±0.04) than unstimulated control (0.07±0.02) over 18 hours (ANOVA test, $p<0.05$). Finally, *in vitro* studies showed the higher microbicidal effect (CFU counts) of the RIF-CUR NP (1μg/ml-1.25μg/ml) versus RIF-NP (1μg/ml) in THP-1 cells infected with MtbH37Rv at 48hours and 4 days (ANOVA test, $p<0.05$). In summary, the RIF-CUR nanocarrier provides a new simple nanotechnological alternative for its potential application in respirable TB therapy.

410. (368) **THE COOPERATIVE ROLE OF *YERSINIA* OUTER PROTEIN (YOP) P AND GALECTIN-1 IN IMPAIRING PROTECTIVE IMMUNITY BY REPRESSING NITRIC OXIDE PRODUCTION**

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Yersinia enterocolitica (Ye) evades the immune response by injecting outer proteins (Yops) into the cytosol of host cells, but the interaction between Yops and host proteins is not completely understood. We have previously described higher expression of Galectin-1(Gal1) in the spleen and Peyers patches (PPs) of mice infected with Ye. Notably, this effect was prevented when mice were infected with a YopP-deficient mutant strain (Ye Δ*yopP*). Considering that Gal1 has an important immunoregulatory role, and has been shown to interact with certain bacterial glycoproteins, we decided to evaluate the role of Gal1 in Ye infection and its potential interaction with YopP. We observed that Gal1 is able to bind Ye and moreover, one of Gal1 ligands in these cells is YopP, as a Ye Δ*yopP* mutant showed decreased Gal1 binding ($p< 0.05$) by flow cytometry when compared to Ye wt. As early control of Ye infection involves activated macrophages (MΦ), we also evaluated Gal1-YopP interactions and their role in the modulation of macrophage function. Quantification of nitrites and TNF production in supernatants of *Lgals1*$^{-/-}$and WT MΦ infected either with Ye wt or Ye *ΔyopP* showed that even though Gal1 and YopP did not influence TNF levels, coordinately inhibited nitric oxide (NO) production ($p< 0.05$). Administration of exogenous recombinant Gal1 (rGal1) was not able to counterbalance the increase in NO levels observed in *Lgals1*$^{-/-}$ MΦ infected with Ye Δ*yopP*. Moreover, *in vivo* experiments showed that *Lgals1*$^{-/-}$ mice orally infected with the Ye Δ*yopP* presented lower bacterial load in PPs when compared to *Lgals1*$^{-/-}$ mice infected with the Ye wt ($p< 0.01$). Exogenous administration of rGal1 was not able to restrain the improved anti-Ye responses generated in absence of Gal1 and YopP. Our data reveal a role for YopP and endogenous Gal1 on macrophage NO production during Ye infection. This could be a promising area to explore the reinforcement of antibacterial responses by targeting Ye-Gal1 interactions.

411. (401) **CHRONIC HEPATITIS B AND CHRONIC HEPATITIS C IMMUNOPATHOGENESIS: SIMILAR BUT NOT THE SAME**

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HBV and HCV are hepatotropic viruses which differ in the way they induce chronic disease. We aimed to compare the hepatic immune response in Chronic Hepatitis B (CHB) and C (CHC) infections and assess their role in liver damage.

Immunostaining was done in 68 formalin-fixed and paraffin-embedded liver biopsies from 26 CHB and 42 CHC treatment-naive patients to characterize liver infiltrate: [Th (CD4+), Th1 (Tbet+), Th17 (IL-17A+), Treg (Foxp3+), and CTL (CD8+)]. Quantification: portal (P)= +/total lymphocytes or lobular= + lymphocytes in 10 fields; (400x). Hepatitis severity and fibrosis were assessed by the modified Knodell (HAI) and METAVIR.

Comparing CHB and CHC lymphocyte prevalence was alike in P areas (Th>CTL>Treg>Th17>Th1). However, CHC patients showed higher frequencies of Treg, Th17 and Th1 cells ($p=0.001$, $p=0.005$ and $p=0.003$, respectively, U-test). In contrast, cell distribution was different in the lobular area (CHB: CTL> Treg>Th17=Th1>Th vs CHC: CTL>Th1>Treg>Th=Th17) with higher frequencies of Th, Th17 and Th1 cells in CHC ($p=0.04$, $p=0.001$ and $p=0.001$, respectively, U-test) compared with CHB. Regarding liver damage, patients with analogous disease stage showed similar cell frequencies but only in CHC P Th17 were associated with advanced fibrosis ($p=0.03$, U-test) and just in CHB P Th ($p=0.04$, U-test) and lobular CTLs and Th17 cells ($p=0.02$ and $p=0.01$, respectively; U-test) were increased in severe hepatitis cases.

Even when all studied populations were identified in CHB and CHC, common and particular features related to liver damage were detected. Lobular CTLs prevalence in both infections implies their contribution in hepatitis pathogenesis. As for CHB, despite the presence of a regulatory microenvironment, CTLs and Th17 cells promote hepatitis severity, suggesting a Treg failure in limiting liver damage but favouring viral persistence. By contrast, CHC showed a highly inflammatory context with CTL and Th1 majority and Th17 cells enhancing liver fibrosis.

412. (402) **GERMINAL CENTER REACTION IN T*RYPANOSOMA CRUZI* INFECTION: CHARACTERIZATION OF FOLLICULAR CYTOTOXIC CD8+T CELLS**

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Germinal Centers (GCs) are specialized structures generated within the B cell follicles in response to T cell-dependent antigens in which B cells differentiate into antibody-secreting cells and memory B cells. Follicular helper T cells (Tfh) are crucial for GCs formation and antibody-affinity maturation. Other GCs-protagonists are follicular cytotoxic CD8+T cells (Tfc), who share gene signatures with Tfh cells but their function is not well established. In some models, Tfc contribute to eliminate infected cells inside the follicle.

Our aim was to study different protagonists of GC reaction and plasmablasts(PB) response along T. cruzi infection. For that, C57BL/6 mice were intraperitoneally infected with 5.000 trypomastigotes of T. cruzi (Tulahuén strain) and the frequency and number of Tfh, Tfc and PB were evaluated by flow cytometry at different days post infection(dpi) in the spleen. Mice injected with PBS were used as controls.

We observed that the peak of the Tfh(CD4+CXCR5+PD-1+ICOS+), Tfc(CD8+CXCR5+PD-1+ICOS+) and PB(B220loCD138+) response was at 18dpi.These responses preceded GC-B cell response (B220+FAS+GL-7+Bcl-6+)which peaked at 28 dpi. Tfc had a higher expression of Bcl-6 and Tcf-1 than non-Tfc CD8+ T cells($p<0,05$). Near 25% of Tfc, but only 3% of non-Tfc, were specific for the immunodominant T. cruzi TSKB20 peptide. Tfc were CD107a+ and IFN-γ, TNF-a, Granzyme B and Perforin-producing cells. By immunofluorescence of spleen sections, at 15 dpi, we detected CD8+T cells inside and around B cells follicles and spatially opposed to follicular dendritic cells; at 23dpi and 28dpi, all CD8+T cells were outside the follicles and some of them in close contact with PB.

To sum up, we observed an activated CD8+T cells subset whose peak of the response was prior to CG, expressed Tfh-related molecules and were observed in close contact with B cells subsets. Tfc could be influencing humoral response, controlling infection more efficiently, or regulating some population of CG.

413. (423) **EFFECT OF PGE2 ON THE FUNCTIONS OF NEUTROPHILS DURING HUMAN TUBERCULOSIS**

Martin C[1,2], Pellegrini JM[1,2], Morelli MP[1,2], Tateosian NL[1,2], Amiano NO[1,2], Ciallella L[3], Palmero DJ[3], García VE[1,2].

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Neutrophils have been associated with tuberculosis (TB) protection but also with excessive inflammatory burden. Previously we showed that PGE2 decreased CD11b expression in *Mtb*-Ag stimulated neutrophils from healthy donors (HD). Here we investigated the potential role of PGE2 on human neutrophils´ response during active TB. We evaluated the expression of immunoreceptors (PD-L1, PD-L2),

the generation of reactive oxygen species (ROS) and the induction of autophagy. Neutrophils were obtained from heparinized peripheral blood from HD and tuberculosis patients (TB) and cultured (2x10^{6}cells/ml) with a *Mycobacterium tuberculosis* lysate (*Mtb*-Ag, 10µg/ml) ± PGE2 (2µM). ROS production and PD-L1/PD-L2 surface expression were determined by flow cytometry. Confocal microscopy and flow cytometry were used to evaluate autophagy levels. P-values < 0,05 were considered significantly different.
We found that *Mtb*-Ag stimulation increased PD-L1 expression on neutrophils from HD (p=0,041, Ag-stimulated *vs.* unstimulated neutrophils) and PGE2 decreased it (p=0,041). Additionally, we measured significantly lower PD-L1 levels on *Mtb*-Ag stimulated neutrophils from TB patients than on HD´s stimulated cells (p=0,014). Besides, neither *Mtb*-Ag nor PGE2 treatment modulated PD-L2 expression on human neutrophils. Moreover, we observed that PGE2 did not modify ROS production in *Mtb*-Ag stimulated neutrophils. Furthermore, significant higher levels of autophagy were detected in *Mtb*-Ag stimulated neutrophils from HD as compared to TB patients (p=0,042) but PGE2 treatment did not modify these levels. Taken together, our findings indicate that PGE2 treatment could alter PD-L1 surface expression on HD´s neutrophils, but had no effect on the levels of autophagy induced by *Mtb*-Ag, at least in our experimental conditions. Therefore, further experiments are required to determine the precise role of PGE2 on human neutrophils during active tuberculosis.

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We found that *Mtb*-Ag stimulation increased PD-L1 expression on neutrophils from HD (p=0,041, Ag-stimulated *vs.* unstimulated neutrophils) and PGE2 decreased it (p=0,041). Additionally, we measured significantly lower PD-L1 levels on *Mtb*-Ag stimulated neutrophils from TB patients than on HD´s stimulated cells (p=0,014). Besides, neither *Mtb*-Ag nor PGE2 treatment modulated PD-L2 expression on human neutrophils. Moreover, we observed that PGE2 did not modify ROS production in *Mtb*-Ag stimulated neutrophils. Furthermore, significant higher levels of autophagy were detected in *Mtb*-Ag stimulated neutrophils from HD as compared to TB patients (p=0,042) but PGE2 treatment did not modify these levels. Taken together, our findings indicate that PGE2 treatment could alter PD-L1 surface expression on HD´s neutrophils, but had no effect on the levels of autophagy induced by *Mtb*-Ag, at least in our experimental conditions. Therefore, further experiments are required to determine the precise role of PGE2 on human neutrophils during active tuberculosis.

415. (448) **PLATELETS-MONOCYTES AGGREGATES IN COVID19 PATHOGENESIS**

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There is an urgent need to understand the pathogenesis of coronavirus disease 2019 (COVID19). In particular, thrombotic complications in patients with COVID19 are common and contribute to organ failure and mortality. It has been reported that COVID19 patients present increased platelet activation. Our aim was to evaluate if activated platelets from COVID19 patients form aggregates with monocytes and modulate their activation and functionality.
Samples from whole blood and PBMCs were obtained from healthy donors or COViD19 patients. PBMCs were purified by ficoll-paque and washed platelets were obtained from plasma by centrifugation with 200 nM of prostaglandin I_2. Phenotype and cytokine production was evaluated by flow cytometry and ELISA. The viral production was measured by cytopathic effect in Vero cells.
We observed that COVID19 patients presented an increased percentage of platelet-monocyte aggregates compared to control samples (7,1 ± 1,9, n=10; 44,3 ± 3,9, n=30, p<0,0001). We did not observe a correlation between platelet-monocyte aggregates and severity of the disease. When we analyzed the functionality of monocytes we found that COVID19 patients presented an increased production of IL8 (n=4, p<0,05) in monocytes aggregated with platelets but not of TNFα and IL6 (n=6).
We also evaluated the role of platelets in the dissemination of the virus. Interestingly, we observed that COVID19 platelets but not control platelets, inhibited the infection of SARS-CoV-2 in Vero cells (n=2, p<0,05).
These results show that patients COVID19 present increased platelet-monocyte aggregates. Further studies are required to evaluate the role of these aggregates and platelets in SARS-CoV-2 pathogenesis.

416. (452) **IMMUNE ALTERATIONS IN ARGENTINE PATIENTS WITH CONGENITAL UREA CYCLE METABOLIC DISORDERS**

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Background. Urea Cycle Disorders (UCD) comprise a group of metabolopathies sharing similar clinical phenotypes, in which acute hyperammonemia (HA) crises often occur. Among others, intercurrent infections have been empirically proposed as the main precipitants. Moreover, acute HA events following infections are clinically different from those triggered by other precipitants, representing a distinct clinical entity with increased morbidity. As infections are concurrent with HA events, we hypothesized that HA may *per se* induce an immunocompromised state that would be causal of the observed recurrent infections.
Methods. Different phenotypic and functional immune function parameters were assessed in UCD patients and healthy control volunteers. *In vitro* lymphoproliferation against different polyclonal and memory recall cell antigens, T helper cell subset frequencies, cytokine secretion in culture supernatants, total immunoglobulin serum levels, and the glycosylation status of leukocyte cell surface proteins were assessed.

Results. A state of marked hypogammaglobulinemia was detected in UCD patients. Conversely, similar lymphoproliferative responses to either polyclonal or memory recall cell antigens were observed in patients with respect to controls. However, patients undergoing HA crises presented significantly reduced lymphoproliferation to different stimuli. Remarkably, significantly reduced counts of Th17 and Th1 cells, together with decreased IL17 and IFNɣ secretion levels, were observed in UCD patients. Moreover, monocytes and lymphocytes seem to display an altered glycosylation pattern in conditions of HA.
Discussion. These preliminary data indicate that UCD patients present alterations in different biomarkers of immune function, especially during HA crises, suggesting a state of immunocompromise that would render patients susceptible to infections. The latter would further aggravate the HA status increasing the morbidity/mortality risk.

417. (453) **METFORMIN REGULATES INFLAMMATORY RESPONSE OF *T. CRUZI*-INFECTED PERITONEAL MACROPHAGES IN AN *EX VIVO* TREATMENT MODEL.**
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During the acute phase of *T. cruzi* infection, high replication of the parasite is controlled by a strong inflammatory response with activation of the innate immune cells due to increase of Th1 proinflammatory cytokines. Macrophages (Mø) have been described to control intracellular parasite replication when iNOS expression and ON production are induced *in vitro*. We previously reported that pretreatment of bone marrow derived Mø with Metformin (Metf) leads these cells to control parasite replication presumably by modulating inflammasome activation without increase of iNOS expression. However, it has been demonstrated that high and continuous ON release by Mø and other cells, is involved in Th1 T cell suppression. In this context, Metf was associated to reduce inflammatory-related ischemic cardiovascular events, prolong lifespan and decrease aged-related inflammation. To determine the Mø activation profile during *in vivo T. cruzi* infection and the possibles effects of Metf treatment, we tested iNOS expression by FACS in Peritoneal Mø (Pe-Mø) subsets, LPM (Large Pe-Mø) and SPM (Small Pe-Mø). We observed increased iNOS expression ($p<0.001$) in LPM and SPM during the acute phase (day 20 post infection) that declines around day 40, despite the high ON levels in plasma at this point. To determine other sources of ON production we assessed iNOS expression in spleen cells by FACS and we found small but consistent $F4/80^+CD11b^+iNOS^+$ cells. After that, we performed an *ex vivo* treatment of total Pe-Mø from infected mice in acute phase and we observed a decrease in supernatants levels of ON and TNF-a levels by ELISA ($p<0,05$) when cells are exposed to Metf. To test if Metf polarize Mø to an antiinflammatory profile, we tested arginase expression by WB and IL-10 production by ELISA but we observe no changes in these parameters. Taken together, these results suggest that Metf could modulate exacerbated Mø activation during high parasitaemia acute phase that could lead to organ damage.

418. (455) **ADJUVANT PROPERTIES OF OUTER MEMBRANE VESICLES DERIVED FROM *BORDETELLA PERTUSSIS*.**
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Pertussis is a vaccine preventable respiratory disease which is currently considered a serious health problem. We have designed a novel pertussis vaccine candidate based on outer membrane vesicles derived from *B. pertussis* (OMVs*Bp*) that is safe, highly immunogenic, and protective in mouse model. OMVs*Bp* includes a wide range of immunogens and PAMPs. Since these PAMPs could have adjuvant properties by themselves we wonder which is the contribution of the OMVs' integrity on their adjuvant properties. To addresses this aspect, we perform comparative studies between intact OMVs (OMV_I) and mechanically disrupted OMVs (OMV_D). Both OMVs were isolated and treated with standardized protocols and formulated with Fim3, a poorly immunogenic protein that is not present in OMVs. Fim3 was expressed and purified from a recombinant *Escherichia coli* strain. An immunization scheme was performed in Balb/C mice consisting of two immunizations with 0,75 μg of OMV_I or OMV_D combined with Fim3 (10 μg). Non immunized mice and OMV_I, OMV_D or Fim3 immunized mice were used as control. Postvaccination serum samples were obtained, and a-Fim3 antibody levels were evaluated by Immunoblot and ELISA. By performing these experiments, we detected that OMV_I+Fim3 induces higher antibody response a-Fim3 than OMV_D+Fim3 (OMV_I+Fim3: 3257 ± 264,5 *vs* OMV_D+Fim3: 236,05 ± 56,63). Moreover, OMV_I+Fim3 induced higher levels of a-Fim3 antibodies in comparison with that detected in Fim3 immunized mice (Fim3: 53,43 ± 11,97). Consistent with the ELISA results, serum samples obtained from OMV_I+Fim3 immunized mice shown the highest reactivity against Fim3 in Immunoblot. The results presented here point out the relevance of OMVs*Bp* integrity for its adjuvant properties.

419. (474) **NEONATAL-MOUSE MODEL TO CHARACTERIZE VACCINES AND STRATEGIES FOR OVERCOMING PERTUSSIS IN EARLY LIFE**
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The recent increase in pertussis cases demands more effective approaches to overcome this health problem. In parallel with maternal immunization, neonatal immunization (NI) is a strategy needing revision. Using the intranasal challenge mouse model (Balb/C), we observed that NI may improve current pertussis epidemiology. For neonates without maternal immunity, mixed vaccination schedules with commercial pertussis acellular vaccine (Boostrix™, GSK) and our novel outer membrane vesicle (OMV)-based vaccine candidate appear to be the most appropriate schedule to induce protection in the pups. For offspring from immune mothers, to avoid blunting effect, NI should be carried out with vaccines other than those applied during pregnancy. Here we detected that after 2-dose vaccination schedules administered subcutaneously at the age of 1 week and 3 weeks, the blunting effect of maternal immunity was lost, regardless of the type of vaccine used. At 7 days after the challenge (at 14 days after the last vaccine dose), any of the 2-dose schedule tested reduced at least 4 logs the *B. pertussis* CFU recovered from the lungs in comparison to non-immunized mice (n=6 per group, $p<0.05$). We also analyzed whether NI is effective in protecting against clinical isolates currently circulating in different countries. Despite NI is effective against several *B. pertussis* strains, it did not reverse the low levels of protection induced by acellular vaccine against circulating strains that do not express pertactin. This result was observed even in neonates with maternal immunity (only a reduction of 2-logs was detected in comparison with non-immunized mice, n=6 per group, $p<0.05$). All the assays were repeated at least 3 times. These results, which complement the previous ones, indicate that although neonatal immunization would contribute to improving the control of the disease, it is inefficient against clinical isolates with increased prevalence in countries that only use acellular vaccines.

420. (489) **HIF-1A/CD73 REGULATORY PATHWAY DETERMINES CHRONIC INFLAMMATORY RESPONSE AND TISSUE DAMAGE IN AN EXPERIMENTAL MODEL OF *TRYPANOSOMA CRUZI* INFECTION**
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The transcription factor HIF-1 regulates the expression of the CD39 and CD73 ectonucleotidases, which drive the catabolism of the pro-inflammatory molecule ATP towards the anti-inflammatory mediator adenosine. We have reported that CD73 activity has a tissue-dependent impact on the host immune response against acute *Trypanosoma cruzi* infection. Here, we explored the effect of CD73 deficiency (CD73KO) on immune cells infiltrating target tissues during chronic *T. cruzi* murine infection. Through flow cytometry, we found that CD73KO mice exhibited higher CD4/CD8 ratio in peripheral blood ($p < 0.001$), in parallel with the lower CD4/CD8 ratio observed in heart and liver at 250 days post-infection (dpi), compared to the wild-type (WT) counterpart ($p < 0.001$ and $p = 0.052$, respectively). The frequency of infiltrating HIF-1α+ CD4+ T cells and CD8+ T cells diminished in CD73KO compared to WT mice ($p < 0.05$). In the heart, a higher frequency of IFN-γ+ CD8+ T cells was observed in CD73-deficient mice ($p < 0.05$) in agreement with the intense infiltrate perceived in histological studies. In addition, the echocardiography (ECO) analysis showed that cardiac functionality was altered in CD73KO mice (left ventricular systolic diameter/weight: $p < 0.05$). In the same way, in CD73-deficient liver, higher frequency of IFN-γ+ CD8 T cells were observed ($p < 0.001$), in accordance with the liver tissue damage evaluated by the analysis of the histology. On the other hand, in visceral adipose tissue, no differences were observed regarding the expression of HIF-1α in T cells in both groups of mice. However, a higher degree of infiltration was observed in visceral adipose tissue from CD73-deficient mice. These data suggest that HIF-1α/purinergic system could drive the inflammatory response against chronic *T. cruzi* infection and its resolution.

Ethics statement

All animal experiments were carried out with approval of the animal handling and experimental procedures by the Institutional Committee for the Care and Use of Laboratory Animals **(CICUAL-Res:736/2018)** of CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina in strict accordance with the recommendation of the U.S Department of Health and Human Services Guide for the Care and Use of Laboratory Animals.

421. (518) **PLASMA "FINGERPRINTS" ACQUIRED BY MALDI-TOF MS AS MARKERS OF PATHOPHYSIOLOGICAL EVOLUTION IN SEPSIS**

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Sepsis constitutes one of the main causes of death in ICUs, presenting a bi-phasic nature with an inflammatory phase followed by an anti-inflammatory response. However, clinically, it's difficult to define a therapeutic approach due to a lack of tools to determine the phase a patient is going through. Our aim was to investigate the usefulness of plasma fingerprints as instrument for discrimination of pro or anti-inflammatory states in sepsis using MALDI-TOF technology. For this, we used cecal ligation and puncture (CLP) murine models, which develop to an inflammatory phase during the 2nd day (D2) and an anti-inflammatory one on the 4th day (D4). The SHAM (SH) group was used as control. To characterize the different phases, hematologic, metabolic and immunologic parameters were determined in plasma. Also, plasma spectra were acquired by the MALDI-TOF technique. Un-supervised (Hierarchical K-means clustering) and supervised analyzes were performed to discriminate the different phases from differential signals (peaks). Peripheral blood leukocytes count was decreased in CLPD2 whereas in CLPD4 was increased associated to neutrophilia (Leukocytes (10^9/l): SHD2=7.7±0.4; CLPD2=3.5±0.6*. Neutrophils: SHD4=0.47±0.11; CLPD4=2.59±0.43&, &*p<0.01). Hepatic enzymes were increased in CLPD2 (ALT(U/L): SHD2=24±2.3*; CLPD2= 92±8.3*,*p<0.01). A marked splenic lymphopenia and decreased antibody titer was observed in CLPD4 (%CD4+cells:SHD4=18.6±2.2; CLPD4=9.2±0.9*. Titer (%): SHD4=100±13; CLPD4= 4.0±2.3&,*& p<0.01). Supervised analyzes were performed to form groups of 5-20 peaks that best differ between groups. Using hierarchical k-means algorithms the best sets of values for each case resulted in an accuracy of 91% comparing CLPD2 and CLPD4; 93% for CLPD2 and SHD4 and 80% for CLPD4 and SHD4. These results show the CLP model can simulate both phases in sepsis, and the potential for plasma fingerprints obtained by MALDI-TOF as a tool to discriminate different phases in sepsis.

422. (522) ***BRUCELLA ABORTUS* RNA INTERFERES WITH M1 POLARIZATION OF HUMAN MACROPHAGES AND DIMINISHES IFN-Γ-INDUCED MHC-I MOLECULES ON OTHER CELLS.**

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**Both authors contributed equally to this work*

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Monocytes and macrophages play a central role in chronic brucellosis. *Brucella abortus* (*Ba*) is an intracellular pathogen which survives inside these cells. Macrophages could be differentiated into classical or alternative profiles, among others. We have previously showed that *Ba* RNA (a PAMP associated with bacterial viability or *vita*-PAMP) activates but does not polarize macrophages *per se.* Given that *B. abortus* can survive inside macrophages once a Th1 response is established, we aimed to evaluate if *Ba* RNA could interfere with M1 polarization. For this, human macrophages were stimulated with *Ba* RNA under M1 conditions (IFN-γ+LPS) for 24 and 48 h. *Ba* RNA diminished the (IFN-γ+LPS)-induced MHC-II and CD64 surface expression at 48 h ($P<0.05$), but it did not modify the secretion of pro and anti-inflammatory cytokines. This phenomenon was not associated with an alternative activation of these cells (M2), as shown by unchanged DC-SIGN, CD163 and CD206 surface expression. The interference of M1 profile mediated by *Ba* RNA could be biologically relevant as it was correlated with a reduced antigen presentation capacity to T $CD4^+$ lymphocytes and Nitrogen Reactive Species production at 48 h ($P<0.05$). Additionally, we have previously shown that *Ba* RNA diminished IFN-g-induced MHC-I surface expression on macrophages. We wondered if this phenomenon could be reproduced in other cells susceptible of being infected with *Ba.* For this, we treated the human brain microvascular endothelial cells (HBMEC) and the renin-expressing human pulmonary adenocarcinoma cells (Calu-6) with *Ba* RNA in presence of IFN-g. This *vita*-PAMP could diminish the IFN-g-induced MHC-I surface expression on these cells with 10 mg/ml of *Ba* RNA ($P<0.05$). This could lead to an inefficient *Ba* infected-cells elimination by cytotoxic T cells. Overall, our results show that *Ba* RNA could alter the proper immune response set to counterattack the bacteria which could persist in the host establishing a chronic infection.

423. (562) **REGULATION OF T CELL RESPONSE IN THE COURSE OF *CLOSTRIDIOIDES DIFFICILE* INFECTION**

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Clostridioides difficile infection (CDI) is the leading cause of antibiotic-associated diarrhoea. The innate immune response during CDI has been extensively explored, however, the phenotype and functions of T cells during CDI is only beginning to be understood.
To characterize the adaptive immunity during CDI, C57/BL6 mice were treated with a cocktail of antibiotics and then infected with 10^5 spores of BI/NAP1/027 *C. difficile* strain. The expression of the co-stimulatory molecules SLAM and ICOS was evaluated at 5 and 8 days post-infection (pi) in lamina propria cells (LPMCs) and in mesenteric lymph nodes (MLNs) lymphocytes stimulated with/without PMA/Inomycin for 4h. Infected mice showed typical CDI symptoms (diarrhea, hunched posture, and weight loss ($p<0.01$)), an increase in bacterial shedding ($p<0.05$) and in clinical score).
On both times studied, ICOS expression and $ICOS^+SLAM^+$ population on $CD4^+$ and $CD4^-$ cells from MLNs ($p<0.01$) increased compared to control mice. SLAM levels were upregulated only at day 8 pi. A further characterization showed that more than 90% of the $CD4^-$ cells are $CD8^+$ cells in MLNs, this was not the case in LPMCs, where less than half of the $CD4^-$ were $CD8^+$. Interestingly, in LPMCs both SLAM and ICOS were downregulated at day 8 pi.
IFN-γ produced by $CD4^+$ and $CD4^-$ cells was only downregulated at day 5 pi in LPMCs. On the contrary, IL-17 production on $CD4^+$ and $CD4^-$ cells from MLNs and LPMCs was increased in infected mice compared to control on day 5 pi, with the highest levels on $CD4^-$ cells from LPMCs. At day 8, we observed a decrease of IL-17 production in $CD4^+$ and $CD4^-$ MLNs cells and in $CD4^+$ LPMCs.
Finally, cecal contents from *C. difficile*-infected mice showed increased IL-10 mRNA levels at 8 days pi.
Our results suggest that *C. difficile* modulates T cells functions. IL-17 is tightly regulated trough the course of infection. The decrease on IL-17 production and the increase of IL-10 could be associated with disease resolution.

424. (564) **EVALUATION OF THE PROTECTIVE CAPACITY OF AN IMMUNOGENIC FORMULATION FOR THE CONTROL OF INFECTIONS CAUSED BY *CHLAMYDIA TRACHOMATIS***

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Chlamydia trachomatis (*Ct*) is the most frequent sexually transmitted bacteria worldwide, therefore, a vaccine development is strongly needed. Our aim was to evaluate the ability to induce a protective immune response of a vaccine composed of a fragment of polymorphic membrane protein D (PmpD) in a murine model of genital infection by *Ct.* A fragment of PmpD was expressed in *Escherichia coli* (rPmpD). The sequence was subcloned in a eukaryotic expression vector (pVAX1). Female C57BL/6 (n=5) mice were immunized with a first intradermal dose of plasmid DNA and three rPmpD boosters, every three weeks, by intranasal and subcutaneous route, simultaneously. Twenty days after the last dose, the immunized mice and not immunized mice (control group, n=4) were vaginally infected with $1.5x10^5$ inclusion forming units (UFIs) of *Ct*-GFP. Anti-rPmpD antibodies in sera (IgG, IgG1, IgG2c) and vaginal washing (IgG, IgA) were measured by indirect ELISA. Infectious bacterial progeny released in cervical secretion was quantified by UFIs assay and fluorescence microscopy. Fourteen days post infection the uterine horns were dissected for morphological analysis. The immunized mice produced a significant systemic IgG2c humoral immune response, indicating PmpD immunogenicity (two-way ANOVA, post-test Bonferroni; $p<0.0001$). In the vaginal mucosa, the immunized mice showed an increase did not reach statistical significance in anti-rFPmpD IgG compared to the control group (Anova; $p>0.05$). The immune sera from vaccinated animals decreased the percentage of *Ct* infected HeLa cells when evaluating their protective ability (Mann-Whitney; $p<0.05$). Moreover, the bacterial load decreased did not reach statistical significance in vaginal secretions of vaccinated mice at day 7 post-infection (Mann-Whitney; $p>0.05$). The uterine horns of vaccinated mice did not show morphological alterations as did those from unvaccinated mice. The PmpD fragment is a potential vaccine candidate to develop a prophylactic strategy to the control of the most common sexually transmitted bacteria.

425. (582) **EARLY TARGETING OF MYELOID-DERIVED SUPPRESSOR CELLS IMPROVES THE PROTECTIVE CAPACITY OF TSf-ISPA VACCINE AGAINST *TRYPANOSOMA CRUZI***

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Introduction: We have previously reported that immunization with a trans-sialidase-based antigen (TSf) formulated with a cage-like particle adjuvant (ISPA) confers protection against *Trypanosoma cruzi* (*T.cruzi*). It has been shown that myeloid-derived suppressor cells (MDSCs) notably increase in several organs during mice acute infection.
Aim: to study whether MDSC depletion could further improve the efficacy of the TSf-ISPA vaccine candidate against *T.cruzi.*
Methods: 3 subcutaneous doses of TSf-ISPA were given to BALB/c mice before intraperitoneal infection with 1000 Tulahuen trypomastigotes. 5-fluorouracil (5FU) (50mg/kg) was given at different times (prior or during infection) to deplete MDSCs. Parasitemia was monitored at days 14 and 21 postinfection (p.i.). Survival was assessed until day 40 p.i. Flow cytometry was used to measure the % and number of MDSCs (CD11b+ GR-1+), CD4 and CD8 T cells, B cells and $CD11c^{high}$ dendritic cells in the spleen after 5FU treatment.
Results: Survival after infection was always higher in TSf-ISPA-immunized and infected mice (TSf-ISPA Tc+ group) than control infected PBS-treated mice (PBS Tc+ group) ($p<0.05$). Early MDSC depletion (5FU given 1 day before infection and at day 5 of infection) increased survival of TSf-ISPA mice that received 5FU (TSf-ISPA 5FU -1 and 5 Tc+) as compared to TSf-ISPA Tc+ mice). For instance, survival of TSf-ISPA 5FU -1 and 5 Tc+: 100%; TSf-ISPA Tc+: 40%; PBS Tc+ 0%; PBS 5FU -1 and 5 Tc+:0%. ($p<0.05$). Parasitemia levels correlated with survival, as PBS Tc+ mice treated or not with 5FU showed higher number of blood parasites as compared to TSf-ISPA Tc+ mice treated or not with 5FU ($p<0.05$). In addition, a trend suggests that early 5FU treatment reduces parasitemia of TSf-ISPA vaccinated mice.
Conclusion: The fact that MDSC depletion improved the efficacy of the TSf-ISPA vaccine candidate raises the possibility of using this population as a target to further potentiate vaccine efficacy against *T.cruzi*.

426. (273) INCREASED LEVELS OF CIRCULATING LPS IN PATIENTS WITH ADVANCED PULMONARY TUBERCULOSIS.

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Tuberculosis (TB) continues to be a global health problem. Our studies indicate that in those individuals where the process evolves to a larger pulmonary involvement, the regulatory action of the endocrine system is likely to promote a potentially unfavorable environment, either for the mounting of protective immunity, or the proper control of the inflammatory mechanisms accompanying such protracted course.
Chronic infectious diseases, as is the case of TB, and its persistent pro-inflammatory response may lead to changes in the mucosal barriers affecting its integrity and hence the translocation of bacteria that line the gastrointestinal tract. As such, individuals with chronic diseases may have slightly increased levels of circulating Lipopolysaccharides (LPS). Given this, we set out to quantify plasma LPS levels (chromogenic assay) in TB patients (n=39) and healthy volunteers (Co, n=38) in addition to analyzing the possible correlations between LPS levels and other inflammatory mediators such as C-reactive protein (CRP, turbitest), interleukin-6 (IL-6, ELISA) and Interferon-gamma (IFNγ, ELISA) as well as the Erythrocyte Sedimentation Rate (ESR).
Compared to Co, plasma LPS levels were found increased in TB patients (p=0.009), as did levels of inflammatory mediators (CRP, p<0.0001; IL-6, p<0.001; IFNγ, p=0.0003) and the ESR (p<0.0001). Further analysis according to TB severity, revealed that severe patients had the highest amounts of circulating LPS (p=0.03 vs Co); with moderate and severe cases showing much higher levels of CRP, ESR, IL-6 and IFNγ (p<0.0001, in all comparisons).
Correlation analysis showed that plasma levels of LPS from severe patients were positively associated with the amounts of inflammatory compounds like IL-6 (p=0.03, r= 0.58) and IFNγ (p=0.01, r=0.8).
The higher levels of circulating LPS depicted by patients with severe TB may emerge as a contributing factor for the persistence of the greater inflammation distinctive of advanced disease.

427. (323) **CHARACTERIZATION OF C1Q MODULATION OF ANTIBODY DEPENDENT ENHANCEMENT OF INFECTION WITH DENGUE VIRUS**

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Antibodies play a critical role in the pathogenesis of Dengue during secondary infection, exerting both pathogenic and protective effects. Poorly neutralizing or sub-neutralizing concentrations of antibodies can bind to the virion and increase the efficiency of viral attachment and entry into myeloid cells through interactions between the Fc portion of the antibody and Fcγ receptor (FcγR), resulting in increased viral replication and the promotion of immune complex-dependent cell signaling that increase cell permissiveness. The extent to which antibodies contribute to enhanced infection with dengue virus (DENV) and other flaviviruses is linked to complement levels. We used monoclonal type-specific and cross-reactive anti-flavivirus antibodies of varying characteristics to assess the effect of FcγR affinity on complement protein C1q modulation of antibody-dependent enhancement of DENV, focusing on the processes of viral entry. We show that physiological concentrations of C1q modulate DENV-immune complex entry into the human monocytic cells lines K562 (FcγRI- FcγRIIhigh) and U937 (FcγRIhigh FcγRIIIow) in an FcγR-dependent manner. Cell-based adsorption and internalization assays show that antibody specificity influences the mechanism of C1q modulation of enhancement. Using pharmacological inhibitors of viral entry, we describe the cell entry routes associated with C1q-mediated protection, varying antibody subclass and specificity. In conclusion, defining the conditions under which C1q interacts with immune complexes and consequently limits ADE may lead the development of vaccines that promote humoral immune responses where protective interactions predominate.

428. (159) **TUMOR MICROENVIRONMENT MAY DRIVE NK CELL EXHAUSTION AND IMPAIRED GLUCOSE UPTAKE THAT LIKELY CONTRIBUTES TO NK CELL SUPPRESSION AND TUMOR PROGRESSION IN HUMAN RENAL CELL CARCINOMA**

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Renal cell carcinoma (RCC) is an aggressive neoplasm, with metastatic potential. Nephrectomy constitutes the gold-standard treatment, and recently, immune checkpoint inhibitors have been approved for the treatment of advanced RCC. As there are no validated molecular targets in RCC, we previously characterized the expression pattern of ligands of the NK cell activating receptor NKG2D on peripheral blood mononuclear cells (PBMC), tumor infiltrating lymphoid cells (TIL) and tumor cells from RCC patients and observed that tumor cells exhibited high expression of MICA, while TIL (but not PBMC from RCC patients), exhibited high expression of MICA, ULBP3 and ULBP4. Additionally, tumor infiltrating NK cells (TINK) and CD8 T cells displayed increased expression of NKG2D and TINK exhibited a pronounced reduced degranulation and IFN-γ production ability compared to PBNK from RCC patients, which indicates a functional impairment. In this work, and to gain a deeper insight into these dysfunctional TINK in RCC, we further characterized TINK in terms of Tim-3 expression (exhaustion marker) and glucose uptake (using the fluorescent glucose analog 2-NBDG in the absence or presence of cytokine stimulation) by multicolor flow cytometry. No differences were observed in the expression of Tim-3 between PBNK from RCC patients and HD. However, TINK expressed higher amounts of Tim-3 compared to PBNK from RCC patients. Moreover, compared to PBNK from HD, TINK and PBNK from RCC patients exhibited an impaired glucose uptake after cytokine stimulation. Although Tim-3 expression and glucose uptake in TINK did not reach statistical significance yet due to the low number of samples so far analyzed, our preliminary results unravel a likely novel tumor microenvironment-driven suppressive circuit that connects Tim-3 expression, NK cell exhaustion, functional impairment and glucose uptake that drives NK cell into dysfunctional TINK and that likely contribute to tumor progression.

429. (174) **THE EFFECT OF SULFATED HYALURONAN ON MONOCYTES/MACROPHAGES IN LUNG CARCINOMA CONTEXT**

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Non-small-cell lung carcinoma (NSCLC) is the most frequent lung cancer and has a medium-low survival rate. There is not a successful single treatment, thus it is necessary to search complementary therapeutic option. Sulfated Hyaluronan (sHA) could be used as an adjuvant therapy due to its antiangiogenic and antitumor action in some cancers. Moreover, monocytes/macrophages (Mo/MØ) are critical modulators of the tumor microenvironment and are able to enhance tumor angiogenesis.
Aim: evaluate the effect of sHA on Mo/MØ in lung cancer context.
M&M: human PBMC cells were isolated by Ficoll-Percoll gradient and were treated with conditioned media (CM) derived from H1299 (NSCLC cell line) plus partial (sHA1) or total sHA (sHA3). Levels of TGF-β1 (total and active) and IL-1β were determined by ELISA as markers of pro or anti-inflammatory Mo/MØ phenotype. Endothelial cell (EC) migratory capacity toward CM of treated Mo/MØ was evaluated in Boyden chamber to evaluate angiogenic modulation. Treated Mo/MØ CM was used to analyze H1299 spheroid formation to analyze antitumoral effect. Statistical analysis: one-way ANOVA and Tukey post-test.
Results: Mo/MØ biosynthesis levels of TGF-β1 total and active and IL-1β were non-significant after treatments. We observed a decrease of EC chemotaxis toward CM of treated Mo/MØ with both sHA in lung carcinoma context ($p<0,05$). Also, H1299 treated with Mo/MØ CM previously incubated with sHA3 formed more aggregates and tended to diminish the number of spheroids ($p<0,05$).
Conclusion: our results indicate that sHA1 and sHA3 could modulate *in vitro* the angiogenic behavior of Mo/MØ over EC migration in lung carcinoma context. Besides, as CM derived from Mo/MØ treated with sHA3 prevented H1299 spheroids formation, suggesting an antitumoral effects. Further investigations are needed to understand the effect of sHA on Mo/MØ behavior and its role in angiogenesis and tumor growth.

430. (211) **BONE MARROW MSC FROM PEDIATRIC PATIENTS WITH B-ALL HIGHLY IMMUNOSUPPRESS T-CELL RESPONSES BUT DO NOT COMPROMISE CD19-CAR T-CELL ACTIVITY**

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Background: CD19-directed chimeric antigen receptor (CAR) T cells (CD19-CAR T cells) achieves high rates of complete response in patients with B-cell acute lymphoblastic leukemia (B-ALL); however, 50-60% of patients treated with CD19-targeted immunotherapy relapse after one year due to poor CAR T-cell persistence or resistance of B-ALL clones. Bone marrow (BM) mesenchymal stem/stromal cells (MSC) are key components of the hematopoietic niche and are implicated in B-ALL pathogenesis and therapy resistance by several mechanisms. MSC exert an immunosuppressive effect on T-cells; however, their impact on CD19-CAR T-cell activity is understudied.
Aim: To characterize *in vitro* and *in vivo* the immunosuppressive and inflammatory properties of BM-MSC from pediatric patients with BALL (B-ALL BM-MSC) and their involvement in CD19-CAR T-cell activity.
Materials and Methods: We performed a detailed characterization of B-ALL BM-MSC, evaluated their immunomodulatory properties and their impact on CD19-CAR T-cell activity *in vitro* using microscopy, ELISA, flow cytometry analysis and *in vivo* using a preclinical model of severe colitis and a B-ALL xenograft model.
Results: While B-ALL BM-MSC were less proliferative than those from age-matched healthy donors (HD), the morphology, immunophenotype, differentiation potential and chemoprotection was very similar. Likewise, both BM-MSC populations were equally immunosuppressive *in vitro* and anti-inflammatory in an *in vivo* model of severe colitis. Interestingly, BM-MSC failed to impair CD19-CAR T-cell cytotoxicity or cytokine production *in vitro* using B-ALL cell lines and primary B-ALL cells. Finally, the growth of NALM6 cells was controlled *in vivo* by CD19-CAR T cells irrespective of the absence/presence of BM-MSC.
Conclusions: Collectively, our data demonstrate that pediatric B-ALL and HD BM-MSC equally immunosuppress T-cell responses but do not compromise CD19-CAR T-cell activity.

431. (262) **RENAL CELL CARCINOMAS INDUCE THE UP-REGULATION OF PD-L1 ON NK CELLS**

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Natural Killer (NK) cells are key effectors against tumor and virus-infected cells; however, evidence of a regulatory role is emerging in different models of autoimmunity and viral infections. In tumors, we have identified a subset of PD-L1-expressing tumor infiltrating NK cells in tumor-bearing mice and in patients with renal cell carcinoma (RCC). When PBMC from healthy donors (HD) were cultured with the HLA class $I^{-/-}$ cell line K562, PD-L1 was up-regulated on NK cells. Direct tumor recognition by NK cells and monocyte (Mo)-derived IL-18 were required for PD-L1 up-regulation. As the cellular and molecular mechanisms that control PD-L1 expression on NK cells in RCC remain unknown, the **aim** of this work was to study PD-L1 expression in human NK cells upon RCC recognition and the underlying mechanisms. To this end, we cultured PBMC from HD with different RCC cell lines (ACHN, SN12c and 786-O) and after 48h we evaluated PD-L1 expression on NK cells ($CD56^+$ $CD3^-$ cells) by flow cytometry. PD-L1 was up-regulated on NK cells cultured with SN12c ($p<0.0001$) and 786-O ($p<0.001$) cells (but not ACHN cells), the use of transwells showed a dependence on cell-to-cell contact. Consistent with previous results, PD-L1 up-regulation was partially reduced by IL-18 blockade ($p<0.05$). The presence of Mo-derived IL-18 in these cultures was confirmed by ELISA ($p<0.05$ for all cases). Finally, aiming to address the cellular mechanisms triggered by tumor cells that result in PD-L1 up-regulation, PBMC were cultured with K562, SN12c or 786-O cells in the presence of pharmacological inhibitors of ROS, NOS or an ATP receptor antagonist, which had no effect on PD-L1 expression. However, the inhibition of autophagy and phagocytosis restricted PD-L1 up-regulation. Our results demonstrate that RCC cell lines, through direct cell-to-cell contact and induction of Mo-derived IL-18, can induce the expression of PD-L1 on human NK cells, and suggest the requirement of autophagy and/or phagocytosis.

432. (270) **A TRANSCRIPTOMIC APPROACH TO UNDERSTAND THE ROLE OF TUMOR-INFILTRATING CD39+ CD4+ FOXP3NEG T CELLS**

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The accumulation of tumor-infiltrating (TI) CD8+ T cells plays a pivotal role in the immune response against tumors. However, the function of CD4+ T cells within Tumor microenvironment (TME) remains uncertain. We previously demonstrated that tumors from tumor-bearing mice showed an important infiltrate of FOXP3neg CD4+ cells (Tconv) expressing CD39. CD39 is an ecto-enzyme involved in the conversion of eATP to immunomodulatory adenosine. We aim to determine the transcriptional profile of TI-CD39+Tconv cells. FOXP3-GFP mice (N=3), were injected with B16F10-OVA cancer cells. On day 17 the following TI-CD4+T cells were isolated by FACS: CD39+Tconv, CD39-Tconv and Treg. Then, we perform the transcriptome sequencing by RNAseq. Principal component analysis showed that all populations analyzed clustered separately, reflecting their differential transcriptional profile. To define the molecular profile of CD39+Tconv cells, we searched for differentially expressed genes (DEGs). We found 449 and 153 significantly up and down-regulated DEGs (p.adj<0.05, fold change=2), respectively, in CD39+Tconv compared to CD39-Tconv cells. Gene-encoding products related with cytotoxicity such as Gzmb, Gzmf, Gzmc, Prf1, Lyz2 and Eomes were up-regulated in CD39+Tconv. Moreover, Gene Ontology Enrichment Analysis revealed an increased expression of genes related to the following pathways: cytolysis, granzyme-mediated apoptotic signaling, lymphocyte chemotaxis and regulation of NK cell mediated cytotoxicity, among others (FDR<0.05). Comparison of CD39+Tconv to Treg identified 244 DEGs up-regulated in CD39+Tconv. Siglech, Gzme, Gzmf, Crtam and Cd160 were found to be among the most increased genes. In addition, we observed that CD39+Tconv were enriched in pathways associated with monocyte chemotaxis as well as cell-matrix adhesion and cell killing (FDR<0.05). Altogether, this data suggests that in TME CD39+Tconv cells could acquire a transcriptional program associated with cytotoxic CD4+ T cells.

433. (329) **MODULATION OF γδ T LYMPHOCYTE FUNCTIONALITY BY GLIOBLASTOMA CELL LINE U251**

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Glioblastoma multiforme (GBM) is the most frequent and malignant cause of primary brain tumors in adults; it has poor prognostic, and median survival after diagnostic is less than a year which makes it a top priority for public health. γδ T cells are a subtype of T lymphocyte that induce apoptosis on stressed and malignant cells by sensing their increased expression of phosphorylated molecules. Their ability to expand after stimulation and exert cytotoxicity over tumoral cells is being used in immunotherapy approaches against cancer. In this work we aimed to evaluate the modulation of γδ T cell functionality by human GBM cell line U251 and how it could impact in their antitumoral response. For that purpose, γδ T cells were purified from human peripheral blood mononuclear cell, by using an anti-TCR γδ MicroBead isolation kit. After purification, γδ T cells were co-cultured with U251 cell line or it's conditioned medium during 24 hours in the absence or presence of phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP, 1 μM). After incubation, we analyzed the activation state of γδ T cells by measuring the expression of CD69 and perforin by flow cytometry, and TNF-a and IFN-g production by ELISA.

Our results indicated that HMBPP-stimulated γδ T cells exhibit an increase in CD69 expression (p<0.01, n= 9) and TNF-a and IFN-g secretion (p<0.05, n= 9) when they were co-cultured with the U251 cell line monolayers. Interestingly, intracellular levels of perforin were higher when γδ T cell were co-cultured with U251, either in the presence (p<0.01, n= 5) or absence (p<0.01, n= 6) of HMBPP. Similar results were observed when γδ T cell were incubated with supernatant collected from U251 cell line monolayers. Our finding suggests that the GBM cell line, U251, can modulate the differentiation towards the Th1-like profile in γδ T cells.

434. (356) **ABSENCE OF CD39 FAVORS INFILTRATION OF TUMORS WITH CD8+ T LYMPHOCYTES WITH EFFECTOR PHENOTYPE.**

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Previously we have demonstrated that tumor-infiltrating exhausted CD8+ T cells exhibit high expression of CD39. CD39 is an ecto-enzyme that participates in the generation of adenosine, an immunosuppressive molecule that interferes with many anti-tumor immune responses. In this work, we aimed to evaluate the impact of CD39 expression on tumor progression and in the cellular composition within tumor microenvironment. For this, C57BL/6 wild type (WT) and CD39 KO mice were injected with B16F10-OVA tumor cells. We evaluated tumor progression by measuring tumor volume at different days post-injection (dpi, 7, 10, 12, 14 and 17). Additionally on day 17 we evaluated different tumor–infiltrating immune cell populations by flow cytometry. We observed no significant differences on tumor volume at any dpi evaluated in both groups. While similar frequencies of tumor-infiltrating (TI) B cells, CD4+ T cells, macrophages, NK, Dendritic cells was observed in CD39KO and WT tumor-bearing mice, CD39 KO mice exhibit higher frequency of TI-CD8+ T cells (p<0.0001) and higher % of TI-CD8+ T cells with effector phenotype (CD44+CD62L-) (p<0.05). Accordingly, CD39 KO tumor-bearing mice show higher frequency of OVA-specific TI-CD8+T cells than WT mice (p<0.01). We also evaluate the expression of inhibitor receptors (iR) (PD-1, LAG-3 and TIM-3) as well as transcription factors (TF) (EOMES, TBET, IRF-4 and TOX) related to exhaustion. We found no statistic differences in the frequency of TI-CD8+ T cells co-expressing 3 IR and FT from CD39KO and WT mice. Together our results suggest that the absence of CD39 may favor the recruitment of CD8+ T cells with effector phenotype into the tumor microenvironment, reinforcing the idea that CD39 could be consider as a promising target in the anti-tumor immunotherapy.
CD39, CD8+ T Lymphocyte, Tumor, Exhaustion.

435. (361) **TRIPLE-NEGATIVE BREAST CANCER SKEWS B CELL RESPONSE TOWARDS AN IMMATURE AND IMMUNOSUPPRESSIVE PHENOTYPE**

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Triple-negative breast cancer (TNBC) is one of the breast tumor subtypes with the worst prognosis due to its aggressiveness and the scarce treatment options available. B lymphocytes are arising as important players in antitumor immunity, but the way in which breast tumors can affect B cell development in distant organs and their resulting effector functions have not been fully addressed. We found that immature proB cells were enriched in the tumor microenvironment of TNBC patients compared to healthy breast tissue, HER2-overexpressed, luminal A, and luminal B breast tumors (p<0.0001) in a cohort of 141 patients (GSE65194). To support this, we analyzed B cell ontogeny in mice bearing 4T1 tumors compared to tumor-free mice. We found a systemic alteration of B cell subsets,

evidenced at different time-points in several organs by an increase in the frequency of immature B cell subsets ($p<0.05$) and a decrease in that of mature B cells ($p<0.05$). These findings might be explained by the downregulation of CXCR4 in bone marrow B cells ($p<0.01$), which promotes B cell retention in the bone marrow. Transcriptomic analysis of Precursor/Transitional 1 (P/T1) splenic B cells from 4T1-bearing mice showed higher expression of immunosuppressive genes like *Lgals1, Arg2, Tgfb2,* and Th2-related genes such as *Il-13 and Il-33* ($p<0.05$). *Lgals1* mRNA was also higher in DLN B cells from tumor-bearing mice ($p<0.05$), suggesting that TNBC could be favoring its own growth and progression via B cell-derived Galectin-1-dependent mechanisms. To summarize, our results show that TNBC induces an accumulation of immature stages of B cell development, both in patients and in a preclinical model. In addition, it tips the balance towards an immunosuppressive B cell phenotype which might favor tumor escape, characterized by high expression of *Lgals1* mRNA that could be targeted to avoid tumor progression.

436. (397) **SETTING UP QUANTITATIVE MULTIPLEX IMMUNOHISTOCHEMISTRY TECHNOLOGY FOR THE MULTIPARAMETRIC ANALYSIS OF THE IMMUNE INFILTRATE OF COLORECTAL CANCER TUMORS**

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The analysis of the immune infiltrate in solid tumors has become a crucial prognostic tool that helps defining clinical outcome and response to immunotherapy. Many strategies have been proposed to properly classify and quantify the immune infiltrate in a standardized manner. Immunohistochemistry (IHC) is a routine technique in a pathology lab, which could provide with a lot of reliable, local information of tumor infiltration. The main goal of this work is to set up a quantitative multiplex IHC (mIHC) technique, developed by Tsujikawa et al. To this end, we used a commercial colorectal cancer (CRC) tissue microarray (TMA) with 90 tumor samples of different pathological stages. Briefly, the TMA was stained with sequential IHC with iterative labeling and stripping steps for a total of 12 markers. After standard IHC preparation, the primary antibodies (Ab) were detected by a peroxidase-labeled secondary Ab, visualized by alcohol-soluble peroxidase substrate 3-amino-9-ethylcarbazole (AEC). Then, the whole-slide was digitally scanned. Iterative staining was achieved by AEC washing slides in ethanol, followed by Ab stripping. Once the 12 stains were obtained, digital images were processed with a computational image analysis workflow and the serial images obtained from the same slide were aligned using MatLab software. Then Fiji software was used to extract the AEC and hematoxylin color information of the coregistered images by color deconvolution algorithms. Finally, images were converted to grayscale and visualized as pseudo-colored images. Our preliminary data has allowed us to sequentially stain the CRC samples with a panel of 12 biomarkers (CD45, CD3, CD8, CD20, CD56, TBET, GATA 3, RORgT, FOXP3, PD1, PDL1, and PANCK), to characterize total leukocytes, total T cells and some subpopulations (CD8+, Th0, Th1, Th2, Th17 and Treg cells), B cells an NK cells. We expect to set up a useful platform that will provide a tool to benefit patient personalized attention.

437. (418) **EFFECT OF SPHINGOSINE KINASE INHIBITORS (SPHKS) SKI-II AND ABC294640 ON LEUKEMIC CELLS AND NON-LEUKEMIC LYMPHOCYTES: RATIONAL TO USE THE COMBINATION OF SPHKS INHIBITORS AND ABT-199 (VENETOCLAX) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)**

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Sphingosine kinases (SphK1 and SphK2) are therapeutic targets for cancer because they regulate the balance between proapoptotic ceramides and sphingosine-1-phosphate. We found that the SKI-II sphingosine kinase inhibitor not only acts on the leukemic clone, it also affects the survival of non-leukemic lymphocytes affecting tumoral microenvironment, mechanism mediated by both sphingosine kinases. Previously, we reported that autologous activation of T cells induces CLL drugs resistance. In this study we evaluated the effect of SphKs inhibitors on the activation and proliferation of leukemic microenvironment. Peripheral blood mononuclear cells (PBMC) from CLL patients were cultured with different doses of SKI-II or ABC294640 (SphK2-selective inhibitor). PBMCs of CLL patients were cultured on immobilized anti-CD3 moAbs (αCD3) to induce activation and proliferation of T cells, or in the presence of a TLR-9 agonist: CpG to induce the activation of B cells. We found that the plasma concentration of 15 μM for both inhibitors reduces the expression of activation markers (CD69 and CD25) of activated T cells ($n=20$ $p<0.0001$), and also reduces proliferation ($n=15$, $p<0.0001$) by CFSE dilution assay. As for B activation, CD69 downregulation was only affected by treatment with ABC294640 ($n=20$, $p=0.0016$). Simultaneous analysis of leukemic B cells demonstrates a correlation between increased survival in response to inhibitor therapy and the worst prognostic clinical profile, Binet B-C ($n=30$, $p<0.0001$). Finally, we tested generation of CLL resistance to ABT-199 (venetoclax), induced by T cells activation, cultivating PBMCs in presence of one or the other SphKs inhibitors and managing to reverse this resistance ($n=8$, $p<0.0001$). We found that the inhibitors affected the activation of the T cells that can give signals of survival to leukemic cells. Our results encourage the use of the combination of selective SphKs inhibitors together with venetoclax in CLL treatment.

438. (488) **EFFECT OF GALECTIN 1 ON MYELOID DERIVED SUPPRESSOR CELLS (MDSC) IMMUNOSUPPRESSIVE ACTIVITY**

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Background: Galectins interact with cell surface glycans and modulate immune cell fate and functionality. However, surface glycosylation of Myeloid Derived Suppressor Cells (MDSC) rests unknown, and the influence of galectins on MDSC function remains unexplored.

Objective: We aim to characterize MDSC glycome and identify Galectin 1 (Gal1) role in their pro-tumor activity.

Materials and Methods: We differentiated mouse bone marrow cells and identify M- and PMN-MDSC subsets with antibodies against surface molecules. We used conjugated lectins and performed a structural study of N-glycans by HPLC. We incubated MDSC with recombinant Gal1 (MDSC-Gal1) and tested their activity by PD-L1 and IDO expression by flow cytometry, cytokines array, and co-culture with mouse T cells. We tested *in vivo* activity by adoptive transfer of MDSC-Gal1 to *Lgals1*$^{-/-}$ mice bearing Gal1 KD CT26 tumors. DMedia±SD is informed.

Results: M-MDSC displayed lower levels of surface α2,6-sialyla-

tion, inhibitory of Gal1 binding, than PMN-MDSC (-2.81±0.42 p≤0.05). In accordance, Gal1 binding to M-MDSC was higher (2451±500 p≤0.05). Yet both subsets had N-glycans ligands of Gal1. Gal1 heightened expression of PD-L1 (3326±687 p≤0.001) and IDO (18±6 p≤0.05) in MDSC. MDSC-Gal1 produced lower amounts of macrophage attracting chemokines and higher levels of regulatory cytokines. MDSC-Gal1 further inhibited T cell proliferation (p≤0.01) and activation. MDSC-Gal1 adoptively transferred to tumor-bearing mice accentuated tumor progression, (301±85 p≤0.05) immune evasion and decreased infiltrating inflammatory macrophages in the tumor compared to MDSC control transferred mice (-0.48±0.29 p≤0.05).

Conclusion: For the first time, MDSC glycosylation profile is described**.** Our results indicate that MDSC glycome renders them sensitive to Gal1, which stimulates the MDSC immunosuppressive and tumor-promoting activity. These findings provide us novel opportunities to develop biomarkers and MDSC-directed therapies in cancer.

439. (510) **IL-17 EFFECT ON TUMOR PROGRESSION MAY DEPEND ON PARTICULAR IL-17 RECEPTORS SUBUNIT EXPRESSION ON TUMOR CELLS AND EFFECTS ON ANTITUMOR IMMUNITY**

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The role of IL-17 signaling in the tumor progression is controversial. Several reports indicate that IL-17 sustain, directly and indirectly, tumor growth and immune-escape. However, IL-17also supports anti-tumoral immunity by potentiating CD8+ T and NK cell responses. Our aim is to determine the role of IL-17-signaling in tumor progression dissecting pro- and anti-tumoral effects. First, we evaluated IL-17 receptor (IL-17Rs) expression in melanoma (B16-SIY) and thymoma (EL4-SIY) tumor cells. While both cells expressed varied amounts of IL-17RA and IL-17RD transcripts, IL-17RC was detected only in B16-SIY. According to the different IL-17Rs expression profiles, exposure to IL-17A in vitro resulted in different outcomes. B16-SIY showed an increase in the amounts of transcripts encoding pro-inflammatory mediators (VEGF, HIF1a and N-cadherin, p<0.05) and no change in others (FGF1, MMP2, MMP9, PDL1). Remarkably, EL4-SIY only showed an increase in the amounts of PDL1(p<0.001). Then, we evaluated tumor growth in vivo in IL-17-signaling-deficient mice and in WT mice to determine the overall role of IL-17 in tumor progression. Interestingly, compared to WT controls, IL-17 deficient mice showed augmented B16-SIY tumor volume (p<0.05,18dpi) but diminished EL4-SIY tumor volume (p<0.001,21dpi) at several days post-injection (dpi) . Finally, we evaluated tumor-infiltrating lymphocytes (TILs) in B16-SIY and EL4-SIY tumors from both mouse strains. The percentages of total and SIY- specific CD8+ cells within TILs obtained at day 18 or 21pi from both tumors were diminished in IL-17 deficient mice compared to WT controls(p<0.05), suggesting that IL-17 signaling is required for the proper development of antitumor CD8+T cell immunity irrespective to its global effect on tumor progression. Our results highlight that IL-17-signaling role in overall tumor progression may be influenced by tumor profiles of IL-17R subunit expression together with IL-17 effects on antitumor inmunity.

Key words: IL-17, IL-17R, anti-tumoral immunity,

440. (530) **REDUCED EXTRAVASATION EFFICIENCY OF *LSP1*$^{-/-}$ LEUKOCYTES COMBINED WITH A DEFECTIVE CD8+ T CELLS PRIMING IN *LSP1*$^{-/-}$ TUMOR DRAINING LYMPH NODE CAUSED AN IMPAIRED ANTITUMOR IMMUNITY RESPONSE IN *LSP1*$^{-/-}$ MICE**

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Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes as well as in endothelial cells. This protein in known as an important regulator of actin cytoskeleton remodeling. LSP1 polymorphisms or downregulation are considered risk factors for some types of cancer.

In order to study the role of LSP1 in antitumor immune response, we employed the B16-OVA melanoma model.

We previously shown that B16-OVA tumor in *Lsp1*$^{-/-}$ mice grow significantly faster and bigger than in wild type (WT) controls. Also, tumors harvested from *Lsp1*$^{-/-}$ mice show a lower frequency of total infiltrating leukocytes compared to WT mice.

Considering that LSP1 is expressed by leukocytes and endothelial cells, an *in vivo* migration assay was performed. WT and *Lsp1*$^{-/-}$ splenocytes were labeled with Cell Proliferation Dye eFluor 670 (3μM and 0.3μM respectively), mixed in a 1:1 ratio and adoptively transferred into WT or *Lsp1*$^{-/-}$ tumor-bearing mice. We observed a lower frequency of *Lsp1*$^{-/-}$ migrant leukocytes in tumors developed in WT and *Lsp1*$^{-/-}$ mice (p<0.01 and p<0.001 respectively) 48hr after transfection. However, no difference in migrant cells was found when tumor draining lymph nodes (dLN) were analyzed.

Taking into account CD8+ T cells priming importance in antitumor immunity, an *in vivo* proliferation assay was performed, by transferring CD8^{+} T cells from OT I mice to WT and *Lsp1*$^{-/-}$ tumor-bearing mice. Transferred CD8+ T cells failed to proliferate in *Lsp1*$^{-/-}$ dLN compared to WT dLN 48hr after cell transference (p<0.01). Additionally, we observed that transferred CD8+ T cells in *Lsp1*$^{-/-}$ mice displayed a significantly lower activation pattern as measured by expression of CD69 and CD44 (p<0.01).

We hypothesize that the impaired control of melanoma growth in *Lsp1*$^{-/-}$ mice could be caused, by a reduced extravasation efficiency of *Lsp1*$^{-/-}$ leukocytes, combined with a defective CD8+ T cells priming in *Lsp1*$^{-/-}$ tumor dLN.

441. (540) **DECIPHERING THE CROSS-RESISTANCE MECHANISMS BETWEEN TARGETED THERAPIES AND IMMUNOTHERAPIES IN BRAFMUT MELANOMA PATIENTS: TUMOR-ASSOCIATED MACROPHAGES AS KEY PLAYERS**

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We aim to understand the mechanisms of cross-resistance between targeted therapies and immune checkpoint blockade (ICB) in melanoma.

We implemented computational tools on public datasets to study the expression and the immune profile of melanoma patients treated with targeted therapies or ICB. In particular, we analyzed single-cell RNA-Seq (scRNA-Seq) data of 48 biopsies from melanoma patients before and during anti-PD-1/anti-CTLA-4 therapy and 6 bulk transcriptomic datasets from melanoma patients treated with BRAFi or BRAFi/MEKi.

In progressing to targeted therapies patient biopsies, we observed that there is an increase in M2 macrophage infiltrate in relapsed biopsies (p<0.05; T-test) estimated by MIXTURE and high expression of the TGF-b/Galectin-1(Gal-1) axis while low levels of T cells activation markers. Moreover, the expression of Gal-1 is increased at both RNA and/or protein levels in 4 pairs of human melanoma BRAFi-resistant cells and 1 pair of BRAFi/MEKi-resistant cells by RTqPCR and immunoblotting, respectively (p<0.05; T-test). On the other hand, our analysis of scRNA-Seq data of melanoma patients treated with ICB revealed that macrophages of non-responding biopsies upregulate a specific glycosylation-related signature and show an immunosuppressive expression profile associated with resistance. We observed that these macrophages have an M2-like

phenotype regulated by the activation of specific transcription factors and signaling pathways such as TGFb, hypoxia, and VEGF. To study the molecular mechanisms and test therapeutic strategies to overcome cross-resistance, we developed an immunocompetent mouse model of resistance to BRAFi/MEKi in BRAFmut melanoma which mimicry the immune profile related to M2 macrophages seen in melanoma patients.
We hypothesize that there are Gal-1/glycan interactions that could promote an immunosuppressive microenvironment in BRAFi-resistant tumors, preventing subsequent response to immunotherapies.

442. (8) **SOLUBLE TNFα BLOCKADE OVERCOMES LAPATINIB RESISTANCE AND UNLEASHES AN INNATE IMMUNE RESPONSE IN HER2+ BREAST CANCER**

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Lapatinib (L) is a dual EGFR/HER2 tyrosine kinase inhibitor used in HER2+ metastatic breast cancer (BC), but its clinical benefit is less than 30%. We showed that soluble TNFα (sTNFα) induces trastuzumab (T) resistance by upregulating mucin 4 (MUC4), a transmembrane glycoprotein that shields the T epitope on the HER2 molecule, and that women with HER2+/MUC4+ BC have worse survival. Here we studied the role of sTNFα blockade in cell migration, tumor growth, and in the innate immune response in JIMT-1, a T and L-resistant HER2+ BC model. To block sTNFα we used INB03, a TNFα dominant-negative protein (DN). JIMT-1 cell migration was not prevented by L or DN treatment alone, but the combination of L+DN inhibited migration by 50% ($p<0.001$ vs control). When MUC4 was knocked down, L alone reduced cell migration and sTNFα blockade did not further enhance this effect. In the in vivo setting, L+DN treatment inhibited JIMT-1 tumor growth by 54% vs IgG, L or DN ($p<0.001$) in nude mice. Tumor immune cell infiltration analysis by immunofluorescence and flow cytometry showed a higher activation and degranulation of NK cells and a decrease in myeloid-derived suppressor cells in the L+DN group, regarding the control groups. Taken as a whole, we proved that sTNFα blockade is able to overcome L resistance, inhibiting cell migration and tumor growth. Moreover, sTNFα neutralization along with L treatment triggers an anti-tumor innate immune response. These findings highlight the potential use of L+DN in HER2+/MUC4+ BC, especially in patients with brain metastasis since L and DN both cross the blood brain barrier.

443. (56) **DECIDUAL FACTORS AND VASOACTIVE INTESTINAL PEPTIDE GUIDE MONOCYTES AND DECICUAL MACROPHAGES TO HIGHER MIGRATION, EFFEROCYTOSIS AND WOUND HEALING IN TERM HUMAN PREGNANCY**

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The recruitment and differentiation of monocytes (Mo) at the decidua are critical events that determine the outcome of pregnancy. Their functional plasticity limits the extent of injury after implantation and promotes tissue homeostasis maintenance. The vasoactive intestinal peptide (VIP) is an immunoregulatory peptide synthesized by trophoblast and decidual cells that promotes trophoblast (Tb) invasion, vascular remodelling and functional shaping of decidual macrophages (dMA) in first trimester placenta. Here we studied the role of VIP in human term placenta as a regulator of Mo and dMA function.
Peripheral blood monocytes were isolated from non-pregnant (NON-P) and pregnant (P) volunteers and normal placental samples were obtained at term. Mo were treated *in vitro* with VIP, or conditioned media from decidual explants (D) or decidual explants cultured with VIP [D (VIP)]. Mo or dMA isolated by positive selection with magnetic beads were used to study efferocytosis with CFSE-labelled autologous apoptotic neutrophils by flow cytometry and their wound healing capacity on human endometrial stromal cell line (HESC) monolayers.
NON-P Mo presented higher percentage of efferocytosis than P (34.5±4.5 *vs.* 21.8±3.3; $p<0.05$) which was accompanied by higher expression of CD36. On the contrary, NON-P Mo were less effective in HESC wound healing than P Mo (24.7±1.7 *vs.* 38.7±3.1; $p<0.05$). Remarkably, conditioned media from D or D (VIP) restored the effects. VIP increased TGF-β secretion without altering TNF-α or IL-1β in dMA, as well as their efferocytic capacity. When Mo and dMA from the same patient were analyzed, D (VIP) increased efferocytosis in Mo and dMA to a similar extent.
The results suggest that VIP might regulate Mo and dMA phenotypes directly or by regulating the secretion of decidual factors favoring efferocytosis and wound healing with an increment in TGF-β without changing pro-inflammatory cytokines at term.

444. (65) **B. ABORTUS DOWN MODULATES INFLAMMATION IN MONOCYTES/MACROPHAGES THROUGH MTOR ACTIVATION**

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Brucellosis, caused by *Brucella* spp, is a disease with a large inflammatory component. *B. abortus* has been shown to activate cells of innate immunity, inducing the secretion of pro-inflammatory factors. However, *B. abortus* has different mechanisms whereby it modulates the immune response, in order to evade it and survive intracellularly. mTOR (mammalian target of rapamycin) is a protein kinase that regulates essential signaling pathways, regulating several cellular functions, such as innate immunity, among others. The aim of this work was to elucidate whether *B. abortus* can modulate the functionality of monocytes and macrophages through the activation of mTOR. *B. abortus* was capable to activate mTOR (evaluated by flow cytometry) during the infection of human monocytes and murine macrophages (RAW 264.7). As heat-killed *B. abortus* recapitulates the effect, we concluded that bacterial viability is not necessary to induce mTOR activation. A significant increase in the expression of TNF-α ($p<0.0005$), IL-6 ($p<0.05$), IL-1β ($p<0.005$) and IL-10 ($p<0.05$), as well as metalloproteinase (MMP)-9 ($p<0.0005$) was observed when infected-cells were pre-treated with rapamycin, a pharmacological inhibitor of mTOR. Together, our results demonstrate that *B. abortus* activates mTOR, which negatively regulates the inflammatory response, potentially contributing to the escape of the immune response, allowing *B. abortus* to survive within monocytes/macrophages for prolonged periods, generating an infection.

445. (69) **MACROPHAGE´S ROLE IN THE MICROENVIRONMENT AGAINST EPASTEIN BARR VIRUS (EBV) IN TONSILS FROM PEDIACTRIC PATIENTS**

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Our aim was to study the macrophage´s role in pediatric EBV infection to help clarify viral contribution to lymphomagenesis.
We studied 74 patients undergoing tonsillectomy. The infection status was assessed by Anti-EBV VCA-IgM, VCA-IgG, EA-IgG and EBNA1-IgG serology, defining 4 status: primary infection (PI), healthy carrier (HC), reactivation (R) and not infected (NI). Viral load, IL1

and TNFα were assessed by PCR and qPCR. Latency pattern was evaluated by immunohistochemistry (IHC) for LMP1, EBNA2 and BMRF1, and by EBERS in situ hybridization (ISH). Macrophage´s characterization was performed by CD68, CD163, and CD169 IHC, expressed as positive cells/mm2. M1 profile was defined as CD68/CD163 >1.5 and M2 as CD163/CD68 >1.5

Fifty-two percent of the patients were HC, 27,0% PI, 14,9% R and 6.8% were NI; 41.5% expressed Latency I, 31.7% Latency II, 12.2% Latency III, 14.6% Latency 0 and 14.6% also expressed lytic antigens; 89% of patients displayed M1 profile. CD68+ cell counts were higher than CD163+ and CD169+ in the series and within groups (ANOVA $p<0.05$). CD68+ and CD163+ cells were statistically higher in IF region in the whole series and in subgroups ($p<0.05$ T test), whereas CD169+ cells were statistically increased at the GC in the cohort, specifically in HC ($p<0.05$ T test). An increase in CD163+ cells was observed in patients with Latency II-III, when patterns were clustered into 0-I and II-III (T test $p=0.01$). No correlation between CD68, CD163, CD169 with TNF-α, IL-1 or viral load ($p>0.05$ Spearman) were proved.

M1 polarization prevailed regardless of the infectious status or viral load. TNFα and IL1 cytokines may not be involved specifically in M1 polarized milieu. In EBV-associated pediatric Hodgkin Lymphomas, we described a M1 predominance, indicating that EBV infection leads to M1 macrophage polarization in spite of the lymphomagenesis process.

446. (71) ***LACTOBACILLUS PLANTARUM* CRL 759-SUPERNATANT REDUCES INFLAMMATION IN AN ENDOTOXIN INDUCED UVEITIS**.

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Uveitis is a term applied to a wide range of conditions characterized by intraocular inflammation. Corticosteroids are the first line therapy for patients with uveitis, but their side effects highlight the need for new therapeutic approaches. Our previous studies demonstrated that *Lactobacillus plantarum* CRL 759 supernatant (LplS) was able to decrease pro-inflammatory cytokines production in LPS-stimulated ARPE-19 cells (human retinal pigment epithelium cell line). The aim of this study was to investigate the effect of LplS in a murine experimental model of uveitis induced by endotoxin.

L. plantarum was cultured in DMEM medium at 37ºC 5% CO_2; LplS was obtained by filtration on 0.22μm membranes. To induce uveitis, 130 μg LPS was injected subcutaneously into C57BL/6 mice. LplS was administered as drops and Prednisolone (P) was used as anti-inflammatory control. The mice were divided into six groups: LPS (LPS injection +PBS drops); LPS+LplS (LPS injection +LplS drops); LPS+P (LPS injection +prednisolone drops) and control groups treated with PBS, LplS or P drops and a PBS injection. Twenty four hours after stimulation, the mice were sacrificed. The ocular inflammation was assessed by slit lamp microscopy and clinical scores were determined. From each mouse, one eye was used for collecting aqueous humor (AqH) and the other was enucleated for histopathological evaluation. Total proteins, TNF-α and leukocyte infiltration were determined in AqH.

The clinical score of mice treated with LplS was significantly lower than the LPS group ($p<0.001$). LplS elicited a marked decrease in the secretions and inflammatory reaction at the anterior chamber, and also the presence of these cells in the posterior segment. In addition, LplS reduced TNF-α (41%) and proteins (61%) concentration in AqH, compared to LPS group ($p<0.05$).

LplS showed a significant anti-inflammatory effect and could be proposed as a potential therapy for inflammatory eye disorders.

447. (73) **DIFFERENTIATION OF MONOCYTES INTO MACROPHAGES IN THE PRESENCE OF *BRUCELLA ABORTUS* GENERATES A DIFFERENT PHENOTYPE**

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Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that triggers a complex host response involving both innate and adaptive immunity. Monocytes and macrophages are not only the first line of defence against *Brucella* infection but also a main reservoir for the bacteria. Monocytes are recruited in large numbers to sites where the inflammatory process exists and the microenvironment will condition their differentiation to distinct functional profiles of macrophages and dendritic cells. How *Brucella* could modulate this process remains unclear. The aim of this study was to characterize the phenotype and functions of monocyte-derived macrophages differentiated in the presence of *B. abortus*. For this, human purified monocytes were cultured with MCSF in presence or absence (control) of heat-killed *B. abortus* (HKBa) for 5 days. Expression of CD40, HLADR and CD54 was quantified by flow cytometry. Cells showed a lower expression of CD54 and HLADR on monocyte-derived macrophages differentiated in the presence of HKBa than in control ($p<0,05$). Moreover, these macrophages were unable to be activated when further stimulated with HKBa or LPS from *E. coli*, as they were unable to up regulate CD54 or HLADR. Next we analysed the ability of *B. abortus* to modulate functional aspects of monocyte-derived macrophages. For this, supernatants from macrophages cultures were collected and the secretion of IL-6 and IL-1β was quantified by ELISA. Monocyte-derived macrophages differentiated in the presence of HKBa expressed higher levels of IL-6 and IL-1β than control ($p<0,05$). Collectively, our results indicate that *B. abortus* affects the phenotype of monocyte-derived macrophages decreasing HLADR and CD54 and increasing the secretion of proinflammatory cytokines. This phenotype could affect the activation of T lymphocytes, cooperating with the evasion of immunity by *B. abortus*.

448. (75) **BYSTANDER ACTIVATION OF MICROGLIA BY *BRUCELLA ABORTUS*-INFECTED NEUROVASCULAR CELLS INDUCES NEURONAL DEATH**

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Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. We have previously demonstrated that *B. abortus* is able to elicit neuronal death by activating microglia (with release of nitric oxide and proinflammatory cytokines) and inducing primary phagocytosis. The aim of this work was to investigate the role of soluble mediators released by infected cells of the neurovascular unit in the modulation of this phenomenon. For this, murine astrocytes and human brain microvasculature endothelial cells (HBMECs) were infected or not with *B. abortus* for 24h. After that, cell free culture supernatants were used to stimulate co-cultures of murine primary neuron/microglia during 48h. Neuronal density was evaluated by fluorescence microscopy. Stimulation of neuron/microglia co-cultures with supernatants from *B. abortus*-stimulated astrocytes or HBMECs induced a significant reduction in the density of neurons comparing with non-treated co-cultured or treated with supernatants from non-infected cells ($p<0.005$ for astrocytes supernatants and $p<0.05$ for HBMECs supernatants). Furthermore, supernatants obtained from infected cells increased several microglia function as proliferation (determined by microscopy, $p<0.05$), secretion of TNF-α (measured by ELISA; $p<0.005$) and phagocytic activity (evaluated by phagocytosis assay with *Escherichia coli*; $p<0.005$). In order to determine possible soluble mediators involved in these phenomenon, we use monoclonal antibodies to neutralize TNF-α and IL-6 cytokines in culture supernatants of infected astrocytes. Neutralization of IL-6 prevented neuronal loss in microglia-neurons co-cultures ($p<0.05$), whereas neutralization of TNF-α did not ($p>0.05$). Overall, these results demonstrate that soluble mediators secreted by *B. abortus*-infected neurovascular cells activate resting microglia and this bystander activation could be involved in worsening the neuronal death induced by *B. abortus*.

449. (84) ***YERSINIA ENTEROCOLÍTICA*-INDUCED MO-MDSC SUPPRESS T-CELL PROLIFERATION THROUGH A NITRIC OXIDE-DEPENDENT MECHANISM**

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Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells with the ability to suppress T-cell responses. Monocytic MDSC (Mo-MDSC) and polymorphonuclear MDSC (PMN-MDSC) are the main subsets described. *Yersinia enterocolitica* (Ye) is a Gram-negative bacterium that causes foodborne gastrointestinal diseases. In previous studies, we demonstrated that Ye oral infection induces the expansion of MDSC. The purpose of this work was to investigate the MDSC sub-population that exerts the suppressive activity and underlying the mechanism behind it. Mice of the strain C57BL/6 were infected with Ye WAP-314 serotype O:8. On day 5 post-infection (p.i), MDSC were analyzed in bone marrow (BM), mesenteric lymph nodes (MLN) and spleen by flow cytometry. Furthermore, Mo-MDSC and PMN-MDSC were purified from the spleen of infected mice and their suppressor activity was evaluated in co-cultures with purified T cells. Nitric oxide (NO) production was analyzed by nitrite quantification in culture supernatants. We found that MDSC expanded in BM and accumulated in both MLN and spleen of infected mice, in contrast with uninfected mice ($p<0.001$, $p<0.01$ and $p<0.01$, respectively). However, mice infected with Ye deficient in YadA, an essential virulence factor that mediates intestine colonization, presented lower MDSC accumulation in MLN and spleen, compared to Ye wild-type infected-mice ($p<0.01$, $p<0.01$, respectively). In contrast to PMN-MDSC, Mo-MDSC from the spleen of infected mice showed suppression activity on T cells. Also, NO production increased in the supernatants of Mo-MDSC/T-cell co-culture, but not in those of PMN-MDSC/T-cell co-culture ($p<0.01$). We conclude that Mo-MDSC is the sub-population that suppress T cell response during Ye infection, and this action is mediated by a NO-dependent mechanism. Moreover, MDSC accumulation in intestinal mucosa and spleen could be dependent of bacterial virulence factors, which are important for Ye invasiveness.

450. (99) **PROKARIOTIC RNA EFFECTS ON PULMONARY EPITHELIAL AND ENDOTHELIAL CELLS MODULATE THE INMUNE RESPONSE**

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Escherichia coli (ECO), *Klebsiella pneumoniae* (KPN) and *Pseudomonas aeruginosa* (PAE) can cause severe pneumonia. Lung epithelial (EpCs) and endothelial cells (ECs) modulate local immune responses. In fact, the reciprocal relation between EpCs and ECs as well as their influence on cells from the innate immunity is determinant in the course of infection.
At the site of infection, cells are exposed to prokaryotic RNA (pRNA). pRNA's immunomodulatory role is relevant, but its effect on lung immune response has not been determined. The aim of this work was to determine the effect of pRNA on EpCs and ECs, and the influence of their response on neutrophil (PMN). For this, human pulmonary epithelial (Calu-6), and microvascular endothelial cell line (HMEC-1) were treated with pRNAs from ECO, KPN and PAE, and the result of this stimulation was evaluated on PMN response.
Activation of Calu-6 and HMEC-1 was induced by ECO and KPN-RNA but not by PAE-RNA, as determined by the increased ICAM-1 expression by flow cytometry (FACS) ($p\leq0.01$). Also, CALU-6 and HMEC-1 released IL-8 after stimulation with ECO and KPN-RNA ($p\leq0.001$). Consistently, conditioned medium (CM) from both cell lines showed chemoattractant activity for PMN after stimulation with ECO and KPN-RNA ($p\leq0.001$). Finally, CM from cells treated with ECO and KPN-RNA induced PMN activation as determined by the increment in CD11b expression by FACS ($p\leq0.01$).
Moreover, when PMN were treated directly with pRNA, CD11b upregulation was induced only by ECO and KPN-RNA ($p\leq0.05$). Also, all pRNA were able to induce ROS generation ($p\leq 0.05$). Finally, PMN migration assay revealed that ECO and KPN-RNA resulted chemoattractant ($p\leq0.01$).
In conclusion, ECO and KPN-RNA are capable of activating EpCs, ECs and PMN. Also, they induce a response on EpCs and ECs capable of activating and attracting PMN to the site of infection. In addition, this study reveals that PAE-RNA constitute a poor stimulus in the context of a pulmonary immune response.

451. (119) **THE EPIDEMIC CLONE OF *KLEBSIELLA PNEUMONIAE* ST258 CARBAPENEM RESISTANT CAUSE A PERSISTENT INFECTION IN A MOUSE MODEL OF PERITONITIS AND SUBVERTS THE RESPIRATORY BURST IN MURINE NEUTROPHILS.**

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Klebsiella pneumoniae (Kp), an opportunistic pathogen, usually affects immunosuppressed patients, and the increase of their resistance to antimicrobials is associated with high mortality. Our previous results showed that Kp sequence type 258, Kp carbapenemase (KPC) producer ($Kp^{258}KPC^{+}$), is able to impair the respiratory burst and release of NETs by human neutrophils (PMN). Now, a murine model of peritonitis was used, in order to evaluate if the subversion of PMN microbicidal functions represents a real advantage for the bacteria. In a lethality test, the highest dose of $Kp^{258}KPC^{+}$ injected (10^8UFC, i.p), did not cause any death 24h post-infection, while 10^7UFC of *Escherichia coli* (Eco), used as control, was lethal in 71% of infected mice. $Kp^{258}KPC^{+}$ induced an important recruitment of leukocytes to the peritoneum within 24h, compared to Eco (Leukocytes/L: $Kp^{258}KPC^{+}= 3.5_x10^9 \pm 0.6$; Eco: $2.1_x10^9 \pm 0.4$; $p<0.05$), mainly PMN (PMN/L: $Kp^{258}KPC^{+}= 2.2_x10^9 \pm 0.5$; Eco= $0.9_x10^9 \pm 0.1$; $p<0.05$). After 72h, mice infected with $Kp^{258}KPC^{+}$ showed a decrease in the number of PMN, but $Kp^{258}KPC^{+}$ counts persisted, even 7d after infection, and spread to liver, spleen and lungs. Depletion of PMN by cyclophosphamide treatment increased by 50% the mortality due to $Kp^{258}KPC^{+}$. However, despite the importance of PMN, immunocompetent mice were unable to eradicate the bacteria and avoid their dissemination. As in human PMN, $Kp^{258}KPC^{+}$ did not induce large amounts of reactive oxygen species (ROS) in murine PMN. Moreover, $Kp^{258}KPC^{+}$ impaired ROS induced by Eco (%DHR^{+} PMN: Ctrl= 15.1 ± 3.4; $Kp^{258}KPC^{+}= 17.7 \pm 3.6$*; Eco= 40.4 ± 4.2; Eco + $Kp^{258}KPC^{+}= 22.4 \pm 3.4$*; *$p<0.05$ vs Eco). Despite the increased mortality in the absence of PMN, that may indicate a possible role of PMN in containing Kp infection, $Kp^{258}KPC^{+}$ managed to escape PMN-mediated killing, and this could be related to the lack of ROS induction. In conclusion, Kp has the ability to evade the immune response, allowing it to persist and disseminate.

452. (179) **PROSTAGLANDIN E2 (PGE2) ENHANCES EFFEROCYTOSIS IN HUMAN MONOCYTE-DERIVED MACROPHAGES**

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Phagocytosis of apoptotic neutrophils by macrophages is one key feature in the resolution of inflammation. Recent studies have shown that prostaglandin E2 (PGE2), an immune mediator with recognized inflammatory properties, is also required for the initiation of the resolution process. Here we investigated the effect of PGE2 on neutro-

phil phagocytosis by macrophages.
Human monocytes were obtained from blood buffy coats and differentiated to macrophages by culture with M-CSF (50 ng/ml) for 7 days. Macrophages were treated or not (control) with PGE2 (10-6 M) during the last 24 and 48 hours of culture. Polymorphonuclear leukocytes (PMNs) were purified and cultured in RPMI medium containing 5% fetal bovine serum overnight to induce apoptosis (>75% annexin V positive). Apoptotic PMNs were stained with CFSE, added to macrophages for 1 hour and washed. Macrophages were then collected and efferocytosis was measured by flow cytometry. We observed that treatment with PGE2 promoted macrophage capacity to engulf apoptotic neutrophils, as demonstrated by an average 15% increase of phagocytic macrophages (treated vs control, n=10, p<0,0001), and also an elevation of the number of engulfed cells per macrophage (MFI 10271 vs 7533 for CFSE positive cells, n= 6, p<0,001). We confirmed this to be actual phagocytosis of neutrophils by confocal microscopy. The effect of PGE2 was specific of apoptotic cells since it was not seen when macrophages were incubated with fresh PMNs. Notably, the effect of PGE2 was also observed even when macrophages were cultured with IL-4 (20ng/ml) to promote a pro-resolution M2 profile.
Our results suggest that promotion of efferocytosis by macrophages could be one mechanism by which PGE2 contributes to resolution of inflammation.

453. (194) **CHOLINERGIC SYSTEM AS AN AUTOCRINE-PARACRINE DENDRITIC CELL MODULATOR: RELEVANCE IN THE PATHOGENESIS OF BRAIN TUMORS**

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Background: Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. Acetylcholine is a neurotransmitter which can also modulate cell survival, proliferation, and differentiation in neuronal and non-neuronal cells such as immune cells, which has been referred to as a "non-neuronal cholinergic system". **Objective:** This work aimed to elucidate the cholinergic system relevance in the interaction between dendritic and GBM cells and its relationship with the pathology. **Materials and Methods:** Human U251 and U373 cell lines were co-cultured with human dendritic cells (DC) in the presence of cholinergic agonists (10-8M of carbachol/24h). We then analysed DC activation marker expression by flow cytometry based on CD86 and HLA-DR. On the other hand, using datasets from The Cancer Genome Atlas (GBM and LGG collections) we analysed the influence of the expression of muscarinic receptors on the immune infiltrate of patients with GBM. **Results**: Cholinergic system increased the expression of CD86 and HLA-DR in DC co-cultured with U251 or U373 cell line, also, the immune infiltrate analysis showed that the expression of muscarinic receptors such as CHRM1, CHRM2 and CHRM4 are associated with the presence of DCs in GBM and LGG in a significative way. **Conclusion:** Our findings suggest that the non-neuronal cholinergic system is present in GBM cells and could modulate their crosstalk with the immune system, influencing the survival of patients with LGG and GBM.

454. (222) **AIM2 INVOLVEMENT IN INFLAMMASOME ACTIVATION TRIGGERED BY OUTER MEMBRANE VESICLES (OMVS) FROM *BORDETELLA PERTUSSIS***

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The resurgence of pertussis has urged the development of new vaccines. We have characterized a vaccine candidate based on outer membrane vesicles derived from *Bordetella pertussis* (BpOMVs). This candidate has shown to be safe and protective with a mixed Th1/Th17/Th2 immune profile. We have demonstrated that our vaccine candidate is able to activate the inflammasome in THP1 and in murine BMDM with a dependence on NLRP3 and Caspase-11. It is known that OMVs from different Gram-negative bacteria can carry genetic material inside itself or membrane associated, regarding BpOMVs there are no reported studies on this issue. One of the intracellular dsDNA sensors capable of activate the inflammasome pathway is AIM2 (Absent In Melanoma 2). We here address the potential role of AIM2 in inflammasome activation by BpOMVs. We stimulated BMDM from AIM2$^{-/-}$ mice with 400ng of BpOMVs and IL-1β secretion was measured in culture supernatant by ELISA. We observed a decrease ($p \leq 0.001$) in IL-1β secretion from AIM2$^{-/-}$ cells under BpOMVs stimulation in comparison to BMDM from C57BL/6 mice. We performed western blot analysis of caspase 1 induction in the same scenario and the levels of Casp1 were also diminished compared to control. We therefore stimulated WT and AIM2$^{-/-}$ BMDM with BpOMVs previously treated with DNAse and the levels of IL-1β secretion were significantly diminished ($p \leq 0.001$). GSDMD was identified as a pore forming protein that is cleaved by caspase-1 or 11 to mediate the release of interleukin IL-1β and IL-1α. We tested IL-1α secretion in culture media as a measure of GSDMD pore formation and found that lack of AIM2 significantly affects its secretion in comparison to control ($p \leq 0.05$). Results indicate that our vaccine candidate activates the inflammasome via the dsDNA intracellular sensor AIM2 in mice BMDM. Future work will focus in demonstrate if this is one of the innate mechanisms that orchestrates the adaptive immune response responsible of the protective capacity of our vaccine.

455. (233) **CERAMIDE 1-PHOSPHATE CONFERS ANTI-INFLAMMATORY AND TISSUE-REPAIR FUNCTIONS TO HUMAN MONOCYTES AND MACROPHAGES**

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Ceramide 1-Phosphate (C1P) is a bioactive sphingolipid released from dying cells and therefore highly augmented in damaged tissues. C1P exerts antiapoptotic effects in several cell types, and it is a well-known chemoattractant for macrophages and progenitor/stem cells. It has been recently reported that C1P, in a murine model of hind limb ischemia, not only improved tissue regeneration by itself but also increased human endothelial colony forming cells (ECFC) regenerative properties. Considering that C1P levels are elevated at injured sites and its effects on human monocytes/macrophages (hMø) remained unknown, here we aimed to study whether C1P instructs these immune innate cells to enhance pro-resolving and repair functions. Human CD14+ monocytes were isolated from PBMCs of healthy donors, cultured with RPMI+10% SFB and stimulated with different concentrations of C1P short chain analog C8-C1P (1 - 20μM). ANOVA, *$p \leq 0.05$.* We found that C8-C1P significantly prevented apoptosis of monocytes, and this effect was accompanied with a higher BCL-2 expression after 24 and 48h. Additionally, LPS and C8-C1P-treated monocytes showed lower expression levels of CD80 and CD44, and CD80 and HLA-DR after 24 and 48h, respectively. Interestingly, hMø differentiated with a higher (20μM) or a lower (1μM) concentration of C8-C1P upregulated genes related

to tissue-repair and resolution of inflammation like VEGFA, MER and PPARG and downregulated IRF1, a pro-inflammatory signature. Moreover, C8-C1P-differentiated hMø supernatants increased ECFC *in vitro* tubule formation only with 20μM, probably due to increased levels of proangiogenic secreted factors. In conclusion, C8-C1P not only augmented monocytes survival and reduced their activation, but also affected hMø differentiation by conferring them pro-resolving and tissue-repair functions. Our results highlight the therapeutic potential of C1P to improve wound healing.

456. (240) **CONSTRUCTION OF FLUORESCENT-TAGGED ADENOVIRAL VACCINE CANDIDATE AS A TOOL FOR STUDYING IMMUNE RESPONSES UPON VACCINATION**

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DNA vaccines are efficient Th1 and CD8 inducers and have shown efficacy to control intracellular pathogens such as *Trypanosoma cruzi*. Live attenuated vectors, like rare serotype Adenovirus, used as vaccine DNA-delivery system, improve immunogenicity and guarantee a strong and long-lasting response.

Considering these facts, we generated a vaccine based on rare serotype human adenovirus (Ad48) carrying Traspain gene, a novel *T. cruzi* chimeric antigen developed in our laboratory. With the aim of studying immune activation by this Ad serotype and the spatiotemporal tracking of the antigen we developed an Ad48 carrying Traspain gene fused with the monomeric red fluorescent protein mScarlet and analyzed its performance.

mScarlet tagged Traspain was constructed by traditional cloning. Ad48-Traspain-mScarlet virus was obtained by homologous recombination in HEK-293 cells, 15 days post-transfection.

Seven clones were isolated by agarose plaque assay and further analyzed. Traspain-mScarlet gene was detected by PCR, *in vitro* expression demonstrated by Western-Blot and Fluorescent Microscopy in infected cells showed full cytopathic effect.

Three brighter clones were compared employing a high-throughput imaging system (*IN-Cell Analyzer 2200*, GE). Clone 2 was selected because it showed a signal/noise ratio of 100 and 2-fold mScarlet MFI compared to other ones. Purification of this clone by sucrose density gradient ultracentrifugation, resulted in titers higher than 2.10^8 TCID50/ml. Low rate of impurities were found by SDS-PAGE and A_{280}/A_{260} ratio = 1.40-1.60.

Traspain specific immune response was assessed by flow cytometry after immunization of C57BL6 mice with two subcutaneous doses of the virus. A strong antigen-specific CTL response was detected by tetramer staining of whole blood from immunized mice.

In conclusion, the recombinant viral vector Ad48 carrying Traspain-mScarlet was generated and its *in vitro* and *in vivo* performance confirmed the feasibility of the vaccine approach.

457. (247) **INNATE CD8⁺ T CELLS: FROM THE THYMUS TO THE SECONDARY LYMPHOID ORGANS (SLO) IN STEADY STATE VERSUS *TRYPANOSOMA CRUZI* INFECTION**

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Simple positive CD8⁺ thymocytes (SP8) that develop in the thymus could give rise either to conventional SP8 or to Innate CD8⁺ T cells (T_{IM}). T_{IM} acquire a memory phenotype during their thymic maturation and are exported to SLO as a conventional T cell. T_{IM} play a protective role during the early phase of infectious processes as reported for certain bacteria, viral and parasite infections. Our previous results demonstrated that during *T. cruzi* infection, a large number of T_{IM} mature in the thymus due to local production of IL-4 and IL-15, 2 cytokines responsible for their maturation/maintenance process. T_{IM} functionally act in a TCR-independent way; instead they are activated through cytokines as IL-12 and IL-18. By using OT-I mice (not RAG2 KO, that carry an OVA specific TCR in most of SP8 cells) we could compare the expression of a large number of markers between OVA tetramer⁺ (OVAt⁺) SP8 cells (not specific for the parasite) and conventional polyclonal SP8 cells simultaneously present in the thymus of control and *T. cruzi* infected mice. Data demonstrate that OVAt⁺ SP8 cells expressed higher levels of CD44, CD122, CD5, CD69, QA2 and decreased levels of CD24 compared to conventional SP8 cells while other markers like CD62L, PD-1 and CD5 seem not to be differentially expressed ($p<0,05$). Moreover, this pattern is even more pronounced after *T. cruzi* infection ($p<0,05$) demonstrating that OVAt⁺ SP8 cells adopt a T_{IM} phenotype in and Ag-independent way after infection. Expression of S1PR1 and S1PR4, that allow mature SP8 thymocyte to be exported to SLO, is downregulated in the bulk thymocyte population from *T. cruzi*-infected compared to control mice. In correlation with these data, exportation experiments performed by labeling thymocytes with CFSE (using intrathymic injection) demonstrated a significant lower number of CD8-CFSE⁺ cells in SLO of *T. cruzi* infected mice ($p<0,05$). Our data contribute to understand the maturation and exportation process of T_{IM} that is still poorly described in the scientific literature.

458. (249) **THYMIC STROMAL LYMPHOPOIETIN (TSLP) IS A NEUTROPHIL CELLS MODULATOR: RELEVANCE IN THE PATHOGENESIS OF BRAIN TUMORS**

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Background: Glioblastoma (GBM) is the most devastating brain tumor, with an associated poor prognosis. Despite the advances in surgery and chemoradiation, the survival of GBM patients has not improved significantly in the past three decades. Thymic stromal lymphopoietin (TSLP) is a cytokine produced primarily by activated epithelial cells and it has been shown to be a key factor in maintaining immune homeostasis and regulating inflammatory responses at mucosal barriers. However, recent studies have found an expanding role for TSLP in inflammatory diseases and cancer.

This work aimed to elucidate the relevance of TSLP in the interaction between neutrophils and GBM cells. First, we evaluated the production of TSLP by tumor cells through RT-PCR and then, we determined whether the TSLP treatment affects the cross-talk between neutrophils and tumor GBM cells. For those purposes, human U251 cell line or tumoral cells obtained from a GBM patient were co-cultured with human neutrophils. We observed that the U251 cell line or the primary cell culture incubated with Epidermal Growth Factor (EGF) produced TSLP ($p<0.05$). Sequencing of the PCR product confirmed it was TSLP. Additionally, the neutrophils obtained from healthy donors and from the GBM patient produced TSLP. Notably, we observed a decrease in the apoptosis of neutrophils when they were co-cultured with the U251 cell line or the primary cell culture in presence of TSLP. Finally, we found that U251 cell line and healthy

neutrophils expressed TSLP receptor (RTSLP) Our findings suggest that TSLP is present in GBM cells and could modulate their cross-talk with the immune system, particularly with neutrophils.

459. (312) **AN ALTERED RESPONSE TO RESISTIN IN MONOCYTES AND LIVER MACROPHAGES CONTRIBUTES TO INFLAMMATION IN ADULT PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE**

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Background: Resistin (Res), a cytokine produced by monocytes (Mo) and macrophages, is highly expressed in blood and liver of patients with nonalcoholic fatty liver disease (NAFLD). Res binds to adenylate cyclase-associated protein 1 (CAP1). CAP1 expression and Res-mediated effects are unexplored in NAFLD.
Aims: to evaluate CAP1 expression and Res-mediated modulation of CD69, IL-6 and phagocytosis in Mo and liver macrophages (LM) from NAFLD.
Methods: we included blood and liver samples from controls (Co, n=40) and NAFLD patients (n=30). CAP1 expression was studied by flow cytometry (FC). To evaluate CD69 expression, peripheral blood mononuclear cells (PBMC) were stimulated with PMA (10 ng/ml) +/- Res (10 ng/ml) for 24 h. IL-6 production was induced using LPS (20 ng/ml) +/- Res for 19 h. Phagocytosis in presence or absence of Res was evaluated by FC using fluorescent beads. Paired t test or Friedman test were used to evaluate effects within experimental groups, and unpaired t test or Mann-Whitney test for comparisons between groups.
Results: CAP1 expression was higher in Mo and LM from NAFLD than Co (p=0.002 and 0.022). Res partially prevented the PMA-induced increase in the frequency of Mo and LM expressing CD69 in Co (p= 0.005 and 0.012). Res partially prevented LPS-induced IL-6 production in Mo from Co (p=0.025). PMA-induced Mo CD69+ frequency and LPS-induced IL-6 production were higher in NAFLD than Co (p=0.016 and 0.001). However, Res-induced modulation of CD69 and IL-6 in NAFLD was not observed. Res induced an increase in phagocytosis percentage in Mo from Co (p=0.008) but not from NAFLD.
Conclusion: Res modulates activation, cytokine production, and phagocytosis in monocytes from controls but not from NAFLD patients. Although CAP1 expression was elevated, Res-induced response is diminished in monocytes and liver macrophages from NAFLD patients. An altered response to resistin in monocytes and liver macrophages contributes to inflammation in NAFLD.

460. (365) **PROBIOTIC LACTOBACILLUS REINFORCE THE INTESTINAL EPITHELIAL BARRIER**

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The epithelial layer not only serves as a passive physical barrier with external environment. Intestinal epithelial cells (IEC) sense external or endogenous danger signals and mount a robust immune response to maintaining intestinal homeostasis. We previously demonstrated that lactobacillus increase Paneth cells and intestinal antimicrobial activity to keep this balance. Our aim was to further characterized the effects of probiotics on the intestinal epithelial barrier (IEB). Mice were fed with *Lactobacillus casei* CRL 431 (Lc431), *Lactobacillus paracasei* CNCM I- 1518 (Lp1518) for 7 and 5 days, respectively, or water. On day 8 mice were killed and samples were removed.
Mice fed with the probiotics increased *ex vivo* intestinal antimicrobial activity against *Staphylococcus aureus*, *Salmonella thyphimurium* and a multi-resistant drugs *Klebsiella pneumoniae* ($p<0.05$). This correlated with increases of Paneth cells ($p<0.05$) and defensin-α5 in the intestinal fluids (15,213 ± 3,252; 25,216 ± 803.0 and 26,724 ± 860.4 pg/ml, Mean±SEM, for Control, Lp1518 and Lc431, respectively). This antimicrobial activity was boosted by the presence of the pro-inflammatory cytokines: IFN-γ, TNF-α, IL-12 and IL-6 ($p<0.05$) and most interestingly, by the immunoregulatory IL-10, in the intestinal fluids of those animals. Animals fed with probiotics did not show increases in the intestinal permeability assessed by FITC-dextran blood levels. The stability and function of the IEB upon probiotics administration was also reflected by increases in the occludin and cadherin proteins in a semi-quantitative histological analysis. Finally, increases in the Goblet cells ($p<0.05$) was determined in probiotics fed animals, regarding a conventional diet.
Probiotics reinforce the IEB barrier and preserve intestinal homeostasis targeting several points, namely, Goblet and Paneth cells, antimicrobial activity of the intestinal fluids, balance between pro-inflammatory/regulatory cytokines, and the joint between IEC.

461. (372) **STUDY OF THE INTERACTION BETWEEN GLIOBLASTOMA DERIVED γδ T LYMPHOCYTES AND NEUTROPHILS**

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γδ T cells constitute a functionally specialized subset of T lymphocytes that act as early sensors of cellular stress and infection. γδ T cells play a critical role in the recruitment and activation of neutrophils at the sites of inflammation. In addition, neutrophils can regulate γδ T cell activity. Glioblastoma multiforme (GBM) is the most lethal primary brain tumor in adults. GBM is clinically highly aggressive because of evolutionary dynamics induced by cross talk between cancer cells and a heterogeneous group of immune cells in tumoral microenvironment. We have previously reported that neutrophils, obtained from healthy donors, regulate γδ T cell activation induced by phosphoantigens. Now, we aimed to investigate whether γδ T cells derived from GBM-bearing patient were susceptible to be modulated by neutrophils. To achieve this purpose, γδ T cells were purified from human peripheral blood mononuclear cells, by using an anti-TCR γδ MicroBead isolation kit. Neutrophils were isolated by dextran sedimentation. After purification, γδ T cells were stimulated or not with (E)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (HMBPP: 10 μM, 60 min), and then were cultured with or without neutrophils. After 24 hours, we evaluated the activation of γδ T cells by analyzing CD69 expression and IFN-α and TNF-γ production by flow cytometry and ELISA, respectively. Our results showed that the response induced by HMBPP on GBM bearing patient-derived γδ T cells, was reverted in presence of neutrophils. This was demonstrated by a decrease in the expression of CD69 and in the IFN-α and TNF-γ production ($p<0.05$). Of note, we did not observe significant differences in the capacity of γδ T cells derived from GBM patient to respond to HMBPP compare to healthy donors. Overall, our results showed that activation of γδ T cells obtained from GBM-bearing patient was modulated by neutrophil in a similar way to that observed in healthy donors.

462. (374) **MOLECULAR INVOLVEMENT OF *IL10* SNP**

RS1800896 GENOTYPES AND IL-10 CYTOKINE EXPRESSION IN THE CLINICAL SEVERITY OF HEMOLYTIC UREMIC SYNDROME.

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Introduction: Although Hemolytic Uremic Syndrome (HUS) is caused by Shiga toxin (Stx)-producing *E. coli*, the inflammatory response is essential for disease progression. Despite antiinflammatory mechanisms are triggered, studies assessing the implication of such mediators, like IL-10, in HUS are scarce. Interindividual differences in IL-10 levels are related to genetic variants. Several reports have linked the single nucleotide polymorphism (SNP) rs1800896 (Legacy: -1082A>G), located in the *IL10* promoter, to several diseases.
Objective: The aim of this work was to evaluate the contribution of IL-10 and SNP -1082A>G to HUS severity.
Methods: Blood samples were collected from HUS patients at the acute period and from healthy children (HC). Plasma was stored and mononuclear cells (PBMC) were isolated and cultured with Medium or LPS for 20h. IL-10 levels were evaluated in plasma and PBMC supernatants by ELISA. DNA was obtained from PBMC and *IL10* -1082A>G analysis was performed by allele specific-PCR.
Results: Circulating IL-10 levels were increased in HUS (Mean±SEM (pg/mL): HUS=60.9±10.0***; HC=9.3±2.1; n=18,17; ***p<0.0001). PBMC from HUS showed a lower capacity to secrete IL-10 (Mean±SEM (pg/x10^5Mo): 764.1±679.7**/2320±2111; **p<0.01). The presence of allele -1082G was associated with higher IL-10 production, both in HUS and HC (Mean±SEM (pg/10^5Mo): AA/AG+GG (HUS=1020±558/1938±620*; HC=2270±1244/7674±4568*; *p<0.05), as well as in circulating levels ((Mean±SEM (pg/mL): AA/AG+GG (HUS=38.9±13.5/76.4±12.5*; HC=5.9±2.63/8.9±2.5*; *p<0.05). Severe patients showed a tendency to be enriched in allele -1082G frequency (OR(CI95%) 3.46(0.7-18)).
Conclusion: We showed that allele -1082G contributes increasing IL-10 levels. Although circulating IL-10 was increased at HUS diagnosis, PBMC from these patients showed a lower capacity to secrete IL-10. Moreover, severe cases showed a tendency towards higher frequencies in alleles -1082G suggesting an association with renal failure

463. (445) ***LSP1$^{-/-}$* DENDRITIC CELLS INDUCE DEFECTIVE ANTIGEN PRESENTATION TO CD4+ LYMPHOCYTES.**

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Leukocyte-specific protein 1 (LSP1) is a 52 kDa 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 CD4+ T cells from *Lsp1$^{-/-}$* mice have a poorer proliferation than CD4$^+$ T cells from wild type (WT) mice after antigen exposure in vivo and in vitro with bone marrow derived dendritic cells (BMDCs).
In order to study the role of LSP1 in DCs to promote a CD4$^+$ T cell-mediated response, we evaluated the capacity of *Lsp1$^{-/-}$* DCs to present antigens in a context of major histocompatibility complex class II (MHC II). In vitro, BMDCs were derived from *Lsp1$^{-/-}$* mice with Flt3-L. BMDCs were pulsed with soluble or particulated Ovalbumin (OVAw or OVAb, respectively), then stimulated with CpG-ODN 1826 and finally cocultured with CD4$^+$ T cells hybridoma (TH3Z OT II). After an overnight incubation, a significantly lower activation of TH3Z OT II cells was observed with *Lsp1$^{-/-}$* BMDCs incubated with OVAw ($p< 0.0001$) and OVAb ($p< 0.001$) compared to *Lsp1$^{+/+}$* BMDCs.
In addition, *Lsp1$^{-/-}$* BMDCs show a lower expression of CD40, CD80, CD86 and MHC II molecules than BMDCs from WT mice after CpG-ODN 1826 stimulus ($p>0.05$, $p<0.05$, $p<0.0001$, $p<0.01$ respectively).
These results suggest that LSP1 deficiency affects DC antigen presentation machinery in MHC II context.

464. (480) **CHRONIC THERMAL STRESS EFFECTS ON INNATE IMMUNE RESPONSE INDUCED BY BACTERIAL CHALLENGE IN RUSSIAN STURGEON**

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The negative impact of chronic stress on fish immunity has been widely accepted although its molecular and cellular basis are almost unknown. This is due, among other reasons, to our poor knowledge on how fish immune system works. Herein, we used Russian sturgeons *(A. gueldenstaedtii*), an ancient non-teleost fish with high economic and ecologic value, to investigate how chronic stress affects the innate immune response, by assessing the effect of long-term exposure to stressing temperatures on fish acute phase response and local inflammation. To that end, sturgeons exposed to tolerable (<24°C) or stressing (>24°C) temperatures were i/p challenged with live *Aeromonas hydrophila* (10^8 UFC/kg), and the serum levels of two acute phase proteins, Serum Amyloid A (SAA) and ceruloplasmin (Cp), were measured by ELISA and an enzymatic assay, respectively. Bacterial challenge caused an increase in SAA and Cp levels in sturgeons exposed to both temperatures, but this increment was lower in chronic stressed fish. Moreover, a positive correlation between SAA and Cp was observed in infected fish. In addition, analysis of the cellular composition of peritoneum by flow cytometry allowed us to identify four distinct populations compatible with lymphocytes (LY), small-peritoneal-leukocytes (SPL), large-peritoneal-leukocytes (LPL) and complex-peritoneal-leukocytes (CPL). Bacterial challenge led to an increase in total peritoneal cells and a decrease in LPL percentage while stressing temperatures rose LY and reduced LPL percentages. The local inflammatory response to bacterial challenge was altered by chronic thermal stress, reducing LPL and LY and increasing SPL percentages. Analysis of the effect of chronic stress on reactive oxygen species production and phagocytic activity of peritoneal cells is in progress. Overall, this work presents novel evidence demonstrating that chronic thermal stress alters sturgeon's inflammatory response at both systemic and local levels.

465. (484) **HIGH SALT CONCENTRATIONS INDUCES A DELAYED ACTIVATION PATTERN IN HUMAN NEUTROPHILS**

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Elevated concentration of salt (sodium chloride) are present in a number of tissues under physiological and pathological conditions. Previous studies have analyzed the effect of high salt concentrations on the phenotype and function of macrophages and T cells. Here, we analyzed the effects induced by high salt on neutrophil function. Neutrophils were isolated from human blood by conventional procedures. We found that exposure of neutrophils to hypernatremic media (400 mOsm) for 30 min induced an early state of neutrophil paralysis characterized by a profound inhibition in the ability of conventional agonists to induce an increased expression of CD11b, calcium transients, the production of IL-8 and the activation of the respiratory burst: % inhibition > 45% for each analyzed response ($n = 9$, $p<0.02$ for each response analyzed, 290 mOsm vs 400 mOsm). By contrast, exposure of neutrophils to high salt for longer periods (18 h) resulted in the activation of neutrophils revealed by a high produc-

tion of IL-8 (35 ± 32 pg/ml vs 3924 ± 329 pg/ml, 290 mOsm vs 400 mOsm, respectively, n =14, p<0.0001) and a dramatic synergistic effect on the ability of LPS-stimulated neutrophils to produce TNF-α: neutrophils cultured at 290 mOsm with or without LPS or neutrophils cultured at 400 mOsm produced TNF-α levels lower than 100 pg/ml, while those cultured at 400 mOsm with LPS produced 938 ± 172 pg/ml (n = 4, p< 0.02). Moreover, long-term exposure of neutrophils to high salt also resulted in the induction of calcium transients and the activation of the respiratory burst (not shown). Pharmacological blockade of p38 MAPK markedly prevented the stimulation of neutrophil responses by high salt without affecting its early inhibitory effects (% inhibition > 65 for IL-8 production, n=6). Our observations reveal that high salt induces a unique biphasic effect on neutrophil functions that includes a second phase characterized by a strong stimulation of neutrophil function.

466. (486) **SEVERE COVID-19 IS ASSOCIATED WITH A NEUTROPHIL ACTIVATED PROFILE**

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Severe COVID-19 is strongly associated to the development of a systemic inflammatory response. It is well established that the activation of monocytes, macrophages and platelets plays a critical role in severe COVID-19. However, the role of neutrophils has not been defined. Here we analyzed the phenotype and function of peripheral blood neutrophils in COVID-19 patients. Neutrophils were purified from peripheral blood by conventional procedures (% purity >97%). In a first set of experiments we analyzed the spontaneous production of IL-8 by neutrophils cultured for 18 h. Neutrophils from severe COVID-19 patients produced higher levels of IL-8 compared with healthy donors: 158 ± 30 pg/ml (n= 12) vs 672 ± 249 pg/ml (n=11, p< 0.05). No significant differences were observed for patients with mild or moderate disease. Neutrophils from COVID-19 patients also showed a higher expression of CD11b (n=10, p<0.0001 healthy vs severe COVID-19 patients) and a higher association with platelets evaluated by flow cytometry: percentage of neutrophils associated with platelets = 6.6 ± 1% vs 18.4 ± 7.1% (n=6, p<0.01). Moreover, we found that plasma from severe, but not moderate COVID-19 patients, induced higher levels of oxidant products by healthy donor neutrophils, compared with plasma from healthy donors: % DHR+ cells at 30 min of culture = 11.71 ± 4.0% vs 34.3 ±7.8% (n=10, p <0.05, evaluated by DHR oxidation and flow cytometry). Interestingly, the production of IL-8 by COVID-19 neutrophils strongly correlated with two recognized markers of disease severity, the plasmatic concentrations of LDH (n=26, p=0.0004, R=0.65) and D-dimer (n=25, p=0.0001, R=0.87), as well as with the national early warning score (NEWS score), a clinical score used to predict in-hospital patient mortality (n=20, p=0.049, R=0.146). Our study suggests that the acquisition of an inflammatory signature by neutrophils might play a role in the development of severe COVID-19.

467. (499) **NEUTROPHILS FROM CHILDREN WITH ACUTE COVID-19 EXHIBIT AN INFLAMMATORY SIGNATURE WITH A DISTINCT EXPRESSION OF HIGH AFFINITY RECEPTOR FOR IgG**

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BACKGROUND: Children can be infected by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), being most pediatric cases mild but the reason underlying are still unknown. This study addressed the immunopathological characteristics of neutrophils from acute COVID-19 infected children.

METHODS: Sixty acute COVID-19 mildly infected children (1-14 years-old) and 9 healthy infants were studied. A multiparametric flow cytometry from neutrophils was performed in whole blood. Levels of IL-1β in supernatant culture from purified neutrophils and serum anti SARS-CoV-2 IgG were quantified by ELISA.

RESULTS: A low expression of CD11b (p<0.01), CD66b (p<0.01) and CD62L (p<0.001), three molecules involved in neutrophil migration, was observed in COVID-19 neutrophils (n=33) compared with healthy controls (n=9). Interestingly, COVID-19 neutrophils also showed a higher expression of two inhibitory receptors LAIR-1 (p<0.01) and PECAM-1 (p<0.01). In addition, a higher expression of two activation markers was also found in COVID-19 respect to healthy controls: HLA-DR (p<0.0001) and the high affinity receptor for IgG, CD64 (p<0.01). According to the functional association between the β2 integrin CD11b/CD18 and receptors for IgG, we found not only that the plasma levels of IgG antibodies directed to the S protein of SARS-CoV-2 inversely correlated with CD11b MFI (p<0.01, r= -0.43), but also directly correlated with CD64 (p<0.05, r= 0.5). CD64 is able to mediate a positive feedback on the activation of inflammasome. Indeed, we found that neutrophils from children with COVID-19 spontaneously produced higher concentrations of IL-1β compared with healthy donors and increase greatly after TLR7/8 agonist stimulation.

CONCLUSION: Neutrophils from infants with acute mild COVID-19 have a distinct proinflammatory signature that could potentially compromise their ability to migrate to peripheral tissues.

468. (549) ***TRYPANOSOMA CRUZI* INFECTION: TRANSCRIPTIONAL ANALYSIS REVEALS A ROLE OF WNT SIGNALING PATHWAYS IN MACROPHAGE POLARIZATION AND FUNCTION.**

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Mo activated by pro-inflammatory cytokines (classically activated or M1 Mo) upregulates different effective defense mechanisms against *T. cruzi* and plays a key role in the control of intracellular parasite growth. However, through the modulation of several signaling pathways, the parasite subverts the Mo polarization to favor its survival. We have reported that *T. cruzi* infection induces β-catenin and Wnt/Ca++ pathways activation in Mo, with these pathways being critical to sustaining intracellular parasite replication. Moreover, the inhibition of β-catenin transcriptional activity (using CCT036477 or iCRT14) or Wnt proteins secretion (using IWP-L6) modulates Mo activation status towards a more microbicidal phenotype, which does not fully fit in the classically activated/inflammatory M1 one.

In the present study we evaluated the transcriptional profiles of non-infected, and infected Mo previously treated with CCT, iCRT,

IWP-L6 or LPS+IFN-γ (M1) (n= 3 replicates), compared their transcriptomes with M1 and M2 signature genes and performed gene set enrichment analysis (GSEA). Hierarchical clustering analysis revealed that infected IWP-L6 Mo are transcriptionally more similar to infected M1 Mo than infected CCT or iCRT Mo, which are clearly separated from control Mo and non-infected ones. Infection with *T. cruzi* up-regulated a M1-specific set of genes, while the inhibition of Wnt proteins secretion or β-catenin transcriptional activity showed down-regulated expression of them. In addition, GSEA revealed that inhibition of Wnt proteins secretion or β-catenin transcriptional activity modulated the transcription of genes related to innate immune receptors (TLR, NOD), cytokine and chemokine signaling, apoptosis, antigen processing and presentation, metabolism, and phagocytosis, between others. These results confirmed through transcriptional analysis our previous findings that during *T. cruzi* infection Wnt signaling contributes to modulate Mo polarization and function.

469. (555) **IMMUNOMODULATORY PROPERTIES OF E. GRANULOSUS LIPOPROTEIN ANTIGEN B**

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Antigen B (EgAgB) is the major lipoprotein produced by *Echinococcus granulosus* s.l. larvae. Its protein moiety is composed by EgAgB8/1-5 apolipoproteins, which belong to a cestode protein family capable of binding hydrophobic ligands. Its lipid moiety comprises several lipid classes, including triacylglycerides, fatty acids and phospholipids. Various reports suggested that EgAgB is involved in parasite immunoregulation based on its ability to modulate LPS-induced activation of innate cells *in vitro*. To go further on EgAgB immunomodulatory properties, we explored EgAgB effects on bone marrow-derived dendritic cells (BMDC) using native EgAgB (nEgAgB, immunopurified from bovine hydatid cyst) and the recombinant EgAgB8/1 (rEgAgB, prepared in insect cells). We found that nEgAgB and rEgAgB exhibited dose-dependent proinflammatory effects on BMDC. At concentrations higher than 1μg/mL, both nEgAgB and rEgAgB induced IL6 and IL1β secretion *per se*. Since saturated fatty acids are capable of triggering inflammatory signaling by a TLR4-dependent pathway, we explored the involvement of TLR4 in EgAgB-induced cytokine secretion using a TLR4 specific inhibitor (TAK-242) and the LPS-inhibitor polymyxin B. Results showed that TAK-242, but not polymyxin B, strongly inhibited EgAgB-induced IL6 and IL12p40 production in BMDC. To analyze a direct interaction between EgAgB and TLR4, we assessed TLR4 dimerization, finding that LPS, but not EgAgB, induced TLR4 dimerization along 120 min stimulation. These results show that EgAgB behaves similarly to palmitate, that even though is unable to dimerize TLR4 it requires its presence to trigger inflammatory pathways. In addition to these effects, nEgAgB and rEgAgB altered LPS-induced cytokine secretion, diminishing CD40 expression and IL12p40 and IL6 secretion, while increasing IL1β production. Overall, results suggest that EgAgB effects on BMDC are TLR4-dependent although might not be a consequence of direct interaction between EgAgB and TLR4.

470. (559) **IDENTIFICATION OF MOUSE MICROGLIAL CELL SUBPOPULATIONS BY HIGH DIMENSIONAL FLOW CYTOMETRY ANALYSIS IN LPS-INDUCED NEUROINFLAMMATION.**

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Introduction: Brain-resident microglia and peripheral leukocytes play essential roles in shaping the immune response in the CNS. These cells activate and migrate during active immune responses and may contribute to the progression of neuroinflammation.
We previously found that systemic lipopolysaccharide (LPS) challenge induced glial activation and an active recruitment of CD45hi leukocytes, close to vascular endothelial cells, in circumventricular organs. However, the phenotype of microglial cells and the recruited leukocytes were not fully characterized.
Methods: In this study, we assessed the phenotype of microglial cells and the recruited leukocytes to the brain, in response to systemic TLR4 stimulation, by applying high dimensional flow cytometry analysis. We used machine learning algorithms to detect changes in morphology and marker expression in microglia, due to activation by systemic administration of LPS (LPS - 1.6 mg/kg, i.p. injections, n=4). After perfusion, we obtained brain cells suspensions, stained the cells and eight parameters where simultaneously analyzed by conventional flow cytometry and bioinformatics algorithms implemented in R.
Results: We detected three populations of microglial cells based on CD45 expression and cell size. After LPS-induced systemic inflammation we observed changes in the microglial cell phenotype and size ($p<0.01$ and $p<0.05$). In addition, we observed increased frequency of CD45hi inflammatory monocytes ($p<0.001$). Dimensional reduction (viSNE) and clustering confirmed these results and suggested additional heterogeneity in the recruited cell populations.
Conclusions: These preliminary results suggest the presence of microglial cell subpopulations that responded to peripheral inflammatory stimulation. Further research is required to better define these populations either by increasing the number of cell markers studied or by morphological and tissular characterization, to identify their pathophysiological relevance.
microglia, LPS, inflammation, TLR4, algorithms

471. (528) **CELL-FREE, IN VITRO RADIOLABELING ASSAYS SENSITIVELY UNCOVERING INFECTION-DEPENDENT ALTERATIONS IN CYTOSOLIC FORMS OF NUCLEOLAR PROTEINS IN HUMAN MACROPHAGES.**

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AIMS: To developed and test cell free, in vitro (iv) radiolabeling (RL) assays detecting infection time-dependent proteome alterations in macrophage cytosols uncovering novel altered proteins and pathways. METHODS: Macrophages were mycobacterial infected comparing live vs killed bacteria and time points. The cytosolic fraction was studied for being the purest. Cell-free, iv RL phosphorylating available sites in proteins and others nucleotide-based assays were used. The iv reactions were resolved in 1D and 2D gels comparing staining vs RL patterns. Reproducibly (>95 % cases) altered proteins were identified by MS and WB. The n for iv assays and treatments was 3 to 9. RESULTS: The assays were sensitive, reproducible and quick. Many proteins were labelled but 2 NUCLEOLAR proteins, C23 and B23, were detected in cytosols with a highly reproducible time-dependency of level alteration. Bacterial viability was not required for triggering that, but >4 days post-infection only live bacteria sustained it. MW alterations in both, but mainly C23, were compatible with partial cleavage to shorter fragments. The same kinase iv phosphorylating both was unaffected in activity by infection. RL assays other than phosphorylation confirmed the altered levels. Coupled iv phosphatase assays and anti-P WB revealed the C23 in vivo phosphorylation status. For detecting minor C23 changes and fragments, RL was more sensitive or linear than gel staining or WB. C23 has reported roles in mRNA translation/degradation, in ribosomes, in hubs for kinases and NEMO, etc. B23 in ribosome, PIDDosome and casp2 regulation. CONCLUSIONS: the assays tracked alterations in low-abundant cytosolic C23 and B23 forms and might be useful for: a) infection studies assessing their multiple roles, b) for cancer (C23 as aptamer target and B23 in leukemia), c) for C23 kinase assays. The RL approach would serve in

tracking their intracellular trafficking and roles in nucleolar, cytosolic and membrane surveillance pathways

472. (552) **INCREASED EXPRESSION OF AUTOPHAGY PROTEIN LC3 IN PATIENTS WITH PROGRESSING CHRONIC LYMPHOCYTIC LEUKEMIA**

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Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in the western hemisphere. It is characterized by a clonal proliferation of a population of CD5+ B lymphocytes that accumulate in the secondary lymphoid tissues, bone marrow, and blood. Some CLL patients remain free of symptoms for decades, whereas others rapidly become symptomatic or develop high-risk disease. Studying autophagy, which may modulate key protein expression and cell survival, may be important to the search for novel prognostic factors and molecules. Here, we applied flow cytometry technology to simultaneously detect autophagy protein LC3B with classical phenotypical markers used for the identification of tumoral CLL B cell clones. We found that two patients with progressing CLL showed increased expression of the autophagy protein LC3B, in addition to positive expression of CD38 and ZAP70 and unmutated status of IGHV. Our data suggest that activation of autophagy flux may correlate with CLL progression even before Ibrutinib treatment.

473. (170) **VIP DEFICIENCY PROMOTES AN INFLAMMATORY OVARIAN MICROENVIRONMENT IMPAIRING PREGNANCY**

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Background: Complex immune regulation is required to ensure a successful pregnancy outcome. Vasoactive intestinal peptide (VIP) is an immunoregulatory peptide known to induce a tolerogenic maternal response. Moreover, VIP regulates folliculogenesis and ovulation as well as ovarian steroidogenesis. VIP KO mice are known to be subfertile, so we aimed to better understand how VIP deficiency affects ovarian physiology and immune homeostasis.
Methods: Animals: VIP Knockout KO (-/-), deficient HT (+/-) and wild type WT (+/+) mice in estrus (defined by vaginal lavage observation) were studied at 3 or 8 months of age. Serum hormones were measured with immulite Xpi Siemens platform. Ovaries were examined after histological staining, hormones quantified using RIA and IL-1 secretion by ELISA.
Results: Young KO mice showed cycling disorders accompanied by an altered P4/E2 ratio in serum and ovary in the day of estrus as compared to WT mice of the same age. We also found histological differences between the ovaries, with an increase in secondary follicles, less corpora lutea, and more atretic follicles ($p<0.05$ Anova and Tukey´s Multiple test) indicating ovarian failure. Moreover, we confirmed that 25% of KO mice fail to ovulate and 50% could ovulate but the oocytes were incompetent to be fertilized. After comparing young WT to reproductively aged WT mice we found, as expected, heavier animals with lighter ovaries and reduced E2 and P4 levels in serum of the aged group. On the contrary, when these parameters were assessed in KO young mice, they were not unlike those of reproductively aged mice, showing an intense inflammatory ovarian microenvironment and foamy macrophages - signs of premature aging. The adoptive transfer of Foxp3+ cells to these animals resulted in selective recruitment of them to the ovary, in relation to other tissues, and an improvement in the pregnancy rate.
Conclusion: VIP contributes to ovarian homeostatic mechanisms required for a successful pregnancy.

474. (343) **EVALUATION OF TIRNAS AS POTENTIAL SEMINAL BIOMARKERS WITH PROGNOSTIC UTILITY IN ASSISTED REPRODUCTION TREATMENTS**

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Infertility affects 15% of the world's population and the male factor is involved in half of the cases. Since, conventional semen analysis provides a limited prediction of male fertility potential, in this work we evaluated the potential use of quantification of abundant seminal sRNAs as markers of assisted reproduction treatments outcomes. These molecules are key regulators of physiological and pathological processes with a great impact on reproductive function.
To accomplish our goal, we performed a prospective study with couples who underwent a ICSI treatments with donated oocytes at PROAR Medical Center between 2015-2019. Seminal samples from these normozoospermic men were collected and seminal $tiRNA^{Lys}$, $tiRNA^{Glu}$, $tiRNA^{Gly}$ and let-7 were quantified by RT-qPCR. The results obtained demonstrated that levels of all three tiRNAs but not let-7 are significantly elevated in seminal samples from cases with failed ICSI cycles, suggesting a potential association between increased seminal tiRNAs and unexplained male infertility.
To further characterize the role of seminal tiRNAs in male infertility, correlation studies were also carried out. Interestingly, they showed a negative association between them and seminal testosterone, highlighting the involvement of these molecules in male endocrinology. Our finding also suggest that these molecules could play a role in modulating male reproductive function in response to physiological stress since they showed significant associations with the degree of sperm DNA fragmentation in fertile men but not in cases with failed ICSI cycles where seminal cortisol levels are altered.

475. (369) **ORAL PROBIOTIC LACTOBACILLUS KEFIRI PREVENTS LIPOPOLYSACCHARIDE-INDUCED PRETERM BIRTH, REDUCES INFLAMMATION AND MODULATES VAGINAL MICROBIOTA IN PREGNANT MICE**

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Preterm birth (PTB) is a complication of pregnancy affecting 5-18% of all births worldwide. Represents 15 million babies born preterm each year with mortality rates 40 times higher than term babies. It is well known that infection-induced inflammation is a contributing factor. This process involves a progressive inflammatory cascade within gestational tissues accompanied by influx of leukocytes. We have reported that treatment with Lactobacillus kefiri CIDCA8348 (Lk48) prevented PTB (88%) in a lipopolysaccharide (LPS)-induced PTB mice model. Given this, we sought to elucidate the mechanisms behind Lk48 protective role in PTB induction.
C57BL/6 females were treated every 48h by oral gavage during a week with Lk48 or vehicle. Next, females were mated with BALB/c males. On gestational day (gd) 16, females were challenged with LPS. Tissue samples from uterus, decidua and placenta were harvest 18h after LPS. Leucocytes influx was evaluated by flow cytometry and histological examination by light microscopy was performed. The impact on vaginal microbiota was evaluated by a qualitative analysis by PCR-DGGE and a gene diversity analysis from vaginal samples. Treatment with Lk48, significantly lowered percentages of CD8+T cells as compared to control (t-test $p<0.01$). Percentages of Ly6G+ neutrophils were also significantly lower in the uterus and decidua of Lk48 mice compared to control (t-test $p<0.001$). Histology confirmed these findings. No sign of inflammatory response was

observed. Furthermore, Lk48 ameliorated changes in placental labyrinths circulation and thickening of interhemal membranes. DGGE profiles allowed differentiation of experimental groups in two separated clusters, suggesting that Lk48 induced changes in the vaginal bacterial community composition.
Our results show that Lk48 treatment prevents LPS-induced PTB in mice by reducing leukocyte infiltration and inflammation-induced damage in reproductive tissue as well as modulating the vaginal microbiota.

476. (403) ***CHLAMYDIA TRACHOMATIS* INFECTION OF THE MALE GENITAL TRACT. ASSESSMENT OF INFECTION PREVALENCE, UROGENITAL INFLAMMATION AND SPERM QUALITY**

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Chlamydia trachomatis (CT) is the most common sexually bacterial transmitted infection worldwide. Although CT urogenital infection and associated pathology in females has been widely described, the study of CT male urogenital infection has been neglected. Herein, we analyzed the prevalence of CT infection of the male genital tract (MGT), seminal levels of inflammatory biomarkers and sperm quality in male patients attending an Andrology clinic.
Semen samples were collected from 107 patients with symptoms of urogenital infection/inflammation or possible infertility. Assessments: uropathogens infections (PCR), spermiograms, leukocytospermia, leukocyte subpopulations (FACS), ROS and inflammatory cytokines seminal levels. Statistical analysis was performed by ANOVA.
CT infection was detected in 41/107 patients revealing a striking prevalence of 38.3%. Either CT mono-infection (26/41) or co-infection with other uropathogens (15/41) was not associated to significant alterations in semen quality (p>0.05). When classifying patients according to inflammatory biomarkers levels, 50% (13/26) of CT-infected patients showed increased seminal levels of inflammatory cytokines, leukocytospermia and reduced levels of morphologically normal sperm (p<0.05). Conversely, CT-infected patients with low levels of inflammatory cytokines in semen (13/26) showed reduced frequencies of CD4+ T cells and increased frequencies of B cells in semen. Strikingly, CT-infection associated with significantly reduced seminal levels of ROS and sperm apoptosis regardless of the state of inflammation (p<0.05).
Our results revealed a significantly high prevalence of CT infection of the MGT in young and middle-aged patients in our setting. Moreover, the infection associated to increased inflammatory biomarkers in semen in 50% of cases and only alters minor semen quality parameters. Altogether, our results indicates that although very prevalent, CT-infection of the MGT does not compromises semen quality.

477. (487) **TWENTY YEARS OF EXPERIENCE WITH PATERNAL IMMUNOTHERAPY: A ROLE AS AN ENHANCER OF THE IN VITRO FERTILIZATION**

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Unexplained Recurrent Spontaneous Abortion (URSA) is a common pregnancy complication that implies loss within the first 20 weeks of gestation. Immunological mechanisms seem to participate in their development. Unexplained infertility has also been associated with these mechanisms. Paternal immunotherapy (PIT) is used worldwide as treatment for URSA, but protocols differ mainly in amount of mononuclear cells, routes of administration, or single versus several doses. Herein we report results from the use of PIT in a cohort of 416 primary URSA, 92 secondary URSA and 298 infertile women. All patients followed a multiple intra-dermal injection of paternal mononuclear cells until detection of more than 50% blocking activity in a mixed lymphocyte reaction (MLR-Bf). Patients and controls were followed during 2 years since their first consultation. Statistical comparisons were done by two-tailed unpaired *t*-test and Fisher's exact test, considering differences significant when P<0.05. Primary URSA and infertile women showed a similar pattern achieving values of MLR-Bf above 50% after 3 PIT in 46% and 49% of patients, respectively. 60% of secondary URSA patients reached the desired value after only 3 injections. When analyzing outcome as succesful pregnancies, a live birth was present in 58% (n=242) of all primary URSA treated women vs. 23% in the control group (p< 0.0001, OR: 4.7, CI 95%: 3.3-6.6). Secondary URSA showed live births in 61% of patients (n=56) vs 28% in controls (p< 0.0001, OR: 4.0; CI 95%: 2.2-7.2). 33% of Infertile patients showed live births (n=99) vs 21% in controls (p=0.0009; OR: 1.9; CI 95%: 1.3-2.8). Analysis showed that best results were obtained in women between 20-35 years old. Treated women requiring IV fertilization became pregnant after 1.37±0.67 procedures (median: 1; range: 1-3) vs controls that required 2.75±0.84 (median: 3; range: 1-5) (p=<0.0001). These results confirm and extend the success of PIT for treatment of URSA and Infertile patients.

478. (514) **MALE GENITAL INFECTION BY HUMAN PAPILLOMAVIRUS (HPV): SEMEN QUALITY, CO-INFECTIONS PRESENCE AND INFLAMMATORY MARKERS**

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Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs) worldwide. The HPV infection in men, its fertility consequences and the presence of concomitant STIs, is largely unknown. This study aimed to characterize the local epidemiology of low-risk (LR) and high-risk (HR) HPV of young adult men from Cordoba, Argentina, further analyzing its relationship with other STIs, seminal quality and their immune response.
Semen and penile swabs specimens were obtained from 109 male patients attending to the andrology clinic. HPV detection and genotyping were performed by PCR-RFLP analysis. *C. trachomatis (Ct)*, *N. gonorrhoeae*, *T. pallidum*, *M. genitalium*, *T. vaginalis*, *M. hominis, U. urealyticum,* HSV1 and HSV2 were analyzed by PCR. Seminal plasma cytokines were quantified by ELISA and semen leukocytes analyzed by flow cytometry. Semen analysis was assessed according to the WHO guidelines. Non-parametric tests and Pearson›s Chi-square test were performed for statistical analysis.
Sixty-one percent of the samples were positive for at least one pathogen. An overall prevalence of HPV infection of 8% (9/109) was found. 56% of these positive cases were LR-HPV and 22% HR-HPV. Remarkably, HPV-6 was the most frequently detected genotype (44%). Other genotypes found were HPV-11, HPV-33 and HPV26/69/66. Strikingly, 67% of HPV+ patients were also positive for *Ct* compared to 37% of HPV- patients, but this difference was not statistically significant (*p*=0.081). No significant differences were found in semen quality, leukocytospermia and IFNɣ, IL-1β, TNF-α, IL-10, IL-17 or IL-6 levels between HPV+ and HPV- groups.
Our study describes the genotype-specific prevalence of HPV genital infection among young adult men from Cordoba, Argentina. No significant semen quality alterations or local inflammation markers were associated with HPV. The high prevalence of STIs found, highlight the need for its routine screening in this population.

479. (459) **ARYL HYDROCARBON RECEPTOR INVOLVEMENT**

IN THE TR1-LIKE CELL DIFFERENTIATION AND IL10 PRODUCTION INDUCED BY AFLATOXIN B_1 INDIVIDUAL AND COMBINED WITH FUMONISIN B_1.

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Co-exposure to aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) is frequent in nature, and has been associated with a high incidence of human hepatocellular carcinoma. AFB_1 toxicity is related with its metabolization by cytochrome P450 1A (CYP1A), which is induced by aryl hydrocarbon receptor (AhR) activation. AhR participates in immune tolerance control, regulating the differentiation of T cells to Tr1 or Treg regulatory T cells, among other mechanisms. Hence, this work was aimed to assess probable changes in Tr1-like cell differentiation due to AFB_1 and FB_1 exposures, and the AhR involvement in such effects. Spleen mononuclear cells (SMC) from mice with different AhR affinities (C57BL/6 and B6.D2N-Ahr^d/J; *background* C57BL/6), were incubated up to 72 h with AFB_1 (0, 5, 25 and 50 µM), FB_1 (0, 25, 125 and 250 µM) and both-toxin mixtures. Later, Tr1-like cell ($CD4^+$, $Foxp3^-$, $CD25^{low/-}$, $IL10^+$) percentages stimulated by TGFβ + IL27 and intracellular IL10 (flow cytometry in both cases), IL10 in culture supernatants (ELISA), and the CYP1A and AhR mRNA levels (qRT-PCR), were assessed.

AFB_1 and its mixtures increased CYP1A and AhR mRNA levels, being greater in the latter; whilst FB_1 did not produce changes. These data were correlated with the highest toxicities caused by the toxin mixtures. Tr1-like cell differentiation were raised by both, AFB_1 (25 µM) and FB_1 (125 and 250 µM), although in a AhR-dependent way, or independently of AhR and TGFB + IL27, respectively. AhR was also involved in the IL10 raise, which was altered only by AFB_1. However, the mycotoxin mixtures did not modify the Tr1-like cell percentage, although they decreased the IL10 production under basal and Tr1-stimulating conditions in an AhR-dependent manner. In conclusion, AhR is involved in the immunosuppression caused by AFB_1, and in the immunotoxicity induced by the AFB_1-FB_1 mixtures; but is not implicated in the FB_1 effects on Tr1-like cells.

SAFIS

BIOINFORMÁTICA, GENOMA, PROTEOMA, Y BLANCOS TERAPÉUTICOS

480. (347) **HOMOLOGY MODELING AND MOLECULAR DYNAMICS SIMULATIONS TO STUDY HUMAN AQP4 ISOFORMS**

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Aquaporin-4 (AQP4) is expressed at the plasma membrane as 2 isoforms, AQP4-M1 of 323 amino acids (aa) and AQP4-M23 of 301 aa. Recently, a new AQP4 isoform with a 29 aa C-terminal (Ct) extension (AQP4-Ex) generated by translational readthrough was described. Crystalized AQP4-M1 (32-254 aa) lacks N-terminal (Nt) and Ct ends. Our aim was to model AQP4-M1 and AQP4-Ex by homology modeling to study and compare their properties by molecular dynamics (MD) simulations. Human AQP4 sequences were obtained from UniProt (entry P55087). CBS-DTU tools were used for post-translational modification analysis. AQP4 crystalized structure was obtained from the Protein Data Bank (entry 3GD8) and each Nt and Ct ends were modeled with PEP-FOLD 3 (RPBS Web Portal). Peptides were linked to the 3GD8 AQP4 model to build AQP4-M1 and AQP4-Ex homotetramers by UCSF Chimera software. A 10 ns MD simulation was run in GROMACS 2019 for both isoforms embedded in a bilayer of lipid POPC molecules and solvated with TIP3P as a solvent model. AQP4-Ex (352 aa) had a 100% identity with AQP4-M1. Ct of AQP4-Ex had only two Serine residues (331 and 335) with high score for phosphorylation motif prediction. Homology modeling of AQP4-Ex showed that the extended Ct is a random coil. MD simulations evidenced that AQP4-Ex has a larger mean square displacement and radius of gyration as compared to AQP4-M1, indicating that AQP4-Ex would be less compact and stable. The distance from His201 to Arg216, representative of the selectivity filter (AQP4-M1: 4.32 ± 0.03 nm vs. AQP4-Ex: 4.27 ± 0.03 nm, n=4, ns), showed that water permeability of these isoforms should be similar. Bioinformatics tools allowed us to model both full-length AQP4 isoforms for the first time. MD simulations of AQP4-Ex provide valuable insights into its water permeation mechanism, which agree with recent experimental observations. This is a promising starting point for performing MD simulations to elucidate the function of this novel extended isoform.

481. (386) ***IN SILICO* PREDICTION OF POTENTIAL ACTIVITIES AND TARGETS OF THE SYNTHETIC FLAVONOID 2'-NITROFLAVONE**

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Flavonoids are polyphenolic compounds considered potential chemopreventive candidates for cancer treatment. We previously described that the synthetic flavonoid 2'-nitroflavone (2'NF) inhibits proliferation, survival and migration of 3 human triple-negative breast cancer cell lines. However, the mechanisms underlying these effects remain elusive.

Here, Molinspiration and SwissADME chemoinformatics software tools were used to evaluate 2'NF pharmacokinetics, drug-likeness and medicinal chemistry friendliness. Prediction of Activity Spectra for Substances (PASS) web tool was used to predict 2'NF biological activities. We also used SwissTargetPrediction, TargetNet, Random Forest QSAR, SEA and SuperPred web services for predicting 2´NF potential targets.

Results from Molinspiration and SwissADME software showed that 2'NF does not violate the "Lipinski´s rule of five" for drugs, has high gastrointestinal absorption and penetrates the blood-brain barrier. PASS tool predicted the following activities (Pa>0.7): inhibition of hypoxia-inducible factor 1-α expression, kinase functions, ubiquinol-cytochrome-c reductase and aldo-keto reductase family 1 C4; enhancement of TP53 and heme oxygenase-1 expression and modulation of cytochrome P450 (CYP1A1, CYP27A1, CYP7B1, CYP2E1) and complement anaphylatoxin (C3a/C5a) receptor activities. Moreover, poly [ADP-ribose] polymerase-1, tankyrase-1 and -2, androgen receptor (AR), aryl hydrocarbon receptor (AhR), estrogen receptor (ER), CYP1A2, CYP2C9, amine oxidase [flavin-containing] A and hepatocyte nuclear factor 4-α were simultaneously predicted by 3 web services as 2'NF potential targets.

Thus, *in silico* predictions revealed that 2'NF has a favorable pharmacological profile as a drug candidate. 2'NF anti-tumoral effects might be associated with apoptosis induction, modulation of CYP activities, oxidative phosphorylation, metabolism of steroid hormones and WNT, AR, AhR and ER signaling pathways, as well as potential anti-angiogenic action.

482. (450) **TEXT MINING ON INDEXED PUBLICATIONS ON LIFE SCIENCES SIGNED BY ARGENTINE AUTHORS**

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Europe PMC (ePMC) is an open science platform that allows access to worldwide Life Sciences publications, from trusted sources. The object of our study was the text of all scientific publications indexed in ePMC, signed by Argentine authors and published between 1955 and 2019 (inclusive). The objectives were: i) to statistically analyze the results of the search; ii) to discover, through data mining, non-explicit - often hidden - information structures and patterns in the text. The analysis was done through text mining (TM), developing auto-

mated workflows in the Knime software platform, and with semantic analysis tools as AntConc software. 75,294 articles were analyzed, published in 5,063 media, signed by 186,410 authors with workplace in Argentina or in collaboration with the country. We also worked with the text of 70,798 abstracts. TM allowed to extract information about journals, authors, and countries participating in the research, and the underlying information contained in titles and abstracts. The number of publications over time was correlated with Argentine economic parameters. The main publication media were detected, the number of authors signing each article was studied, and the countries sharing authorships with Argentina were analyzed. The pathologies that were mentioned in abstracts were detected; also the substances used for their treatment, grouping them by action site or by action mechanism. The topics that were especially covered by authors were found with unsupervised digital detection algorithms. The automated workflows (specially developed for this study) can be applied to other scientific or biomedical databases, to improve planification on research in prevention and treatment of illness. The unsupervised topic detection could serve to analyze decisions of authors on research subjects and to detect advances and deficits of an organized scientific policy.

483. (451) **EFFECT OF MEMBRANE COMPOSITION ON EPITHELIAL SODIUM CHANNEL (ENAC) AND AQUAPORIN 1 (AQP1) FUNCTION. ALL-ATOM AND COARSE-GRAINED MOLECULAR DYNAMICS APPROACH**

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ENaC is a major regulator of salt and water reabsorption in several epithelial tissues. AQP1 is a membrane integral protein with key role in transcellular water transport in response to osmotic gradients. Both proteins are present in vascular endothelial cells but their functions in this tissue would not be related to their canonical functions. Our *in vitro* experimental results strongly suggest that there is a relationship between them.
The characterization of lipid-lipid, lipid-protein and protein-protein interactions is a key factor in understanding the modulation effects that membrane lipids and proteins can have on each other. Using molecular dynamics simulations (MDS) as a "computational microscope" we can characterize the interactions of membrane proteins with lipids, matching, incorporating, and extending the information which may be obtained from experimental, structural and biophysical studies. Homology modeling of ENaC was carried out with the Uniprot sequence (P37088, P51168, P51170) with 6BQN as template on the Swiss-Model server. Subsequent refinement was carried out through MDS. The system consisted of a lipid bilayer of POPC:DOPC:DPPC:CHOL at different relative concentrations, the AQP1 tetramer (PDB code 4CSK) and the modeled ENaC, in the presence of TIP3P type water molecules. It was prepared using the CHARMM-GUI Web-based graphical interface and CHARMM36 force field. The MDS were performed with GROMACS 2019 package and GROMACS tools were used for analysis of the following bilayer properties: the surface area per lipid, compressibility modulus, mass density profile, two-dimensional density maps of lipid distribution and lateral diffusion coefficients. The bilayers properties were used to correlate the permeability of ENaC and AQP1 on electrophysiology models with the GROMACS package. Additional studies involved the molecular docking technique to analyze the possible binding modes of aldosterone with ENaC and its influence on channel functionality.

484. (454) **GPP SGRNA DESIGNER VS CHOP CHOP: IMPLICATIONS OF THE GENOMIC CONTEXT IN CRISPRA SGRNA DESIGN FOR ANGIOGENIC GENES.**

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Introduction: Angiogenic gene overexpression has been the main strategy in numerous cardiovascular regenerative gene therapy projects. However, most have failed in clinical trials, due to, among other reasons, dose inadequacy and lack of potency. CRISPRa technology enhances gene overexpression levels based on the identification of sgRNAs with maximum efficiency and safety. GPP sgRNA Designer and CHOP CHOP are the most widely used platforms for the prediction of sgRNAs, the scope of efficiency and sensitivity of their algorithms being partially uncertain. The objective of our study was to analyze the performance of GPP sgRNA Designer and CHOP CHOP for the design of sgRNAs in a panel of angiogenic genes, in a changing genetic context such as successive versions of the human genome.
Materials and methods: The top 20 ranked sgRNAs were provided by GPP sgRNA Designer (GPP) and CHOP CHOP (CHOP) for each of the following VEGFA, KDR, EPO, HIF-1A, HGF, EGF, PGF, FGF1 genes from both GRCH 37 and GRCH 38 human genomes.
Results: The mean ranking variation in the 20 positions was greater for GPP than CHOP in EPO ($p < 0.05$), EGF ($p < 0.005$), HIF-1A ($p < 0.005$), PGF ($p < 0.001$) and HGF ($p < 0.001$), whereas it did not reach statistical significance in KDR, FGF-1 and VEGFA (Wilcoxon Test). Accordingly, the global accumulative change analysis for all genes was significantly greater with GPP than CHOP (14.5±8.6 vs 4.05±2.28 AUC, $p < 0.001$, paired t-test). The rearrangement analysis of ranking positions was clearly different between platforms (GPP:-0.3187±0.2698 vs CHOP:-0.0437±0.0563, $p < 0.05$, paired t-test).
Conclusion: GPP sgRNA Designer proved to be more sensitive in establishing the best sgRNAs in relation to genomic context modifications. Second, CHOP CHOP shows a narrower classification reordering. Therefore, GPP exhibits the best performance in sgRNAs design for a panel of angiogenic genes.

485. (515) **DATA PROCESSING FOR COMPARISON OF GENE EXPRESSION LEVELS FROM RAW DATASETS USING FREE SOFTWARE R IN HEPATOCELLULAR CARCINOMA PATIENTS.**

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Many datasets on mutations, changes in the number or expression levels of genes, as well as clinics are now available for different cancers. Thus, organization in data banks, with comparison and visualization tolos, is growing and useful for biomedical research. Moreover, the use of raw data has a great potential for specific biostatistical studies.
Hepatocellular carcinoma (HCC) is the second most lethal cancer and it lacks effective therapy. Data analysis from patient databases can be useful to identify molecular markers, targets for potential treatments, and to investigate hypothetical mechanisms.
The aim of this study was to process raw data from the "The Cancer Genome Atlas-Liver Hepatocellular Carcinoma" project (TCGA-LIHC) dataset to analyze them with the free software for statistical computing R, and to compare the expression levels of genes involved in an antimigratory AMPK-p53 axis that we study at the cellular level: the different genes for the three AMPK subunits (PRKAA1, A2, B1, etc.); TP53; and EMT transcription factors SNAI1 and 2, which are p53 targets.
First, bioinformatic analyses of TP53 mRNA expression with open source online tools showed an increase in the overall survival in patients with low versus high TP53 expression: 55.1 months versus 61.7 months, respectively (P=0.03). Besides, TP53 showed mutations (61% missense and 31% truncating) in 30.5% of patients.
To better analyze mRNA levels, the whole raw data was organized with R, leaving only those of the genes of interest, separating in samples of healthy and tumor tissue and matching those corresponding to the same patient. mRNA (RNA-seq Illumina) data were selected and compared. As an example, the median value of mRNA levels for SNAI2 increased from 144.7 in healthy to 205.7 in tumor tissue (P=0.01, Wilcoxon Signed Rank Test).
This methodology can be systematized to compare gene expression

in HCC patients with respect to non-tumor tissue, and association in their changes can also be analyzed.

CARDIOVASCULAR Y RESPIRATORIO

486. (49) **SUBCELLULAR DISCORDANT ALTERNANS IN CARDIAC MYOCYTES: CHARACTERIZATION AND SUSCEPTIBILITY TO PHARMACOLOGICAL RYR2 MODULATION**

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Objectives: To characterize discordant calcium release in ventricular cardiomyocytes, and evaluate the impact of pharmacological inhibition of Ryanodine receptors (RyR2) on the occurrence and magnitude of discordance.
Materials and methods: Ventricular cardiomyocytes were obtained from wistar rats (8-12 weeks old) after heart excision and enzymatic digestion. Cells were loaded with Fluo-4 and line-scanned in a confocal microscope to detect cytosolic Ca.2+ Pharmacological inhibition of RyR2 was tested with 5 μM of both VK-II-86 and Dantrolene. Tree groups of cells (control, VK-II-86 and dantrolene) were field stimulated at 1 and 5 hertz. After 1 minute at each frequency electrical pacing was stopped to record spontaneous Ca2+ release (Ca2+ waves).
Results: We found previously undescribed features of subcellular discordant alternans such as its reversibility after returning to basal pacing frequency and its instability promoted by the recruitment of out-of-phase Ca2+ release zones by its neighbors. A discordance index (DI) is proposed (Standard deviation of local alternans ratio) and correlated with the degree of negative staircase with a Pearson r of -0.66 (n= 15).
The equipotent RyR2 inhibition obtained with VK-II-86 and dantrolene was evident when Ca2+ waves frequency was significantly reduced compared to control (ANOVA with tukey post-test). At both basal and high pacing frequency only VK-II-86 promoted a significant increase of DI (n=14-22 per group. ANOVA with tukey post-test).
Conclusions: Subcellular discordant alternans are a reversible process induced by rapid pacing and can be characterized with a Discordance index which correlates with transient amplitude-frequency relationship and is sensitive to pharmacological RyR2 modulation. Interestingly, differential response to VK-II-86 and dantrolene was observed, suggesting that RyR2 modulation and prevention of Ca2+ waves occurrence can be reached with normal or high chance of promoting discordant Ca2+ release.

487. (67) **APELIN IS A MEDIATOR OF THE MYOCARDIAL ADAPTATIONS INDUCED BY SWIMMING ROUTINE**

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Introduction. Apelin is a short-length peptide that increases in response to exercise training in the myocardium of spontaneously hypertensive rats (SHR) improving cardiovascular vasodilation, antioxidant capacity, and mitochondrial function. However, the molecular mechanisms underlying these actions have not been elucidated yet. We hypothesize that aerobic training, through the apelin pathway, may attenuate oxidative stress and mitochondrial structural and functional alterations in the hypertrophied myocardium of the SHR. **Aim**. To explore whether a swimming routine or the exposure to exogenous apelin produces adaptations in the SHR myocardium. **Methods.** The experimental group ?SR? performed an 8-week swimming routine and was compared with non-trained group, ?NT?. Results were expressed as mean±SEM and analyzed by Student's t test or ANOVA, as appropriate ($p<0.05$). **Results.** Swimming training increased both apelin and APJ mRNA (apelin: SR: 121.7 ± 3.3 vs NT: 100 ± 5.3; APJ: SR: 117.8 ± 5.6 vs NT: 100 ± 5.29), and APJ protein expression (%, SR: 211.2 ± 38.3 vs NT: 100 ± 3.1) in the myocardium. The mitochondrial membrane potential (DYm) was less depolarized in response to training compared with NT (mV, SR: -173.04± 2.09 vs -155.13± 6.36). Exposure to exogenous apelin increased myocardial superoxide dismutase activity (U/mg, 59.9±5.19 vs C: 41.6±3.07) and reduced reactive oxygen species production in isolated cardiomyocytes (%, 74.7±6.8 vs C: 100±9.14). The H_2O_2-induced drop of DYm in isolated cardiomyocytes from NT was prevented by 10 min-apelin exposure (0.98±0.025 vs. control: 0.83± 0.025), effect canceled by the APJ antagonist, ML221, (0.82±0.071). **Conclusions.** The swimming routine seems to up-regulate the apelinergic pathway in the myocardium of SHR. The effects produced by exogenous apelin promoting antioxidant capacity and probably improving the mitochondrial function resembles aerobic training adaptations.

488. (76) **ADIPOSE TISSUE'S METABOLIC MODIFICATIONS IN RESPONSE TO AEROBIC TRAINING IN SPONTANEOUSLY HYPERTENSIVE RATS**

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There is an association between hypertension (HT), insulin resistance (IR), and oxidative stress (OE). In previous studies, we have demonstrated the presence of IR and increased EO in spontaneously hypertensive rats (SHR). Also, it is known that physical training delays the progression of vascular disease and improves insulin sensitivity. These effects possibly involve modifications in adipose tissue (AT).
Objective: to determine the effects of aerobic training (AE) on IR, OE and AT phenotype in SHR.
Materials and methods: SHR were randomly assigned to a sedentary group (S) and another one subjected to a swimming routine for 8 weeks (Ex). Oxidative stress was determined by T-Bars and O_2^- production measured by the lucigenin method. To study the insulin response, Glut4 expression and phosphorylation were measured in adipocyte membrane fractions and a glucose tolerance curve (GTC) was performed. To determine changes in the phenotype of AT, histological sections were analyzed by light microscopy. Results are expressed as mean ± SE and statistically analyzed by T-test, considering $p<0.05$ as a significant difference.
Results: AE decreased the OE determined by O_2^- production (23.12 ± 11.27% S vs. Ex), accompanied by a downward trend in T-Bars. On the other hand, AE increased the insulin- induced translocation of pGlut4 (46.29 ± 21.44% (6), while no effect was observed in S(2). This effect correlated with a decrease in the area under the GTC curve (29.59 ± 9.58 compared to group S). Finally, a transition from unilocular to multilocular AT was observed in group Ex, although the results obtained so far, did not reach statistical significance.
Conclusion: we propose that AE is an effective strategy to reverse metabolic parameters that are altered in a murine HT model, through mechanisms involving changes in AT.

489. (78) **DECREASED CA^{2+} LEAK FROM THE SARCOPLASMIC RETICULUM (RS) PROTECTS FROM EARLY DEVELOPMENT OF PATHOLOGICAL CARDIAC HYPERTROPHY**

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Abnormal Ca^{2+} release from the SR, associated with Ca^{2+}-calmodulin kinase II (CaMKII)-dependent phosphorylation of RyR2 at Ser2814, has been linked to cardiac diseases, such as pathological cardiac hypertrophy (PCH) and its progression to heart failure. The increase in SR Ca^{2+} uptake by phospholamban (PLN) ablation (PLNKO), to restore the decrease in SR Ca^{2+} load produced by RyR2 phosphorylation, ameliorates SR Ca^{2+} handling but exacerbates cardiac disease.
In the present study, we tested the hypothesis that the abrogation of CaMKII-induced Ca^{2+} loss by RyR2, induced by CaMKII phosphorylatable site mutation (S2814A), protects the heart from RS Ca^{2+} overload produced by PLN ablation and delays the development of PCH.
We used hearts from 10 weeks old WT, S2814A, PLNKO mice and a crossbreed strain of S2814A and PLNKO generated in our laboratory, named SAKO. WT and SAKO strain were subjected to transverse aortic constriction (TAC), an experimental surgical model for pressure overload-induced PCH, known to be associated with CaMKII overexpression.
Cardiac hypertrophy parameters (transthoracic echocardiography) were studied one month after the intervention. SAKO mice were monitored up to 180 days.*$p<0.05$ according to paired t-Student test.
Our data demonstrated that PLNKO mice had higher contractility, increased relaxation and SR Ca^{2+} load, while S2814A mice have a contractile behavior similar to WT.SAKO mouse myocytes showed an increase in RS Ca^{2+} load under basal condition.
Moreover, WT mice developed CPH after 1 month of TAC (LVMI: d0: 3.26-0.23 vs d30: 4.47-0.37*; n=20), while SAKO mice only showed an increase in LVMI 180 days after TAC (d0:3.78-0.28; d30:4.88-0.43 and d180:5.30-0.35*; n-6).Sham mice hearts showed no change.
The results allowed us to suggest that, in spite of sustained increase of SR Ca^{2+} uptake, prevention of diastolic RS Ca^{2+} loss during pressure-overload insult is sufficient to protect the heart by delaying maladaptive cardiac mass remodeling.

490. (97) **HEART CARDIOLIPIN CONTENT IN THYROID DISORDERS**

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Several studies have pinpointed that induced thyroid dysfunction leads to cardiac and metabolic disturbances and might result in long-lasting effects upon cardiovascular function in rats. Functional mitochondrial network is crucial to preserve cell viability and cardiac performance in response to thyroid disorders. Located in the inner mitochondrial membrane, cardiolipins are vital for the optimal function of numerous membrane proteins, playing an important role in energy metabolism. There is evidence of the regulating effect of thyroid hormones on cardiac lipidic profile. Recently, it has been reported that IMM cardiolipin levels affect calcium handling by regulating MCU stability and abundance. The aims of this study were to examine: (1) whether thyroid hormones affect cardiac function by altering cardiac calcium handling in young rats and (2) whether these thyroid disorders alter heart mitochondrial cardiolipin.
Male Sprague–Dawley rats weighing approximately 50g were randomly assigned to one of the experimental groups: (1) euthyroid rats (received SC injections of 0.9 NaCl (0.1 ml/100 g body weight), (2) hyperthyroid rats (received SC injections of T3, 20ug/100g body weight) and (3) hypothyroid rats (received 0.02% methimazole in drinking water). T3 and methimazole treatments lasted 60 days. Animals were sacrificed by cervical dislocation and hearts were rapidly excised. Cardiomyocytes from each group were isolated by collagenase-based enzymatic digestion. Free cytosolic Ca2+, sarcoplasmic-reticulum Ca2+ content, cardiomyocyte shortening and relaxation measurements were performed. Cardiolipins were isolated and identified by TLC. Cardiolipin quantification was carried out by measuring the levels of free orthophosphate (Pi) using the Bartlett's technique.
Contractility alterations induced by thyroid disorders were associated with altered free cytosolic and sarcoplasmic-reticulum Ca2+ content. While hypo rats showed a reduced contractility associated with a reduced intracellular Ca2+ content, hyper rats exhibited an increased contractility correlated with higher levels of Ca2+. Hormonal imbalances also affected cardiomyocyte relaxation as revealed by alterations in both time to 50% relengthening and time to 50% Ca2+ decay. While hypo rats showed an impaired relaxation associated with an increased time to 50% Ca2+ decay, hyper rats exhibited an increased relaxation correlated with a faster Ca2+ decay. The number of spontaneous releases per minute was significantly increased in cardiomyocytes of both hypo and hyper rats. Cardiac cardiolipin content was reduced in hypothyroid rats but it was increased in hyperthyroidism.
Our results confirmed that thyroid disorders affect cardiovascular performance parameters such as contractility and heart relaxation time. Furthermore, cardiomyocytes isolated from both hypo and hyper rats had an enhanced proarrhythmogenic substrate. This could be partially attributed to an increased Ca2+ leaking from the mitochondria associated with a phospholipidic imbalance. Increased cytosolic calcium levels could activate electrogenic NCX which might generate a depolarizing current resulting in DADs and arrhythmias. Maintenance of euthyroidism is fundamental to preserve cardiac performance. An imbalance in phospholipidic profile of the mitochondrial membrane such as cardiolipin, is related to defects in the mitochondrial and cardiac function. T3-dependent cardiolipin signals contribute to the maintenance of mitochondrial homeostasis and involved Ca+2 handling.

491. (102) **EFFECTS OF CANNABIDIOL ON INFARCT SIZE AND POSTISCHEMIC MYOCARDIAL DYSFUNCTION: MECHANISMS INVOLVED**

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Background: Cannabidiol (CBD) is a non psychoactive phytocannabinoid with recognized anti-inflammatory activity. Our aim was to determine the effects of acute treatment of CBD on myocardial postichemic alterations and the mechanisms involved.
Methods: Isolated Wistar rats hearts were isovolumically perfused through Langendorff system with Ringer´s solution (pH=7.4, 37°C) and paced at 280 ± 10 beats/min. After 20 min of stabilization, the following experimental protocols were performed: Non-ischemic control (NIC):110 min of perfusion; Ischemic control (IC): 30 min of normothermic global ischemia and 60 min of reperfusion (R);CBD group: 0.25μM CBD was administered during the first 10 min of R. Infarct size (IS) was determined by TTC staining. Systolic function was assessed by left ventricular developed pressure (LVDP) and $+dP/dt_{max}$ and diastolic function by left ventricular end diastolic pressure (LVEDP) and $-dP/dt_{max}$. The expression of phosphorylated forms of eNOS, PKCε, Akt and the content of cannabinoid receptor 2 (CB2) were determined by western blot.
Results: CBD significantly decreased IS (7 ± 1 % vs. 31 ± 2 % in IC) and improved the post-ischemic recovery of myocardial function. At 60 min of R, LVDP was 56 ± 8 % and $+dP/dt_{max}$ 55 ± 8 % vs. 17 ± 3 % and 15 ± 4 % in IC, respectively; LVEDP = 18 ± 3 mmHg vs. 52 ± 4 mmHg in IC; $-dP/dt_{max}$ = 58 ± 9 % vs. 14 ± 4 % in IC. The expression of P-eNOS and P-Akt decreased approximately 30% of NIC value (considered as 100 %) in IC and increased approximately 60% in CBD group. The expression of P-PKCε decreased approximately 50% in IC and increased a 40% in CBD group. The content of CB2 receptors diminished 30 % in IC hearts and increased 20 %

in CBD treated hearts.
Conclusions: The data demonstrate that CBD reduces the cell death and systolic and diastolic post-ischemic dysfunction induced by ischemia-reperfusion. These beneficial actions appear mediated by Akt/PKCε/eNOS-dependent pathways through CB2 receptors.

492. (129) **CARDIAC NBCe1 OVEREXPRESSION EXERTS A DIRECT EFFECT ON VENTRICULAR ELECTRICAL ACTIVITY**

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Introduction: Intracellular pH maintenance in cardiomyocytes is crucial for the correct function of electrical activity and contractility. Prevention of acidosis relies on two sarcolemmal acid extruders: Na^+/H^+ exchanger and Na^+/HCO_3^- cotransporter (NBC). There are two known cardiac isoforms of NBC, an electroneutral NBCn1 and an electrogenic NBCe1, ($1Na^+:2HCO_3^-$). We have previously demonstrated in rat and cat ventricular myocytes the existence of an anionic current generated by NBCe1 that contributes to the cardiac action potential shape and duration.
Aim: The principal aim of this research was to determine the contribution of NBCe1 in heart electrophysiology in an *in vivo* mouse model with NBCe1 overexpression.
Experimental design: We attempted NBCe1 overexpression by using a cardiotropic adenoassociated virus vector (AAV9-NBCe1) delivered through a retro-orbital injection in male 3 months old mice and then performed a series of studies to assess ventricular electrical activity including patch clamp technique on isolated cardiomyocytes and electrocardiogram (ECG), using an AAV9-mCherry as control.
Results: We performed ECG studies on both mice groups before and after 28 days virus injection and we found a significant reduction in QTc interval duration of AAV9-NBCe1 injected mice compared to control, that was achieved after 14 days of treatment and sustained through time (ms; AAV9-mCherry: 61.2±2.4,n=5; AAV9-NBCe1*:53.8±0.5, n=6; *p<0.01 vs AAV9-mCherry). Moreover, action potential duration at 50% of repolarization (APD50) was also significantly reduced on these isolated mice ventricular cells (ms; AAV9-mCherry: 17.7±3.2, n=6; AAV9-NBCe1*: 10.4±1.5, n=9; *p<0.05 vs AAV9-mCherry). No signs of cardiac hypertrophy were found in any of studied animals.
Conclusion: We have demonstrated for the first time that *in vivo* cardiac NBCe1 overexpression has a direct impact on heart electrophysiology by decreasing APD of ventricular cardiomyocytes and reducing QTc interval duration of ECG.

493. (215) **INTERACTION OF ZINC WITH AN ANGIOTENSIN RECEPTOR ANTAGONIST. IMPROVEMENT OF THE AT1R BLOCKADE AND THE ANTIHYPERTENSIVE ACTIVITY**

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Structural modification of drugs is a strategy for improving their pharmacological properties. Candesartan (Cand) is an angiotensin receptor antagonist widely used for hypertension treatment. We have previously modified its structure by the addition of Zinc (ZnCand), an essential trace element that contributes to the maintenance of cell redox balance.
In this study, we analyze the antihypertensive effects and cellular mechanisms of action of ZnCand in comparison to Cand.
For *in vivo* experiments, SHR male rats were treated with 10-13 mg/kg/day Cand or ZnCand for 6 weeks and the systolic blood pressure (SBP) was measured. For hypertrophy response, rats were monitored echocardiographically, then hearts (HW) and body (BW) were weighed to calculate HW/BW, LVMI (left ventricular mass index) (LVMI) and LVM/TL (TL: tibial length). For *in vitro* procedures, transfected HEK293 cells with the angiotensin type 1 receptor (AT1R) were coincubated with 0.1, 1 and 10 μM of Cand or ZnCand and TAMRA-Ang II, for competitive binding assay, or Ang II for ROS and calcium determinations.
ZnCand produced slightly higher SBP reduction (33.5±2.9%, p<0.05) than candesartan (23.03±3.5%, p<0.05) as well as the reduction of HW/BW (12.11±0.11%. p<0.05), LVMI (14.76±0.29%, p<0.05) and LVM/TL (15±0.61%, p<0.05).
The competitive assay showed, at 10 μM, ZnCand, a higher binding effect on the AT1R (97.93±0.39%, p<0.05) in comparison with Cand (81.13±1.5%, p<0.05). Besides, ZnCand decreased ROS (87.58±1.3%, p<0.05) and inhibited the calcium intracellular flux (62.75±3.6%, p<0.05) more effectively than Cand (20.98±4.35% and 44.12±4.32%, p<0.05, respectively).
We demonstrate that the introduction of zinc in the structure of candesartan improved its effect on SBP reduction through a strong interaction with the AT1R, ROS inhibition and decrease in calcium release. Besides, ZnCand regressed the hypertrophy. We conclude that this drug might be a candidate for the treatment of hypertension.

494. (225) **CARDIOMYOCYTES ACUTE EXPOSURE TO HIGH GLUCOSE PROMOTE ARRHYTHMOGENIC EVENTS INDUCED BY CAMKII**

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The regulation of glycemia is disrupted under certain metabolic conditions as impaired glucose tolerance, metabolic syndrome and diabetes mellitus; or even stress context, i.e. surgery, cardiac infarct. Given that arrhythmias and calcium (Ca2+) mishandling due to, in part, Ca2+-Calmodulin Kinase II (CaMKII) hyperactivation, are present in prediabetic and diabetic hearts we hypothesized that an acute increase in glycemia causes Ca2+ handling abnormalities capable to trigger arrhythmogenic events in a CaMKII dependent pathway.
We used isolated mice cardiomyocytes from wild type (WT) and S2814A, (which have the S2814 residue of RyR2 mutated to A, unphosphorytable by CaMKII), loaded with Fura 2-AM to evaluate intracellular Ca2+ handling by epifluorescence, Langendorff perfused hearts to measure developed pressure (DP) and monophasic action potential (MAP) and hearts homogenates to perform [^{3}H]Ryanodine binding assay and western blot to evaluate ryanodine receptor 2 (RyR2) activity and activation.
The change from normal glucose (NG) buffer (11 mM, 325.61mOsm) to high glucose (HG) buffer (25 mM, 339.26 mOsm) induced an increased amplitude of Ca2+ transient (CaiT, 72.59±17.29% of NG, p=0.001) and 73.30% of cells presented arrhythmogenic events during HG perfusion. To prove that it was not an osmolarity effect we use a buffer supplemented with sucrose or choline chloride in the same proportion. The change in osmolarity by sucrose or choline chloride did not produce any effect either in CaiT or rhythmicity. The effects of HG were prevented by treatment with a specific CaMKII inhibitor; AIP (CaiT 5.56±3.89% of GN+AIP) and only 35.0% of cells presented arrhythmogenic events, and in S2814A mice myocytes (CaiT 23.99±7.39% of NG and arrhythmogenic events 27.27%). Moreover, HG perfusion in the hearts increased DP (34.29±10.02%) and produced ectopic beats with respect to NG perfusion. HG [^{3}H] Ry binding assays showed a significant increase in RyR2 sensitivity (HG; 1.05±0.27 vs NG; 2.30±0.38 μM) and maximal activity (HG; 48.09±2.35 vs NG;31.58±1.98). We conclude that acute administration of HG induces changes in Ca2+ handling and arrhythmic events, dependent on RyR2 activation by CaMKII.

495. (258) **DAM EARLY FREE ACCESS TO HYPERTONIC NACL SOLUTION INDUCES A LONG-TERM EFFECT ON BLOOD PRESSURE RESPONSE OF ADULT OFFSPRING.**

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Osmoregulatory mechanisms can be vulnerable to electrolyte and/or endocrine environmental changes during the perinatal period, affecting their responses even in adulthood when the stimulus has disappeared. The aim of this study was to evaluate whether the availability of hypertonic sodium chloride solution during the perinatal period may induce a differential programming on cardiovascular responses of adult offspring after an osmotic challenge.
We studied Wistar rats, with a perinatal manipulation (M) that covered dams from 1 week before conception until the 28 postnatal days (PN) of the offspring. The experimental groups were: -M-NaCl: Free access to 0.45M NaCl solution, food and water); and -M-Ctrol: Free access to food and water. We evaluated in male and female adult offspring (60PN) the changes (Δ), in the mean arterial pressure (MAP), the heart rate (HR), and diastolic-systolic arterial pressure (DAP and SAP) induced by 2M NaCl infusion.
The repeated measures ANOVA indicated that both, M-Ctrol and M-NaCl males increased the MAP ($F7.133=36.786$; $p<0.001$) after 2 M NaCl. However, M-NaCl male offspring kept the MAP significantly higher than the M-Ctrol during 25 min post-infusion ($F7.133=3.007$; $p=0.006$); this pattern was also observed in DAP ($F7.119=4.038$, $p=0.001$). In females, the 2M NaCl infusion induced an equal increase of MAP in both groups (M-Ctrl and M-NaCl). The analysis of ΔHR in response to ΔMAP induced by 2M NaCl infusion showed a significant effect of perinatal programming, thus, M-NaCl females present a greater bradycardic response than M-Ctrol females (r=-0.80 vs r = -0.57; p=0.04); showing also a significant effect of ΔHR at 18 mmHg increase (t_6= 3.44, p = 0.014).
The data indicate that the availability of a rich source of sodium during the pre/postnatal period induces a long-term and dimorphic effect on sodium overload induced cardiovascular responses of adult offspring.

496. (277) **NOVEL EFFECT OF CANNABIS SATIVA OIL ON THE MYOCARDIUM OF HYPERTENSIVE RATS**

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Cannabis sativa oil has been used for different medical purposes like pain relief or refractory epilepsy treatment. Its main pharmacological components are Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD, through the endocannabinoid-system receptors (CB1 and CB2) has cardioprotective effects against inflammatory and oxidative damage. However, the effect of THC and CBD on hypertensive cardiac hypertrophy (CH) remains unclear. In CH dysfunctional mitochondria may produce deleterious effects on heart function. Thus, we tested the hypothesis that activation of the endocannabinoid system attenuates CH and improves myocardial mitochondria function in spontaneously hypertensive rats (SHR). To this aim, 3-month old male SHR were randomized into treated (TR) (N=4) and control (CR) (N=4). Cannabis sativa oil was orally administered to TR for 1 month. Data are presented as mean±SEM and Welch?s t-test was used for statistical differences ($p<0.05$). CH, determined by left ventricular weight/tibia length ratio, was reduced by treatment (mg/mm, TR: 28.28±0.58; CR: 32.31±1.1, p<0,05). Analysis of cardiac ultrasounds at the beginning and end of treatment showed in TR: 24.7% reduction in LV mass ($p<0,05$) and a significant decrease in LV wall thickness (from 1,85 ± 0,01 to 1,58 ± 0,02) without changes in LV diastolic dimension and arterial pressure. Mitochondria membrane potential was improved by treatment (in mV, TR: -165.9±3.05; CR: -150.6±4.47, p<0,05). Based on these results we propose that a 1-month treatment with Cannabis sativa oil in SHR is effective to reduce CH and improve the mitochondrial membrane potential, possibly traducing into better mitochondrial function.

497. (456) **CELLULAR MECHANISMS UNDERLYING THE LOW CARDIOTOXICITY OF ISTAROXIME**

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Pharmacological Na+/K+ ATPase (NKA) inhibition induces Ca2+ calmodulin dependent kinase II (CaMKII)-dependent cardiomyocyte death and arrhythmias. Istaroxime is a novel inhibitor of NKA with low risk of Ca2+ triggered arrhythmias due to its additional capacity to accelerate Ca2+ uptake via sarcoplasmic reticulum Ca2+ ATPase (SERCA). We aim to test if therapeutic concentrations of istaroxime impact cardiomyocyte viability and to gain insight into its mechanisms.
2 μM ouabain and 10 μM istaroxime promoted an equivalent inotropic effect by perfusing rat ventricular myocytes and measuring cell shortening (T test. n= 15 and 23 respectively).
Cell viability was evaluated by morphological parameters after 24 hours of culture at 37ºC in the absence and presence of 2 μM ouabain and 10 μM istaroxime (percentages of cell viability: Control 52 ± 2.5%; Ouabain 2 μM 33 ± 3%; Istaroxime 10 μM 46 ± 3% respectively. ANOVA with Tukey post-test. n=7 per group).
The apoptotic index BAX/BCL-2 and CAMKII activity were quantified by western blot in homogenates of rat cardiomyocytes incubated during 1 hour in control, ouabain 2 μM or istaroxime 10 μM. In contrast to ouabain, istaroxime did not promote significant CaMKII activation or cardiomyocyte apoptosis (ANOVA with tukey post-test. N=4 per group)
We explored diastolic Ca2+ release by confocal microscopy in Fluo-4 loaded myocytes after incubation with the indicated drugs. In contrast to ouabain, istaroxime did not significantly increase Ca2+ spark and wave frequency, but increased the proportion of aborted Ca2+ waves. This lower Ca2+ wave incidence remains present in cells from PLB-KO mice, suggesting that relief on PLB-dependent SERCA inhibition is not the only mechanism underlying istaroxime`s low arrhythmogenesis as previously suggested.
We conclude that istaroxime reaches a significant inotropic effect without inducing CaMKII-dependent cardiomyocyte death, and new insights are provided to explain low arrhytmogenesis.

498. (521) **EVALUATION OF MOTOR CAPACITY AND CARDIAC PERFORMANCE IN A PARKINSONISM MODEL IN DROSOPHILA MELANOGASTER**

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Although Parkinson Disease (PD) is characterized by motor disorders, it has been described that some of these patients exhibit cardiac perturbations. However, the relationship between the pathophysiology of PD and cardiovascular complications remains unclear. This work aims to evaluate the survival, motor capacity, and cardiac performance in a model of parkinsonism in Drosophila melanogaster that harbors a loss of function microdeletion in the DJ-1-encoding gene. The absence of DJ-1 induces early-onset PD in humans.
Canton-S flies (control) and parkinsonism model flies (park) were used. We carried out survival analysis and motor ability assessment by climbing tests. Heterozygous individuals of control and Park flies expressing the reporter protein GFP in cardiac and pericardial cells (GFP flies) were used for heart contractility assessments by confocal microscopy. Heart rate and arrhythmicity index (AI) were evaluated in 8 and 40 days-old flies. Survival curves were obtained using

the Kaplan?Meier method and analyzed using the log-rank (Mantel-Cox) test. Student?s t-test (two-tailed) was utilized for comparison between two groups and ANOVA followed by post hoc Tukey?s test was utilized for comparison among three or more groups.
The lifespan of control and park flies did not differ significantly. However, the climbing ability of the park flies decreased with age, especially after day 25. Heart rate in 8 days-old park flies was lower than in aged-matched control flies. Age-dependent reduction in heart rate was very similar in both groups. The AI only increased in 40-day park individuals. The described alterations in these parameters do not seem to be associated with alterations in the activity of proteins involved in intracellular calcium handling, e.g. SERCA and NCX.
Conclusions: individuals with parkinsonism show decreased motor capacity and manifest changes in heart rate and increased arrhythmogenesis.

499. (538) **CARDIAC EFFECTS OF NICOTINE EXPOSURE IN DROSOPHILA MELANOGASTER ARE MEDIATED BY SUBUNITS ALPHA1 AND 7 OF THE NICOTINE RECEPTOR**

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The relationship between substances consumed by humans and their impact on health has been explored in different animal models in an attempt to deepen on mechanisms of absorption, distribution, metabolization, and effects exerted in the organism. Drosophila melanogaster has been widely used as a model to study the pathophysiology induced by several substances, e.g. nicotine. This work aims to provide information about the effects of this component of tobacco in the heart. We explored the genetic relevance of the two subunits (α1 and α7) of the nicotine receptors in the cardiac response of Drosophila melanogaster exposed to nicotine. We evaluated intracellular calcium handling, the phenomenon underlying of heart performance, by means of a cardiac targeted genetically-encoded reporter system, TinC-Gal4-UAS-GCaMP3. Seven-days-old adult flies expressing a siRNA targeted to the α1/7 subunits of the nicotine receptor were compared to wild-type individuals to evaluate the impact of subunits downregulation. A pulse of nicotine was spiked to the semi-intact preparation of the heart (1.69 mM, final concentration) to exert acute effects on heart activity.
The addition of nicotine incremented the heart rate (89.14±9.36 vs 107.86±11.18 beats/min n=9-10) and accelerated the maximal velocities of contraction and relaxation (dΔF/dtmax: 0.164±0.02 vs 0.213±0.02; -dΔF/dtmax: -0.118±0.015 vs -0.181±0.015 n=9-10) and relaxation time in wild-type flies (0.187±0.025 vs 0.12±0.004 n=8). These effects were abolished when α1/7 subunits of the nicotine receptor were downregulated. Paired Student´s t-test was used for statistical analysis, where p values<0.05 were considered statistically significant.
These results suggest that nicotine´s effect on cardiac performance might be mediated by α1/7 subunits of the nicotine receptor in the Drosophila Melanogaster´s heart. We planned further experiments to test if this can be reproduced in mammalian hearts, as for example in mice.

500. (542) **PARTICIPATION OF MITOCHONDRIA IN THE CARDIOPROTECTIVE EFFECT MEDIATED BY 6-ETHOXZOLAMIDE**

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Previous data from our laboratory show that anhydrase carbonic (AC) inhibition with 6-ethoxzolamide (ETZ) reduces the infarct size and improves the post-ischemic recovery of myocardial function. However, the participation of mitochondria in this intervention has not been clarified yet. Our objective was to study the effects of ETZ on mitochondrial alterations produced by ischemia-reperfusion (Is-R). Isolated rat hearts, perfused by the Langendorff technique and after 20 min of stabilization (E), were assigned to the following groups: 1) Control (C): they were perfused until completing 110 min; 2) Ischemic control (IC): 30 min of global Is and 60 min of R; 3) ETZ: 100 μM ETZ was administered during the last 10 min of E and the first 10 min of R. To evaluate the participation of p38MAPK and PKCε, experiments were carried out in the presence of their inhibitors SB202190 and celerythrine (Che), respectively, administered during the first 10 min of R. Mitochondrial state was determined by changes in light scattering decrease (ΔDL) by the addition of 200 μM Ca2+, Ca2+ retention capacity (CRC, Calcium Green 5N) and membrane potential (ΔΨm, Rhodamine123) in isolated mitochondria from the different groups. Mitochondrial fission was assessed by the content of P-Ser637-Drp1 by western blot (WB). The expression of P-p38MAPK, P-PKCε, calcineurin Aβ and P-HSP27 were also evaluated by WB.
ETZ significantly increased ΔDL (1.3±0.2 vs 0.3±0.1 a.u.) and CRC (266±10 vs 5±1 nmol/mg protein) and preserved ΔΨm (-141±5 vs -95±6 mV). ETZ increased the content of P-p38MAPK, P-PKCε, P-HSP27 and P-Drp1 but decreased calcineurin Aβ expression. SB202190 and Che treatment abolished these changes and the parameters reached values similar to those observed in IC.
These data show that ETZ decreases the mitochondrial state post-ischemic alterations and the fission process through intracellular signaling pathways that involve p38MAPK/PKCε/HSP27 activation and calcineurin Aβ inactivation.

501. (554) **AORTIC LARGE-CONDUCTANCE CALCIUM ACTIVATED POTASSIUM CHANNEL AND POTASSIUM CHANNEL WITH INWARDLY RECTIFICATION ARE DIFFERENTLY EXPRESSED IN NORMOTENSIVE AND HYPERTENSIVE RATS**

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Potassium channels and C-type natriuretic peptide (CNP) play key roles in the regulation of vascular tone. Objective: to evaluate the aortic expression of BKCa and Kir channels in spontaneously hypertensive rats (SHR) and their role in the vasodilator effect of CNP. Experimental design: Male Wistar rats and SHR (14 weeks-old) were sacrificed and aorta was removed to determine: BKCa and Kir2.0 protein expression and vascular reactivity after CNP addition (1pmol/L - 1umol/L). Denuded aortic rings were pre-contracted with phenylephrine (1umol/L) in the presence or absence of tetraethylammonium (TEA, non-selectively potassium channel blocker, 1mmol/L), iberiotoxin (IbTx, BKCa blocker, 30nmol/L), or BaCl2 (Kir blocker, 30 umol/L). Statistical analysis: Results are expressed as mean±SEM, Student t-test or 2-way ANOVA, followed by Bonferroni posthoc test; n=4-6/group.
Results: Protein content of BKCa and Kir2.0 was lower in SHR compared to Wistar rats (BKCa: Wistar=1.1±0.1 vs. SHR=0.7±0.1*; Kir2.0: Wistar=0.8±0.1 vs. 0.4±0.1*; *p<0.05). The maximal relaxant response of CNP (Emax: Wistar=54.8±7.3 vs. SHR=57.6±3.1) and the negative logarithm of the concentration that induces the 50% of Emax (pEC50: Wistar=6.7±0.1 vs. SHR=6.8±0.1) were similar in denuded rings between both groups. TEA abolished the vasodilator effect of CNP. IbTx and BaCl2 decreased Emax similarly (IbTx: Wistar=36.1±4.8**; SHR=33.5±3.2##; BaCl2: Wistar=35.5±3.6**; SHR=34.0±3.2##; **p<0.01 vs. Wistar; ##p<0.01 vs. SHR) in both groups. However, the pCE50 with IbTx was lower in Wistar (Wistar=6.3±0.1**; SHR=6.8±0.1; **p<0.01 vs. Wistar), while CNP pEC50 was more affected by opening of Kir channels in SHR (Wistar=6.7±0.1; SHR=6.4±0.1##; ##p<0.01 vs. SHR).

Conclusion: The endothelium-independent vasodilator effect of CNP appears to be more sensitive to Kir blockade and less sensitive to BKCa blockade in SHR, although the expression of both channels is decreased in this model of essential hypertension.

502. (513) **THE IMPACT OF AEROBIC TRAINING IN CARDIOVASCULAR ALTERATIONS IN OVARIECTOMIZED RATS**
Alejandro Martín Ibañez, Joshua Godoy Coto, Alejandra Yeves, Omar Velez Rueda, Irene Ennis, Ernesto Alejandro Aiello, Verónica Celeste De Giusti.
Centro de Investigaciones Cardiovasculares

Resveratrol (RSV) is a polyphenol present in several plants. Nowadays it is sold as an over-the-counter dietary supplement mainly due to its antioxidant, anti-inflammatory and antitumoral effects. Animal renal injury models describe RSV benefícial effects, while studies with chronic intake of RSV demonstrate nephrotoxic effects. Hence, RSV effects on renal tissue are still controversial. Due to the urinary concentrating mechanism, renal medullary interstitium presents an elevated osmolality that can abruptly vary depending on the hydric state of the body, reaching values up to 800-1200 mOsm/kg H2O. To survive in this environment, renal cells activate protective pathways. We demonstrated that renal epithelial cells (MDCK) subjected to high osmolarity undergo an adaptive process during the first 24h, in which the transcription of the osmoprotective gene cyclooxygenase 2 (COX-2) is activated, among others. After 48h, these cells are already adapted and acquire a polarized epithelium morphology. In the present work, we evaluate the effect of RSV on adaption and differentiation mechanisms, focusing particularly on COX-2 role. To do this, MDCK cells were pretreated with different concentrations of RSV (1, 5, 10, 25μM) and then cultured in hyperosmotic medium (NaCl 125mM, 512 mOsm/kg H2O) for 24 and 48h. Cells were then harvested to obtain cell number and viability. Immunofluorescence (IF), western blot and RT-PCR analysis were performed. RSV significantly decreased cell number in a concentration-dependent manner at 24 and 48h ($p<0.0001$). Cells treated with RSV did not reach typical epithelium morphology; moreover, 10 and 25μM RSV showed a mesenchymal phenotype. COX-2 mRNA ($p=0.0003$) and protein levels ($p=0.0033$) were surprisingly upregulated by RSV at 24 and 48h. COX-2 IF revealed an increase of the protein in cytoplasmic granules. These results suggest that in renal cells RSV modulated osmoprotective-COX-2 expression and impeded monolayer differentiation.

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503. 513 **RESVERATROL EFFECT ON RENAL OSMOPROTECTION: MODULATION OF COX-2 EXPRESSION**
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Resveratrol (RSV) is a polyphenol present in several plants. Nowadays it is sold as an over-the-counter dietary supplement mainly due to its antioxidant, anti-inflammatory and antitumoral effects. Animal renal injury models describe RSV benefícial effects, while studies with chronic intake of RSV demonstrate nephrotoxic effects. Hence, RSV effects on renal tissue are still controversial. Due to the urinary concentrating mechanism, renal medullary interstitium presents an elevated osmolality that can abruptly vary depending on the hydric state of the body, reaching values up to 800-1200 mOsm/kg H2O. To survive in this environment, renal cells activate protective pathways. We demonstrated that renal epithelial cells (MDCK) subjected to high osmolarity undergo an adaptive process during the first 24h, in which the transcription of the osmoprotective gene cyclooxygenase 2 (COX-2) is activated, among others. After 48h, these cells are already adapted and acquire a polarized epithelium morphology. In the present work, we evaluate the effect of RSV on adaption and differentiation mechanisms, focusing particularly on COX-2 role. To do this, MDCK cells were pretreated with different concentrations of RSV (1, 5, 10, 25μM) and then cultured in hyperosmotic medium (NaCl 125mM, 512 mOsm/kg H_2O) for 24 and 48h. Cells were then harvested to obtain cell number and viability. Immunofluorescence (IF), western blot and RT-PCR analysis were performed. RSV significantly decreased cell number in a concentration-dependent manner at 24 and 48h ($p<0.0001$). Cells treated with RSV did not reach typical epithelium morphology; moreover, 10 and 25μM RSV showed a mesenchymal phenotype. COX-2 mRNA ($p=0.0003$) and protein levels ($p=0.0033$) were surprisingly upregulated by RSV at 24 and 48h. COX-2 IF revealed an increase of the protein in cytoplasmic granules. These results suggest that in renal cells RSV modulated osmoprotective-COX-2 expression and impeded monolayer differentiation.

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504. 155 "**Alterations of L-type calcium current and the Action Potential restitution do not constitute a requisite for CRR"**
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It has been described that one of the triggers of a wide spectrum of ventricular arrhythmias is the abnormal intracellular Ca^{2+} handling during the excitation-co-contraction coupling (ECC) in the cardiomyocyte. One of the possible abnormalities is the alteration of the recovery of refractoriness between heartbeats, known as Ca^{2+} release restitution (CRR). Although the control of CRR has been associated with the sarcoplasmic reticulum (SR) Ca^{2+} loading and ryanodine receptor (RyR2) Ca^{2+} sensitivity, an intriguing point is whether the restitution of the action potential (AP) and/or the L-type calcium current (ICa) are involved in the determination of CRR. To assess these interrogates, we used mouse isolated cardiac myocytes with higher CRR velocities respect to control myocytes (WT, 2mM external Ca^{2+} concentration), obtained by increasing SR Ca^{2+} load by using two different maneuvers, 1. ablation of phospholamban (PLNKO myocytes) and 2. Increasing extracellular Ca^{2+} concentration (WT myocytes, 4mM external Ca^{2+} concentration). Restitution of cytosolic Ca^{2+} transient (Fura-2 AM), L-type Ca^{2+} current (ICa, patch-clamp) and action potential (AP, microelectrodes) were evaluated with a two-pulse protocol (S1/S2). CRR, ICa and AP restitution percentages increased as a function of the coupling interval (S2-S1), following an exponential curve. CRR was accelerated in PLNKO vs. WT myocytes and in WT myocytes at 4 vs. 2 mM Ca. In both cases there was a greater ICa Ca^{2+}-dependent inactivation induced by the enhanced RyR2 release of Ca^{2+}. However, whereas ICa and AP restitution did not differ between PLNKO vs. WT myocytes, they were slightly but significantly accelerated in WT myocytes at 4 vs. 2 mM Ca^{2+}. Similar results were obtained with a mathematical model of human myocyte. We conclude that an acceleration of ICa restitution recovery may influence but is not a requisite for the occurrence of a faster CRR.

505. (169) **REGULATION OF CELL VOLUME IN HUMAN ERYTHROCYTES EXPOSED TO *ESCHERICHIA COLI* ALPHA-HEMOLYSIN.**
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Escherichia coli α-hemolysin (HlyA) triggers sublytic and lytic processes on human erythrocytes (RBCs). We studied how HlyA affects volume regulation in RBCs.
RBCs were treated with 0.1 ng/μl HlyA or vehicle at 37 °C for 1, 5 and 10 min. Cell volume (Vt) was measured using the Coulter Counter principle. Rapid kinetics of Vt was assessed by light scattering using a stopped flow rapid mixing equipment. Cells were preexposed 1, 5 and 10 min to HlyA, and Vt changes were measured over 4 s after 140 mosM osmotic gradient. Exponential curves were fitted to data. Best fit values of the exponential coefficient (k) were obtained, which estimates osmotic water permeability (P_f). Intracellular contents of Na^+ and K^+ were measured by flame photometry.
HlyA-treated RBCs underwent a slight shrinkage, followed by continuous swelling up to 12% over control values after 10 min. Vt changes were accompanied by parallel intracellular [Na^+] increase and [K^+] decrease.
Exposure to HlyA decreased k values from 7.6 ± 0.1 seg^{-1} to 3.4 ± 0.2 seg^{-1} after 10 min. In hyposmotic media (190-280 mosM), Vt increased over 10 min. Preexposure to these media showed that k values decreased as Vt increased, so that a 54% reduction of k values was obtained when Vt was increased by 30%.
In the presence of HlyA and suramin (blocker of purinergic receptors), Vt did not change, but k values were reduced by 30%.
Comparing HlyA and hyposmotic media, the reduction of k values may be partly assigned to cell swelling. However, results using suramin+HlyA, where changes in Vt and k were uncoupled, show that another factor is affecting k, and therefore Pf of RBCs.
Paradoxically, HlyA causes RBC swelling, which ultimately leads to lysis, while simultaneously reduces water permeability.
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506. (192) **ROLE OF AQUAPORIN-2 AND TRPV4 IN RENAL CELL MIGRATION**

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We have previously shown that Aquaporin-2 (AQP2) promotes renal cell migration. This promigratory effect is due, at least in part, to the modulation of Na^+/H^+ exchanger (NHE1) activity, responsible for lamellipodia alkalinization, which would generate the appropriate microenvironment for actin and focal adhesion dynamics. Taking into account that we have demonstrated a physical and functional interaction between AQP2 and Ca^{2+} channel TRPV4, and that NHE1 activity is modulated by Ca^{2+}, we propose to investigate the contribution of TRPV4 in the AQP2-dependent renal cell migration. We used two renal cell models: one WT not expressing AQPs and another one expressing AQP2. First, we determined TRPV4 expression in lamellipodia of migrating cells by immunofluorescence assays. Then, we evaluated TRPV4 participation in collective cell migration through wound healing assays in presence of a specific activator (GSK1016790A, 3nM). Finally, we characterize focal adhesion complexes by revealing the mechanosensor Vinculin. Our results showed that TRPV4 is present in lamellipodia of both cell types, but AQP2-expressing cells have a higher intensity ratio per area analyzed (WT: 794±84, n=32; AQP2: 1371±79, n=74; ***p<0,001). In AQP2-expressing cells, the activation of TRPV4 produces a decrease in migration indicating that, probably, TRPV4 is already in an activated state and overactivation results in a harmful excess of Ca^{2+} (Control: 29.65±1.25%, n=10; GSK: 19.14±1.18%, n=6; ***p<0,001). Moreover, lamellipodia of AQP2-expressing cells have focal adhesions of small size evidencing the rapid turnover of active migrating cells (WT: 6.68±0.78μm^2, n=29; AQP2: 2.50±0.23μm^2, n=10; *p<0,05). These results let us to propose that during lamellipodia protrusion the presence of AQP2 activates it's partner TRPV4, leading to a regulated Ca^{2+} entry. Furthermore, we propose that Ca^{2+} entering in the vicinity of focal adhesions would favor the assembly/disassembly cycles of these adhesive sites.

507. (309) **ELECTRICAL OSCILLATIONS OF ISOLATED BRAIN MICROTUBULES**

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Microtubules (MTs) are important cytoskeletal structures engaged in a number of specific cellular activities, including vesicular traffic and motility, cell division, and information transfer within neuronal processes. MTs also are highly charged polyelectrolytes. Recent *in vitro* electrophysiological studies indicate that different brain MT structures, including two-dimensional (2D) sheets (MT sheets) and bundles, generate highly synchronous electrical oscillations (Cantero et al. Sci Rep, 2016 & 2018). Although taxol-stabilized isolated MTs are capable of amplifying electrical signals, no information is heretofore available as to whether isolated MTs also engage in electrical oscillations. Herein we tested the effect of voltage clamping on the electrical properties of non-taxol stabilized isolated brain MTs. Electrical oscillations were observed at holding potentials between ±200 mV. Mean oscillatory currents were linear with respect to holding potential, with a change in conductance from 59.6 ± 3.6 nS to 160.8 ± 7.6 nS (n = 3) after loose-patch correction. This average change in conductance was much higher than previously reported for more complex MT structures. The frequency domain spectral analysis also disclosed a richer oscillatory response as compared to that observed in voltage clamped MT sheets from the same preparation. This interesting finding is consistent with the possibility that more structured MT complexes (i.e. bundles, sheets) may render more coherent responses at given oscillatory frequencies and raise the hypothesis that combined MTs may tend to entrain and oscillate together. The electrical oscillatory behavior of isolated brain MTs is consistent with that of "ionic-based" transistors whose activity is synchronized in higher MT structures. The ability of MTs to generate, propagate, and amplify electrical signals may have important implications in neuronal computational capabilities.

508. (497) **INDUCTION OF OXIDATIVE STRESS IN LYMPHOID TISSUE FROM MURINE MODELS OF HYPOTHYROIDISM: TREATMENT WITH PROPYLTHIOURACIL VERSUS THYROIDECTOMY**

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Introduction: Hypothyroidism induced by antithyroid drugs increases reactive oxygen species (ROS). Some authors attribute the increase in ROS to a decrease in the intracellular concentration of non-enzymatic antioxidants and other authors to a cytotoxic effect of the drug in the liver. **Objective:** 1- Analyze whether the increase in ROS is a pathological condition induced by hypothyroidism or is due to the direct action of antithyroid drugs; 2- Evaluate the effect of hypothyroidism on lymphocyte functionality. **Methods:** Hypothyroidism was induced in Balb/c mice by treatment with propylthiouracil (PTU; 0.5 g/l drinking water for 15 days) or thyroidectomy. Thyroid hormone levels were quantified by RIA. ROS were evaluated by DCFH-DA staining and flow cytometry. Liver damage was evaluated in tissue sections stained with hematoxylin-eosin and Masson's Trichome. The number of lymphoid follicles was determined in sections

of lymph nodes or spleen stained with hematoxylin-eosin. Apoptosis of lymphoid cells was quantified by flow cytometry and cell proliferation was evaluated by incorporation of [^{3}H]-thymidine into DNA. **Results:** We found that both, hypothyroid mice by treatment with PTU and thyroidectomized (Thy) had an increased production of ROS in lymph nodes (LN) and spleen (S) compared to euthyroid mice [% increase (LN) PTU: 68.5±8.4, Thy: 62.2±6.5 and % increase (S) PTU: 50.3±5.8, Thy: 62.6±7.3 ($p<0.05$; n=9)]. We did not observe liver tissue damage in the mice treated with PTU. Hypothyroid mice had smaller lymphatic organs, a lower density of lymphoid follicles/field and a decreased proliferative capacity against mitogenic stimuli compared to euthyroid controls. Apoptosis in hypothyroid mice was similar to controls. **Conclusions**: Hypothyroidism induced by PTU or thyroidectomy increases ROS production in lymphoid tissue and has negative effects on lymphocyte functionality. PTU did not induce liver cytotoxicity in our study model.

509. (498) **EFFECTS OF OXIDATIVE STRESS ON LYMPHOCYTE FUNCTIONALITY IN A MURINE MODEL OF HYPERTHYROIDISM. ROLE OF THE CELLULAR ANTIOXIDANT SYSTEM IN THE SCAVENGING OF OXYGEN FREE RADICALS**

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Introduction: Thyroid hormones increase cellular metabolism and the production of reactive oxygen species (ROS). ROS could act as a signaling molecule involved in cell functionality or exert cytotoxic actions depending on its intracellular concentration. The harmful effects of ROS are counteracted by the cellular antioxidant system. **Objective:** To study the effects of oxidative stress on lymphocyte functionality in hyperthyroid mice, analyzing the role of antioxidant enzymes in the scavenging of ROS. **Methods:** Balb/c mice were treated with placebo (euthyroid) or T4 (12 mg/l drinking water for 30 days-hyperthyroid). ROS were evaluated by DCFH-DA staining and flow cytometry. The expression of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) was determined by qPCR and WB. Apoptosis was evaluated by Annexin V-PI, Rhodamine-123 or Hoechst-33342 staining followed by flow cytometry or fluorescence microscopy. The number of follicles in lymphoid tissue sections stained with hematoxylin-eosin was determined by microscopy. Cell size and granularity was evaluated by flow cytometry. Proliferation was evaluated by incorporation of [^{3}H]-thymidine. **Results:** We found an increased production of ROS in the lymph nodes (LN) and spleen (S) of hyperthyroid mice (% increase LN:60.6±5.1; S:63.5±5.9, $p<0.05$) that was correlated with an increased genomic and protein expression of CAT and GPx ($CAT_{RNAm\%}$ LN:85.1±6.2, S:147.2±9.7; $CAT_{protein\%}$ LN:154.3±9.7, S:65.7±5.8; $GPx_{RNAm\%}$ LN:43.7±3.1, S:39.8±4.7; $GPx_{protein\%}$ LN:47.6±5.1, S:71.1±6.6; $p<0.05$). We did not find significant differences in the expression of SOD. Apoptosis was similar in both groups. Hyperthyroid mice had hypertrophied lymphatic organs, more lymphoid follicles and lymphoid cells in active cell proliferation. **Conclusions**: Antioxidant enzymes partially decrease ROS, avoiding their cytotoxic effect on lymphoid cells. ROS could participate in the signaling pathways involved in lymphocyte functionality.

510. (511) **THE IMPACT OF AEROBIC TRAINING IN CARDIOVASCULAR ALTERATIONS IN OVARIECTOMIZED RATS**

Alejandro Martín Ibañez, Joshua Godoy Coto, Alejandra Yeves, Omar Velez Rueda, Irene Ennis, Ernesto Alejandro Aiello, Verónica Celeste De Giusti.
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Menopausal women show an increase in cardiovascular diseases. Sodium/proton exchanger (NHE) and sodium/bicarbonate cotransporter (NBC) are the main alkalinizing mechanisms of the cardiomyocytes. Last year we reported that the NHE activity is increased, whereas the NBCe1 is decreased in ovariectomized (OVX) rats. Ennis et al. have demonstrated that an aerobic routine is able to transform a pathological cardiac hypertrophy (CH) to a physiological one, possibly related to an inhibition of the NHE activity. Additionally, it was described an increase in NBCe1 activity in mice after a swimming routine. Here we test the hypothesis that swimming is able to prevent the cardiovascular modifications previously described by our team in OVX rats. Bilateral ovariectomy was performed in 10 weeks old Wistar rats. OVX rats were randomly assigned to a sedentary group (OVXs) or to an aerobic swimming routine (8 weeks/5 d a week) (OVXe).
Body weight and visceral adipose tissue were increased in OVXs rats, and both changes were prevented by the swimming training. Left ventricular mass index (echocardiography) was higher in OVX rats in comparison with Sham. (LVMI (mg/mm) Sham 20.14±2.35 n=2; OVXs 23.99±2.17, n=5; OVXe 26.94±4.53 n=4). Ongoing experiments such as fibrosis, capillary density and molecular markers will determine the type of CH.
NHE activity was increased in OVXs myocytes, and swimming showed a tendency to prevent it (JH at pH=6.8 (mmol/min) Sham 1.63±0.28 n=4; OVXs 2.04±0.18, n=3; OVXe 1.73±0.28 n=4). NBCe1 activity was diminished in OVXs myocytes, whereas OVXe recovered the impaired activity (DpH= Sham: 0.25±0.02, n=4; OVXs 0.10±0.02*, n=8; OVXe 0.14±0.01*, n=13; *$p<0.05$ vs Sham)
Our results suggest that swimming exercise might prevent some of the cardiovascular deleterious modifications in OVX rats. Future experiments are required to fully elucidate the molecular targets of this intervention, which may represent a valuable therapy for the menopausal women population.

511. (533) **THYROTROPIN MODULATES CALCIUM HANDLING AND CONTRACTILITY IN ADULT RAT CARDIAC MYOCYTES**

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Hypothyroidism (Hypo) is associated with cardiac fibrosis, myocardial edema and contractile dysfunction. However, the subcellular mechanisms underlying contractile dysfunction are not completely understood. We hypothesized that an increase in the concentration of Thyrotropin (TSH), which occurs in the context of Hypo, could alter intracellular Ca2+ handling and contribute to the negative inotropic and lusitropic effects associated with Hypo. Our results show that TSH does not have direct acute effects on euthyroid adult cardiac myocyte contractility. However, when myocytes were exposed to TSH for 24 hours we observed a decrease in cellular contractility associated with a decrease in the amplitude and relaxation rate of the Ca2+ transient. A similar impact of TSH con contractility was observed in cardiomyocytes derived from human IPS cells. These effects were abolished by the PKA inhibitor H89, suggesting that the TSH receptor through its Gsa/PKA singling underlies the contractile effect of TSH in adult cardiac myocytes. Cardiomyocytes incubated with TSH showed profound alterations in the expression of Ca2+ proteins SERCA, RyR2 and the Na+/Ca2+ exchanger (NXC) which were reverted by H89. Importantly, TSH fails to further reduce contractility in myocytes isolated from a rat model of Hypo with elevated TSH. We conclude that TSH has direct negative effects on cardiac mechanical activity by altering the expression of Ca2+ handling proteins by a PKA-dependent-signaling pathway. We speculate that elevated TSH contributes to contractile dysfunction associated with Hypo.

512. (152) **MICROTUBULES´ ROLE IN THE INITIAL STAGES OF LYTIC IMMUNE SYNAPSE (LIS) DEVELOPMENT IN NATURAL KILLER CELLS (NK)**

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Natural killer cells (NK) are the cytotoxic cells from the innate immune system, which eliminates viral-infected and neoplastic cells. They form a specialized junction with their target cells referred to as Lytic (L) Immune Synapse (IS). LIS maturation is characterized by the local reorganization of actin filaments and NK receptors, and centrosome (Ct) and Golgi apparatus (GA) translocation towards this site. AKAP350 is an A-kinase anchoring protein that participates in MT nucleation at the Ct and at the GA. Our previous results showed that AKAP350 participates in NK cytotoxic activity. The aim of this work was to characterize MT nucleation during NK activation and to evaluate MT´ role in the initial events of LIS maturation. In order to characterize MT nucleation, YTS cells were activated through LFA-1 and CD28 receptors for 30 minutes at 37°C and then subjected to an ice-recovery assay for detection of newly nucleated microtubules. Confocal microscopy analysis of a-tubulin and GM130 (a GA marker) showed that not only the Ct (commonly regarded as the exclusive microtubule organizing center, MTOC, in lymphocytes) but also the GA were responsible for MT nucleation. Our results further showed that MT nucleation at the GA was diminished in AKAP350 knockdown cells (-50%*). Previous works indicated that NK cytotoxicity is reduced by Nocodazole (Noc) or Taxol (Tx) treatment. YTS cells were subjected to Noc or Tx treatment and incubated with KT86 target cells at 37° for 30 minutes. Confocal microscopy analysis of LFA-1 staining of those cells showed that LFA-1 organization was inhibited both by disruption of MT filaments (Noc: -42%*) and by MT stabilization (Tx: -40%*). On the other hand, actin organization at the LIS was exclusively impaired by Noc treatment (-30%*). Overall, our studies characterize for the first time the GA participation as a MTOC in NK cells and demonstrate the relevance of MT dynamics for LFA-1 clustering at NK LIS. (*p<0.05)

GASTROENTEROLOGÍA

513. (63) **INTESTINAL MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) IS DOWNREGULATED BY OXIDATIVE STRESS (OS) VIA A POSTTRANSLATIONAL MECHANISM. IMPACT ON ITS MEMBRANE BARRIER FUNCTION**

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The intestinal tract is a major site of pro-oxidant agent's production, as a result of continuous exposure to food additives and contaminants. Homeostatic control of intestinal oxidative environment is crucial in nutrient digestion and absorption, and barrier function. Intestinal Mrp2 is an ABC transporter that limits the absorption of xenobiotics orally ingested, thus acting as a biochemical barrier. We here evaluated the short-term effect of OS on intestinal Mrp2 subcellular localization and its barrier function by treatment of isolated intestinal sacs with 250 μM of tert-butyl hydroperoxide (TBH 250) for 30 min (N=6). We first confirmed that TBH 250 generated OS in intestinal tissue as indicated by increasing lipid peroxidation products (+61%) and catalase (+44%) and glutathione peroxidase (+28%) activities, and by decreasing GSH content (-19%), the GSH/ GSSG ratio (-29%) and superoxide dismutase activity (-21%), respect to controls (C, p<0.05). Assessment of Mrp2 distribution between brush border (BBM) and intracellular (IM) membrane fractions by western blotting, showed that Mrp2 protein decreased in BBM (-44%) and increased in IM (+75%) after treatment with TBH 250 compared to C (p<0.05), consistent with an internalization process. In line with this, efflux of the Mrp2 substrate dinitrophenyl-S-glutathione in everted intestinal sacs decreased in TBH group (-35%, p<0.05), indicating that Mrp2 internalization is of functional significance. Also, treatment with Gö6976, a selective inhibitor of cPKC isoforms, was able to completely block the decrease in Mrp2 protein expression in BBM (-45%, p<0.05) and its increase in IM content (+74%, p<0.05), as well as the impairment in Mrp2 activity (-35%, p<0.05) induced by TBH. In conclusion, we demonstrated a posttranslational regulation of rat intestinal Mrp2 by short-term exposition to OS. This process consisted of a rapid Mrp2 internalization to IM, likely mediated by cPKC, with impairment of its barrier function.

514. (116) **GENISTEIN (GNT) AMELIORATES PARAQUAT (PQ)-INDUCED HEPATIC INJURY IN RATS**

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Previously, we demonstrated that GNT induces expression of hepatic P-glycoprotein (P-gp), a canalicular ABC transporter that extrudes toxicants such as PQ into bile. Aim: To evaluate the hepatoprotective role of GNT in a model of acute intoxication with PQ in rats. Methods: Treatment I: Control (vehicles), GNT (5 mg/kg/day sc, 4 days), PQ (50 mg/kg ip, last day) and GNT+PQ. Hepatic malondialdehyde (MDA) levels were measured by thiobarbituric acid reactive substances methodology. Levels of 4-hydroxynonenal protein adducts (4-HNEp-add) and glutathione-S-transferase alpha (GSTα) protein expression were evaluated in liver by Western blotting. Treatment II: Control (vehicle), GNT (5 mg/kg/day sc, 3 days). Biliary excretion of PQ was studied in two models: *in vivo* excretion and isolated perfused liver (IPL). PQ (50 mg/kg iv) was administered at day 4, before bile sampling. Bile was collected each 10 min, during a 40 min-period. PQ was quantified by HPLC. Results (*p<0.05 vs all other groups; #p<0.05 vs C and PQ): PQ significantly increased hepatic MDA and 4-HNEp-add levels, whereby pretreatment with GNT ameliorated this effect [MDA (pmol/g protein, n=3): C: 55±2; GNT: 55±7; PQ: 76±3*; GNT+PQ: 64±4. 4-HNEp-add (% of C, n=4-6): C: 100±33; GNT: 172±48; PQ: 258±71*; GNT+PQ: 126±33]. PQ biliary excretion remained unchanged after treatments in both experimental models. Hepatic GSTα protein expression was augmented in GNT-treated rats, while PQ did not modify the hepatic GSTα levels (% of C, n=3): C: 100±33; GNT: 359±86#; PQ: 73±40; GNT+PQ: 425±98#. This result agrees with the lower content of 4-HNE adducts in the GNT+PQ group respect to PQ group. Unexpectedly, the increased activity of P-gp induced by GNT did not enhance PQ biliary excretion. Thus, GNT protective mechanism is likely through the induction of GSTα which rapidly metabolizes 4-HNE before formation of protein adducts.

515. (125) **MITOCHONDRIAL EXPRESSION OF HUMAN AQUAPORIN-8 IMPROVES AMMONIA-DERIVED UREAGENESIS IN THIOACETAMIDE-TREATED HEPATOCYTES**

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Hepatocyte ammonia metabolism is critical for prevention of hyperammonemia and neurological damage. We previously found that in hepatocytes, mitochondrial aquaporin-8 (AQP8) channels facilitate ammonia diffusion and its metabolism into urea. In this study, we investigated whether the gene transfer of human AQP8 (hAQP8)

to hepatocyte mitochondria is able to recover the impaired ammonia-derived ureagenesis in hepatocytes treated with the model hepatotoxin thiocetamide (TAA). Primary cultured rat hepatocytes were treated with TAA (0-30 mM) for 24 h. We previously reported that the urea synthesis from ammonia is significantly inhibited by TAA at 20 and 30 mM concentrations without affecting cell viability. Hepatocytes were transduced with the recombinant adenoviral vector encoding hAQP8, AdhAQP8, or a control adenovector. Mitochondrial subfractionation followed by immunoblotting confirmed that hAQP8 was rightly expressed at mitochondrial level, and that its expression was unaffected by TAA. Mitochondrially-expressed hAQP8 was able to significantly restore ureagenesis from TAA-treated hepatocytes (Control: 100± 17%; TAA-20mM: 49 ± 19%*; TAA-20mM+hAQP8: 94 ± 15%; TAA-30mM: 25 ± 7%*; TAA-30mM + hAQP8: 114 ± 20%; data are means ± SEM; n=6; *P<0.05 from control). Nuclear magnetic resonance studies using ^{15}N-labeled ammonia confirmed that hepatocyte ^{15}N-labeled urea synthesis was reduced by TAA and fully restored by hAQP8 transduction (Control: 100 ± 13%; TAA-30mM: 26 ± 16%*; TAA-30mM + hAQP8: 168 ± 16%*; data are means ± SEM; n=3; *P<0.05 from control). In conclusion, our data indicate that the mitochondrial expression of hAQP8 improves ammonia conversion to urea in TAA-treated hepatocytes thus providing further support for a key role of AQP8 in hepatic ammonia metabolism. This knowledge might contribute to the understanding and treatment of certain hyperammonemic conditions.

516. (131) **FURTHER EVIDENCE FOR THE INVOLVEMENT OF MITOCHONDRIAL AQUAPORIN-8 IN HEPATOCYTE CHOLESTEROL AND FATTY ACID SYNTHESIS**

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Hepatocyte mitochondrial aquaporin-8 (mtAQP8) is a multifunctional channel protein able to facilitate the diffusional release of H_2O_2. We recently provided evidence suggesting that mtAQP8, via a H_2O_2 signaling, plays a role in the modulation of hepatocyte cholesterogenesis. To expand that study, we cultured human hepatocyte-derived Huh-7 cells in medium with lipoprotein-deficient serum (LPDS) to reduce sterol content which in turn induces the *de novo* synthesis of cholesterol and fatty acids, and assessed the involvement of mtAQP8 by gene silencing. Lipid synthesis was determined by following the incorporation of radiolabeled acetate. AQP8 silencing was performed by using two different siRNAs and corresponding scrambled control siRNA. Protein expression was assessed by immunoblotting. Cell culturing in LPDS medium induced mtAQP8 expression by 80% ($P < 0.05$) as well as the *de novo* synthesis of cholesterol and fatty acids by around 100% ($P < 0.05$). Accordingly, expression of the key enzymes for cholesterol and fatty acid synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoAR) and fatty acid synthase (FAS), was also significantly increased by 300 and 60% ($P < 0.05$), respectively. AQP8 silencing (-50% protein expression) down-regulated the LPDS-induced synthesis of cholesterol and fatty acids by around 30% ($P < 0.05$). HMGCoAR and FAS expressions were also down-regulated by around 50% ($P < 0.05$). In conclusion, our data suggest that human hepatocytes cultured in LPDS media up-regulates mtAQP8 expression and that mtAQP8 gene silencing down-regulates the LPDS-induced *de novo* cholesterol and fatty acids synthesis by decreasing expression of the key lipogenic enzymes HMGCoAR and FAS. These data further support a regulatory role of mtAQP8 in hepatocyte lipid homeostasis.

517. (149) **TRANSCRIPTIONAL REGULATION OF P-GLYCOPROTEIN BY PROLACTIN IN FEMALE RAT LIVER.**

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P-glycoprotein (Pgp), a canalicular transporter encoded by *Mdr1a* and *Mdr1b* genes in rodents, plays an important role in the excretion of xenobiotics into bile. Prolactin (PRL) plasma levels are greatly increased in lactating females and the classic PRL way of action implies interaction with its receptor and activation of Stat5 transcription factor. Previously, we have reported that the activity and protein expression of hepatic Pgp were up-regulated in 15 days postpartum (PP) rats, and the same effect was observed in rats after exogen PRL treatment. Aim: To elucidate the mechanism involved in the modulation of hepatic Pgp expression by PRL. Methods: *Mdr1a* and *Mdr1b* mRNA levels were quantified by qRT-PCR in 1- livers of lactating rats at 15 days PP *vs* virgin females (VF), 2- primary cultured hepatocytes isolated from female rats. Cells were incubated for 4, 6 and 12 h with 0.1 μg/mL PRL (concentration mimicking plasma levels in PP rats) or vehicle. Also, hepatocytes were pre-treated with 5 μg/mL actinomycin D (ActD, RNA polymerase II inhibitor) or vehicle for 30 min, before PRL treatment (0.1 μg/mL) for 12 h or vehicle. Results (% of control, p<0.05): 1- *In vivo* we observed that *Mdr1b* mRNA levels were significantly up-regulated (+240%, n=5-6) in PP group. 2- In line with this, in isolated hepatocytes treated whit PRL, both *Mdr1a* (+110%) and *Mdr1b* (+80%) mRNA abundance was significantly increased at 12 h, without changes at 4 and 6 h (n=3-5), while PRL effect on *Mdr1a* and *Mdr1b* mRNA levels was completely abolished by ActD (n=3-5). Conclusion: These findings demonstrated a transcriptional mechanism by which PRL up-regulates hepatic Pgp expression. This effect could be mediated by Stat5 since the biological functions of PRL are mainly derived by its activation. Increased hepatic Pgp expression and activity suggest altered biliary excretion of its substrates with potential consequences on their efficacy or toxicity.

518. (321) **MAPPING OF SCIENTIFIC PRODUCTION ON SALIVARY CORTISOL**

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Salivary cortisol can replace free serum cortisol measurements. In recent years, a growing number of related researches have been published. In fact, salivary cortisol concentrations have been used as a biomarker of physiological and pathological conditions. The aim of the present study was to map the scientific landscape related to salivary cortisol research worldwide between 2014-2019. This study adopted a bibliometric method. An observational, descriptive, retrospective investigation was carried out, with quantitative description of the data. The search was made in PubMed database. “Salivary cortisol” were used as the keywords to reach the relevant publications. VOSviewer software was used for data visualization. SPSS and Microsoft Excel were employed for data analysis. A total of 12510 authors of 8301 organizations, have been identified in the 2779 articles. The top 10 authors have contributed with 249 (8.96%) of the papers. Granger DA (University of California-Irvine, Johns Hopkins University), was the most productive author (73 papers) with a total link strength of 333. Humans and hydrocortisone appeared as the most frequent keywords, with a total link strength of 36666 and 33907, respectively. The journals in which the main articles were published came from various genres, including neurology, medicine, psychology and psychiatry. Most of the articles (22.88%) were from the United States of America, followed by Germany (11.41%) and United Kingdom (5.18%). During the study period, international research on salivary cortisol was increased, indicating the importance of their use to prognosis, diagnosis and monitoring of patients with both oral and systemic diseases.

HEMATOLOGÍA

519. (209) **ALPHA-HEMOLYSIN INDUCED HUMAN ERYTHROCYTES ADHESION TO VASCULAR ENDOTHELIUM. A BIOMIMETIC APPROACH.**

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The α-haemolysin (HlyA) is an exotoxin produced by several strains of uropathogenic *E. coli*, one of the most important etiological agents of urinary infections. HlyA irreversibly binds to human erythrocytes (RBCs), initiating a degenerative process called eryptosis, characterized by biochemical and morphological changes such as phosphatidylserine (PS) exposure to the external layer of the plasma membrane of RBCs, shrinkage, and swelling. HlyA-induced PS externalization can lead to adhesion of RBCs to vascular endothelial cells (VECs).
We studied the capacity of HlyA-treated RBCs to adhere to: 1- activated endothelial HMEC-1 cells under different flow conditions (dynamic adhesion); 2- surfaces homogeneously covered with extracellular matrix components in static conditions.
Results showed that HlyA induced adhesion of RBCs to VECs at low flow (0.2 dyn/cm^2), although higher flows induced rapid detachment. On the other hand, HlyA treatment also induced static adhesion of RBCs to collagen or fibrinogen. Thus, HlyA-treated RBCs displayed high but weak adherence to VECs under the experimental conditions.
Additionally, to study the molecular mechanism of the HlyA-induced adhesion of RBCs we designed a biomimetic device to emulate the conditions of the blood vessels.
The device was built by coupling a microfluidic chip to a nanopatterned surface (NPS) coated with gold nanoparticles (AuNPs). Different adhesion molecules from the VECs could be anchored to the AuNPs to mimic exposure of adhesion molecules of an activated endothelium whereas, the architecture of the capillaries was emulated by a network of microfluidic channels built in polydimethylsiloxane (PDMS). The synthesis of the device was optimized by following the process with quartz microbalance and fluorescence microscopy. Future experiments using the device will allow investigating HlyA-induced adhesion of RBCs to specific adhesion molecules under flow conditions.

INFECTOLOGÍA Y PARASITOLOGÍA

520. (345) **STRUCTURAL ALTERATIONS OF HUMAN CHORIOAMNIOTIC MEMBRANES TREATED WITH ALPHA-HEMOLYSIN OF *E.COLI***

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α-hemolysin (HlyA), toxin secreted by uropathogenic strains of *Escherichia coli* (*E.coli*), has a fundamental role in urinary tract infections (UTIs). In pregnancy, UTIs are very frequent, being *E. coli* the etiological agent of almost the 80% of the cases. Considering that UTIs are related with premature rupture of fetal membranes, we proposed to analyze structural tissue changes of human chorioamniotic membranes treated with HlyA *in vitro.*
Methods: Chorioamniotic membranes (n=6) were obtained from deliveries by elective cesarean section (>37 weeks). All included women had normal pregnancies, without evidence of active labor or infection. Membrane explants were mounted and insured to a *Transwell* device to generate two independent chambers. To simulate an ascending infection, explants were incubated in the chorion-side with 5nM/50nM HlyA during 24h. HlyA was detected by immunohistochemistry and histological signs of damage (like edema, vacuolization, early/late apoptosis, extracellular matrix thickness, and number of fibroblast) were evaluated from paraffin-embedded tissue sections stained with hematoxylin/eosin. Necrosis was tested by LDH release and the transepithelial electrical resistance (TEER) was measured using a Millicell-ERS unit (n=3). Groups were compared using *t-*, U Mann-Whitney or Chi-squared test as correspond.
Results: HlyA interaction with chorioamniotic membranes caused structural alterations and a slight diminish of TEER after 24hs of incubation. The main tissue alterations were observed for the highest toxin concentration tested (50nM HlyA). Epithelial layer remained practically unaltered, while chorion cells showed an increment of vacuolization and necrosis. Extracellular matrix thickness was higher and fibroblast number lower in treated groups compared to control ones.
Conclusion: HlyA by itself is capable to introduce structural modifications in human chorioamniotic membranes, suggesting a role of this toxin in premature rupture of membranes.

MEDICINA REGENERATIVA Y TERAPIA CELULAR

521. (59) **EFFECT OF LIVER OVEREXPRESSION OF BRECEPT ON A RAT MODEL OF NAFLD**

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in developed countries, affecting up to 20% of the global population. Treatments for NAFLD patients are limited. Thus, the development of treatments to improve NAFLD patient's health, is of clinical relevance. TGF-β pathway is related to hepatic steatosis and fibrogenesis in NAFLD. We have described a new soluble variant of TGF-β type II receptor, called TGFBR2-SE, that was fused to the Fc portion of human IgG (TGFBR2-SE/Fc), leading to the development of Brecept (Br). The aim of this work was to study, in a high fat high sucrose diet (HFHSD)-induced NAFLD rat model, the effect of lentiviral-mediated liver overexpression of Br (Lv-Br). We compared three experimental groups: Control, HFHSD, and HFHSD+Br that received an intrahepatic injection of Lv-Br at week 17. Before Lv-Br injection, we observed insulin resistance in the HFHSD group ($p<0,05$) by Glucose Tolerance Test. At week 21, animals were sacrificed. Liver rats from the HFHSD+Br group showed a coloration and consistence similar to the Control group. Also, we found a slight tendency of liver weight improvement in the HFHSD+Br group (Control vs HFHSD $p<0,05$; Control vs HFHSD+Br, and HFHSD vs HFHSD-Br non-significant differences). In liver sections stained with H&E, Masson's trichome and Reticulin we observed that administration of Lv-Br, compared with the HFHSD group, significantly decreased microvesicular steatosis, improved hepatocyte cord's organization and hepatocellular ballooning close to central veins. In addition, we also observed a slight decrease in microvesicular steatosis near portal triads (PT). Moreover, Lv-Br decreased type III collagen deposition in perisinusoidal/pericellular space, type I collagen deposition in periportal/portal zones and the size of inflammatory foci in PT. Therefore, these results suggest that liver overexpression of Br exerts a beneficial effect against NAFLD induced by a HFHSD in rats.

METABOLISMO Y NUTRICIÓN

522. (237) **CARDIOMETABOLIC AND HEPATIC CHANGES PRODUCED BY CHRONIC ADMINISTRATION OF VITAMINS AND IRON OVERLOAD DURING EXPERIMENTAL METABOLIC SYNDROME**

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Consumption of a high-fructose-fat diet (HFFD) in rats promotes the development of metabolic syndrome (MS). The aim of this study was to evaluate the effect of antioxidants supplements (AS) and iron overload (FeO) on hepatic and cardiometabolic parameters.
Material and Methods: Male rats were exposed for 12 weeks to a standard diet (SD) or HFFD with or without AS or FeO (each subgroup n= 8-10). AS was composed of selenium, zinc, vitamins E, C and α-lipoic acid. Body weight (BW) and systolic blood pressure (SBP) were measured every 4 w. Metabolic variables (blood glucose, triglyceridemia -Try-, cholesterolemia -Cho-, serum HDLc, uricemia) were assayed at the end of the experiment, and thiobarbituric acid reactive substances (TBARS) in plasma were estimated. After euthanasia, abdominal white adipose tissue (AWAT) and liver were extracted and weights expressed as a percentage of BW. TBARS and triglycerides were evaluated in liver homogenates. Data were analyzed by two-way ANOVA tests adjusted by Bonferroni correction. Results: SBP increased at 4 w in HFFD, SD-Fe ($p<0.05$), and in 8-12 weeks in HFFD, SD-Fe, HFFD-Fe vs controls groups ($p<0.001$). AS decreased SBP in HFFD, HFFD-Fe ($p<0.02$) rats and also BW in HFFD ($p<0.01$). BW and AWAT were decreased in HFFD-Fe ($p<0.01$) groups. Try increased in HFFD ($p<0.05$); HFFD-AS ($p<0.01$) and in HFFD-Fe vs controls rats ($p<0.05$). Cho was lower in SD-Fe, HFFD-Fe ($p<0.01$) and HDLc in HFFD-AS ($p<0.01$), SD-Fe ($p<0.01$) and HFFD-Fe ($p<0.01$) groups. TBARS in plasma and liver and triglycerides in liver increased in HFFD rats ($p<0.01$), while AS decreased TBARS ($p<0.01$) -data only available in these groups-. Conclusions: This study show some evidence that AS intake counteracts SBP increase in HFFD and HFFD-Fe groups while FeO increase SBP in SD and HFFD rats. AS may also have a role in counteracting HDLc and TBARS increase probably through the scavenging property of O2- accumulation. At last FeO diminished Cho and HDLc in SD and HFFD rats

523. (363) **DAM EARLY FREE ACCESS TO HYPERTONIC NACL SOLUTION INDUCES A LONG-TERM EFFECT ON RENAL GENE EXPRESSION IN THE ADULT OFFSPRING.**

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Exposure to hyperosmotic environment during a pre/postnatal period can differentially program the fluid intake and excretion pattern in a way that persists until adulthood. Our results indicated that maternal voluntary ingestion of hypertonic NaCl solution during pregnancy and lactation until one-week post-weaning affect the sodium and water intake and the brain angiotensinergic type 1a receptor (AT1a) and vasopressin (AVP) mRNA expression induced by osmotic challenges. In order to analyze possible renal alterations, we studied the effect of this perinatal hypertonic sodium exposure on adult offspring kidney mRNA expression of AT1a, AVP type V2 (AVP2) and the TRPV1 channel, during basal condition and after an osmotic challenge.
We studied Wistar Rats, with a perinatal manipulation (M) that covered dams from 1 week before conception until the 28 postnatal day of the offspring. The experimental groups were: -M-NaCl: Free access to 0.45M NaCl solution, food and water; and -M-Ctrol: Free access to food and water. We analyzed the kidney weigh and mRNA expression of adult offspring in basal and after 2M NaCl infusion challenge conditions.
We did not find any significant difference in the kidney weights between the groups. However, the M-NaCl group present a significant increase in the At1a expression in relation to M-Ctrol ($F= 8.09$; $p=0.017$, program effect). By the other hand, we found that 2M NaCl infused animals, present a significant decrease ($F=6.31$; $p=0.026$) of the TRPV1 mRNA expression (postnatal treatment effect). Finally, the AVP2 receptor gene expression did not show a significant difference but had a tendency to increase in M-NaCl animals ($p=0.08$).
Together these and our previous results indicate that the availability of a rich source of NaCl during the perinatal period induces long-term changes at brain and renal angiotensinergic and vasopressinergic systems modulating the behavioral, endocrine and renal response to achieve the hydroelectrolyte homeostasis.

524. (466) **QUERCETIN MITIGATES HIGH-FAT DIET-INDUCED GLUCOSE INTOLERANCE IN PART INDUCING FNDC5/IRISIN IN MUSCLE AND IN L6 MYOTUBES AND WHITE ADIPOSE TISSUE BROWNING**

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Irisin is an exercise-induced myokine that can induce browning of white adipose tissue (WAT) and other metabolic benefits. We previously observed that supplementation with the flavonoid quercetin (Q) mitigated high-fat diet (HFD)-induced glucose intolerance and adipose hypertrophy. In this study, we investigated whether these beneficial effects could be related to Q capacity to activate muscle FNDC5/irisin and uncoupling protein-1 (UCP-1) and others brown markers in the WAT of HFD fed rats. Also, the role of Q on FNDC5/irisin was further investigated in vitro in L6 myotubes triggered with palmitate. Co-administration of Q (20 mg/kg body weight/d) to the HFD during 6 weeks significantly increased FNDC5/irisin pathway and p-AMPK and p-p38 in skeletal muscle compared to the control and HFD groups. In addition, Q significantly upregulated proteins involved in WAT browning (PRDM16, PGC1-α, PPARγ and UCP-1) compared with control and HFD groups. Moreover, in the HFD+Q group we observed a partial and significant BAT weight increase compared to the HFD and Ctrl groups, respectively. In L6 myotubes Q prevented palmitate-decrease GLUT4, PGC1-α and irisin secretion. Q 1 μM also prevented palmitate-downregulated mRNA levels of PGC1α and FNDC5. In addition, PGC-1α siRNA transfection in L6 myotubes abrogated the effects of Q on FNDC5 protein expression. These data suggest that Q activated FNDC5/irisin pathway, in part via PGC-1α activation. Overall, Q supplementation enhanced FNDC5/irisin pathway in L6 myotubes and muscle, activated AMPK, p38 MAPK and GLUT4 in muscle and induced adipose tissue browning in the WAT of rats fed a HFD. These findings support the potential relevance of consuming Q-rich foods or supplements to attenuate high fat diet-induced metabolic alterations.

525. (535) **DOES ZINC DEFICIENCY EXACERBATE METABOLIC DISORDERS INDUCED BY HIGH-FAT DIET?**

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Introduction: Moderate zinc deficiency during fetal and postna-

tal life is associated with cardiovascular and metabolic disorders in male adult rats. **Objective:** We evaluated if fetal and postnatal zinc deficiency exacerbates the extent of adiposity and metabolic dysfunction induced by high fat diet (HFD) in male adult rats. **Methods:** Female Wistar rats received low(L:8ppm) or control(C:30ppm) zinc diets from pregnancy to offspring weaning. C male offspring continued with C(C) or HFD (60% of total calories)(CH) diets. L offspring were fed L (L) or L and HFD(LH) diets. At day 81, plasma adiponectin levels, oral glucose tolerance test (OGTT)and morphology and oxidative state of retroperitoneal adipose tissue (RAT) were measured. **Results:** CH and LH had higher body weight (C:418±13;CH:505±9*;L:401±10;LH:444±5ŧ‡g) and showed an increase of RAT weight, a decrease of adipose cells density and adipocytes hypertrophy (C:4958±388;CH:9621±586*;L:8130±448*; LH:11833±440ŧ‡μm²). LH showed lower body weight and higher adipocyte area than CH. HFD induced a decrease of plasma adiponectin levels(C:8.3±0.6; CH:6.5±0.4*;L:8.6±0.9; LH:6.4±0.1ŧμg/ml) and functional changes in RAT like a decrease of SOD and catalase antioxidant activities and an increase of TBARS (C:0.21 ±0.02;CH:0.38±0.04*;L:0.40±0.06*;LH:0.43±0.05*pmol MDA/mg prot).L rats showed an increase in oxidative stress in RAT. LH and CH showed an increase of OGTT curve area(C:27797±504;CH:30827±971*;L:27826±809; Lh:34851±1344ŧ‡min.mg/d). L, LH and CH showed higher plasma glucose levels after 3 hours of glucose overload. Zinc deficiency exacerbated alterations induced by HDF. Two way ANOVA, Bonferroni post-test,mean±SEM,*p<0.01 Vs C ,ŧp<0.01 Vs L and ‡p<0.01 Vs Ch. N=8 per group **Conclusion:** Zinc deficiency and HFD induce alterations in glucose metabolism and RAT, increasing the predisposition for the development of metabolic diseases in adult life. Zinc deficiency during fetal and postnatal life exacerbates some of these changes.

NEFROLOGÍA

526. (118) **ANGIOTENSIN II EFFECT ON MICROTUBULE DYNAMICS IN RENAL TUBULAR CELLS**

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Tubular remodeling in response to acute kidney injury (AKI) involves the dedifferentiation and regeneration of the remaining epithelial tubular cells. Microtubules (MT) dynamic instability plays a central role in renal repair after AKI. Angiotensin II (AGII) has two main receptors, AT1R and AT2R, which mediate dissimilar effects. During ischemia reperfusion (IR), AT1R mediates a pro-fibrotic response, whereas AT2R facilitates the recovery of the kidney function (our unpublished data). The aim of this work was to investigate AT1R and AT2R participation in the regulation of factors associated with MT dynamic instability that could affect the epithelial tubular cell-response to an AKI. MDCK cells were grown in conditions that assure a well-defined epithelial polarity and treated with 0.5 μM AGII (AGII), AGII plus the AT1R antagonist losartan (5 μM) (AGII + los), or 1 μM C21, AT2R agonist (C21). EB1 is a central regulator of MT dynamic instability that participates in tubulogenesis. AGII induced an increase in EB1 levels which was mimicked by C21 (+50%*, n=3) and was not prevented by Los. α-tubulin acetylation is linked to the presence of stable MT. Our preliminary data showed that activation of AT2R, but not AT1R, decreased the fraction of acetylated α-tubulin (Control (C): 0.55 ± 0.05; AGII: 0.25 ± 0.05; AGII + Los: 0.06 ± 0.03; C21: 0.14 ± 0.03, n=2). Primary cilia are organelles of tubular cells that are down-regulated during AKI-tubular remodeling, whose length directly correlates with the levels of acetylated α-tubulin. Analysis of the primary cilia showed that through AT1R AGII increases whereas through AT2R it decreases the cilia length (in μm: C: 2.8 ± 0.1; AGII: 3.1 ± 0.1; AGII + Los: 2.4 ± 0.1 *; C21: 2.3 ± 0.1 *, n>80). Overall, our results indicate that AGII increases MT dynamic instability through AT2R, which would favor tubular remodeling. Our future studies will evaluate the relevance of these effects in the response to IR induced AKI. * p<0.05 vs C.

527. (190) **RENAL EXPRESSION OF Na-K-Cl COTRANSPORTER 2 AND AQUAPORIN 2 IN RATS WITH ACUTE OBSTRUCTIVE CHOLESTASIS**

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Na-K-Cl cotransporter 2 (NKCC2) and Aquaporin 2 (AQP2) are proteins localized in the apical membrane of the renal thick ascending limb of Henle's loop (TAL) and the collecting duct (CD), respectively. NKCC2 performs active transport of NaCl in the TAL, which contributes to create the corticomedullary concentration gradient. The interstitial hypertonicity achieved in the renal medulla leads to water absorption through the AQP2 water channel in the CD. In acute obstructive cholestasis (OC), increases in urinary flow and in the fractional excretion of osmolytes were observed. In order to evaluate the roles of NKCC2 and AQP2 in the modifications of renal handling of water and osmolytes detected, renal expression of NKCC2 and AQP2 was evaluated in rats with OC. Bile duct ligation of 21 h (BDL, n=4) was performed in Wistar rats. Sham-operated rats served as controls (S, n=4). Apical membranes were isolated from renal cortical (C_{AM}) and medullary (M_{AM}) tissue. The expression of NKCC2 and AQP2 was evaluated by immunoblotting and immunohistochemistry. %NKCC2: C_{AM}: S=100±8 BDL=71±8*; M_{AM}: S=100±4 BDL=91±4. %AQP2: C_{AM}: S=100±10 BDL=98±13; M_{AM}: S=100±3 BDL=81±4* (*p<0.05). The immunohistochemistry confirmed the data obtained by immunoblotting. In BDL rats, NKCC2 protein expression remained unchanged in M_{AM}, while it decreased in C_{AM}. AQP2 protein expression decreased in M_{AM} of BDL rats, where it is mostly localized. The decrease in the expression of NKCC2, in C_{AM} of BDL rats, could lead to a decrease of the interstititial hypertonicity by reducing the reabsorption of sodium, potassium and chloride and, consequently, avoiding the subsequent reabsorption of water. The decrease in AQP2 expression could contribute to increase the urine output, by decreasing the reabsorption of water in the CD.

528. (546) **EFFECTS OF THE ANGIOTENSIN II TYPE 2 RECEPTOR (AT2R) AGONIST, COMPOUND C21, ON THE EXPRESSION OF APELIN AND ITS RECEPTOR APJ IN THE POST-ISCHEMIC KIDNEY. POSSIBLE INTERACTION BETWEEN RENIN-ANGIOTENSIN AND APELINERGIC SYSTEMS.**

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In previous experiments we observed that pretreatment with the AT1R antagonist, losartan, or the AT2R agonist, C21, prevents glomerular filtration drop, loss of sodium reabsorption, and loss of brush border in a renal ischemia/reperfusion (I/R) model. On the other hand, we have found that I/R decreases the expression of the adipokine apelin; and in turn the pretreatment with apelin prevents the impairment of proximal tubular transport and has anti-inflammatory effects in this model. We also demonstrated that losartan prevents the decreased expression of apelin and its receptor APJ. The aim of this study was to evaluate the effects of the AT2R agonist, C21, on apelin and APJ mRNA levels in an I/R model. Male Wistar rats (5-6 per group) underwent unilateral renal ischemia for 40 min followed by 24 h of reperfusion (I/R) or sham surgery (C). C21, 0.3mg/Kg/d i.p., or its vehicle was administered for two days prior to I/R. mRNA levels were analyzed by qRT-PCR. Apelin mRNA levels decreased in I/R. This decrease was prevented with C21 pretreatment (%change fold C: C+C21=+15; I/R= -92*; I/R+C21= -12#; *p<0.05 vs. C, #p<0.05 vs. I/R). Changes in APJ mRNA expression were: C+C21=+490*; I/R= -90*; I/R+C21= +750*#. These data indicate that C21 promotes the synthesis of both the nephroprotective adipokine apelin and its receptor APJ in I/R, suggesting the participation

of APJ and its ligand in the beneficial effects previously described for C21 in I/R model. This data, reinforce the idea of an interaction between renin-angiotensin and apelinergic systems in post-renal injury repair processes.

NEUROCIENCIAS

529. (375) **ROLE OF DIETARY OMEGA-3 PUFA IN DEPRESSION AND MEMORY IN A RODENT MODEL**

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Background: dietary intake of omega-3 polyunsaturated fatty acids (ω3 PUFA) is reflected in the brain fatty acid composition. These essential nutrients can modify cellular and behavioral processes related to anxiety, depression and memory. In a previous work, we observed that dietary ω3 PUFA deficiency in mouse produced changes associated with the development of depressive-like behavior -like hypermotility and up-regulation of AVP and AVPr1 genes in the hypothalamus and pituitary, respectively- and reduced the hippocampal gene expression of NMDA1, which plays a role in memory formation. On the other hand, ω3 PUFAs excess increased the LTP-related gene CREB1a. Objectives: to assess the effect of dietary ω3 PUFA level on depressive-like behavior and nitric oxide (NO) level in the hippocampus, molecule related to memory formation process. Material and methods: albino Swiss mice were exposed to ω3 PUFA Control (C; soybean oil, 7%), Deficient (D; sunflower oil, 7%) or Excess (E; blend oil; 4.2% cod-liver+2.8% soybean) diet before conception till adulthood, and at this time point, the depressive-like behavior was evaluated through the tail suspension test (TST) in male animals. After that, animals were euthanized and their hippocampi dissected to determine NO level by Griess. Statistics: ANOVA and LSD post hoc test. Results: the immobility time (s) in TST was higher in D group (79.8±14.7) compared to C (43.3±8.7) and E (59.1±10.8) groups ($P<0.05$ indicated by LSD post hoc). Furthermore, there were no differences in NO level among dietary groups. These results support the hypothesis that ω3 PUFA deficiency induces depressive-like predictive effects, and suggest that the hippocampal NO pathway should not be implicated in the changes on memory gene expression produced by both ω3 PUFA deficiency and excess. Further investigations are needed to better understand the relevance of dietary PUFA ω3 in depression and memory.

530. (496) **ASSESSMENT OF OLFACTORY FUNCTION IN A CLINICAL SETTING**

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Smell detects, recognizes and identifies environmental volatiles. The aim of this work was to evaluate smell performance of a group of 350-450 health workers as part of a screening task of acute loss of smell in COVID-19 pandemic context.

Smell identification (SI) and self reported judgments of odor (SRO) and taste (SRT) were evaluated. A battery of 12 commercial food odorivectors (peach, pineapple, berries, banana, mint, cherry, vanilla, coffee, vinegar (V) and others) were smelled by means of filter paper strips impregnated in 20 % V/V aqueous solutions (V at 4,5 % V/V) in four sessions (S1 to S4). In each session, SI was evaluated with a set of four randomized odorivectors: (V) and other three essences to avoid sensory adaptation. A multiple choice paradigm (MCP) with five randomized odorivector options was always offered with the algorithm of google forms (GF).

SI was quantified by number of hits: 0 (anosmic, A), 1-2 (hyposmic, H) and 3-4 (normosmic, N). SRO (How do you consider to detect or discriminate odors?) and SRT (How do you consider to detect or discriminate tastes?) were self categorized as poor (I), normal (II) or high sensitivity (III). Gender (G), age (AG), smoking status (SS) were reported and body mass index (BMI) was calculated. Collected data were analyzed by descriptive statistics, categorical chi-square and one-way analysis of variance tests (AOV).

For SI judgments very few values for A and H categories were found (S1=0 and 7; S2=1 and 21; S3=4 and 60; S4=1 and 22). Instead N encompassed 403, 378, 390 and 315 assessors respectively. SRO (I=2,9%, II=89,9% III=7,2%) and SRT (I=1,6%, II=92% III=6,4%) revealed predominantly normal self reported chemosensory functions. SI status was independent of G, AG, SS or BMI levels (chi-square values with $p> 0.05$) and hits depend of odorivector (AOV $p< 0.01$), where V, mint, cherry and vanilla were the most identified odors. MCP, several food odors and GF give a useful tool to elucidate acute loss of smell.

531. (461) **VENLAFAXINE RESTORES MEMORY IMPAIRMENT IN AN ANIMAL MODEL OF DEPRESSION.**

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Background: major depressive disorder is a prevalent, chronic, disabling, and multidimensional mental illness. Cognitive dysfunction represents a core diagnostic and symptomatic criterion of MDD, and is a principal determinant of functional non-recovery. Venlafaxine (VEN), a serotonin-norepinephrine reuptake inhibitor (SNRI), is an antidepressant drug commonly used to treat a wide spectrum of mood disorders including treatment-resistant depression. Despite decades of clinical use, the impact of VEN on memory processes is still incompletely understood. Objectives: in the present study we investigated VEN effect on memory and nitric oxide (NO) level in the hippocampus in an animal model of depression (olfactory bulbectomy). Material and methods: thirty-two adult male albino Swiss mice were divided into sham and olfactory bulbectomized (OB) groups, and orally treated during 28 days with saline (S) or VEN (10 mg/kg/day). The last day of treatment, the tail suspension test (TST) was performed and then, long-term memory retention was evaluated using the object recognition test (ORT). After that, animals were euthanized and their hippocampi dissected to determine NO levels by Griess. Statistics: two-way ANOVA test followed by LSD *post hoc* test whenever appropriate. Results: sham-VEN and OB-S animals showed a memory impairment compared with sham-S animals ($F(1,31)= 12.46$, $p\leq0.05$), and OB-VEN animals exhibited enhanced memory performance in ORT compared to OB-S ($p\leq0.05$). Furthermore, a significant reduction in NO levels was detected in OB-S but VEN treatment reversed this effect in OB group (OB-S 1.25 nmol/μg protein vs. OB-V 3.79 nmol/μg protein). These results suggest that VEN counteracts memory impairment in OB animals with participation of the NO pathway. To improve the diagnosis and treatment of depression and its associated cognitive impairments, a better understanding of the neuropathophysiological basis of this disabling illness is required.

ONCOLOGÍA

532. (3) **SPECIFIC ISOTYPES OF THE RETINOIC ACID RECEPTOR (RARS) DIFFERENTIALLY MODULATE PARAMETERS ASSOCIATED WITH METASTATIC SPREAD IN TRIPLE NEGATIVE MAMMARY CANCER CELLS.**

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Migration and adhesion are highly related to metastatic dissemination and retinoid system is implicated in their modulation.
Objective: Evaluate the effect of activating each retinoic acid receptor (RAR) isotype in migration, soluble MMPs activity, adhesion and metastatic potential in LM38-LP and 4T1 triple negative murine cell lines. Both cell lines express all RAR isotypes with the exception of RARβ in 4T1 cells.
Cells were treated with the RARα agonist AM580 (200 nM), RARβ agonist AC55649 (2 μM), RARγ agonist BMS961 (50 nM) or vehicle (DMSO). Migratory potential was evaluated by wound healing assays. AM580 and AC55649 reduced LM38-LP migratory capacity ($p<0.05$) while AM580 increased migration in 4T1 cells ($p<0.05$). MMPs were analyzed by zymography. AM580, AC55649 and BMS961 decreased soluble MMP2 activity in LM38LP. On the contrary, AM580 increased both MMP2 and MMP9 activities in 4T1. Besides, AM580 and AC55649 diminished LM38LP adhesive capacity while AC 55649 increased this potential in 4T1. Finally, a lung colonization assay was performed. Cells were pretreated with the agonists and then inoculated into syngeneic BALB/c mice. While AC55649 increased metastatic potential of LM38-LP cells ($p<0.05$), BMSS961 increased this potential in 4T1 cells ($p<0.05$).
We hypothesize that the differences in RARβ expression between the cell lines could be responsible of opposite biological effects observed. The increase in metastatic potential after RARβ/γ activation could be associated with the selection of a minority population with high plasticity to colonize the target organ.

533. (85) **INSULIN-LIKE GROWTH FACTOR 2 mRNA-BINDING PROTEIN 1 (IGF2BP1) UPREGULATION BY SORAFENIB IN HEPG2 CELLS. ROLE OF SIRTUIN 1 AND 2 (SIRT1/2).**
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Resistance to sorafenib (Sfb) still remains one of the major cause of treatment failure in Hepatocellular carcinoma (HCC). This condition often involves ABC-transporters upregulation, like multidrug resistance-associated protein 3 (ABCC3), that extrudes Sfb from hepatic cells. IGF2BP1 is an oncoprotein highly expressed in HCC which modulates its target genes by affecting their stability, translatability or localization. In a previous work we showed that IGF2BP1 knockdown results in downregulation of ABCC3 in HepG2 cells and that SIRT1/2 inhibitors suppress Sfb-mediated induction of ABCC3. **Aim**: To evaluate the effect of Sfb on expression of IGF2BP1 and ABCC3 proteins and potential mediation by SIRT1/2. **Methods**: EX-527 was used as SIRT1/2 inhibitor. Experimental designs were: I) HepG2 cells were treated with 0.75, 2 and 5 μM Sfb (selected from a previous dose-response curve) for 48 and 72 h. II) HepG2 cells were treated for 72 h with Sfb (2μM), EX-527 (40μM) or both. **Results:** All results are expressed as % of control (C) and presented as mean±SD, n=3-6, $p<0.05$. Sfb (2 μM) increased IGF2BP1 protein levels at 48 h (135±10* vs C: 100±13). After Sfb treatment for 72 h, IGF2BP1 protein level was increased at all tested concentrations: 0.75, 2 and 5 μM (167±9*, 189±30*, 163±12*, respectively, vs C: 100±22). ABCC3 protein levels were increased by Sfb 2 μM both at 48 h (154±3* vs C: 100±13) and at 72 h (151±15* vs C: 100±20). Inhibition of SIRT1/2 prevented Sfb from inducing IGF2BP1 (72±18* vs C: 100±10). **Discussion:** Our data suggest that SIRT1/2 proteins are involved in IGF2BP1 upregulation by Sfb. It remains unclear if the induction of IGF2BP1 is responsible for ABCC3 upregulation by Sfb. Experiments silencing IGF2BP1 are currently being performed to elucidate this hypothesis. Targeting IGF2BP1 could be a useful tool to modulate multidrug associated resistance proteins, at least ABCC3, in order to minimize the resistance to sorafenib in HCC.

534. (90) **CD13 EXPRESSION AND INTERACTION WITH ITS BINDING-PARTNER GALECTIN 1 IN LIVER TUMOR AND SINUSOIDAL ENDOTHELIAL CELLS**
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CD13/aminopeptidase N is a glycosylated membrane exopeptidase whose expression is increased in endothelial cells during tumor-related angiogenesis. The β-galactoside-binding protein galectin-1 (Gal1) is up-regulated in hepatocellular carcinoma (HCC), the most common type of liver cancer. Previously we found that hepatocyte-derived Gal1 promotes liver sinusoidal endothelial cell (LSEC) proliferation and migration, suggesting Gal1 contribution to angiogenesis in liver tumors. Recently, using a proteomic approach we identified CD13 as a novel ligand for Gal1 in human SK-HEP-1 LSECs.
Here, we analyzed CD13 expression in a wide set of human cancer tissues using Oncomine and The Cancer Genome Atlas (TCGA) databases and in liver tumor samples through UALCAN platform. CD13 expression was decreased in HCC samples (n=371) compared with normal tissues (n=50) ($p<0.001$). No statistical difference was found between individual cancer stages. Kaplan-Meier plotter indicated a better survival for low CD13 mRNA expression in liver cancer patients ($p<0.01$). Conversely, high levels of Gal1 correlated with poor overall patient survival ($p<0.05$).To further understand the potential involvement of CD13 in HCC progression and angiogenesis, we performed gene ontology (GO) analysis. GO annotation, using GOrilla platform indicated that CD13 co-expressed genes are mainly involved in cell surface receptor signaling pathways related to cell-cell and canonical Wnt signaling and regulation of cytokine secretion ($10^{-5} > p < 10^{-3}$). By Western blot we confirmed CD13 expression in human HepG2 liver cancer cells and SK-HEP-1 LSECs and by Far Western Blot we observed Gal1/CD13 interaction in SK-HEP-1 cells. Also, a SK-HEP-1 cell membrane fraction was applied to a Gal1-affinity column. Gal1-binding proteins were eluted with lactose and by Western blot CD13 was confirmed as a ligand for Gal1. Our findings encourage the study of the relevance of this interaction in angiogenesis during liver tumor progression.

535. (400) **EFFECT OF ALKALINE GRADIENT ON CLEAR RENAL CELL CARCINOMA PROLIFERATION: ROLE OF ISOFORM 1 OF NA^+/H^+ EXCHANGER FUNCTION.**
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The association between proliferation and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancer, is probable that pHe affects differently cancer or normal cells. Our previous studies showed that cells derived from renal cell carcinoma (RCC) were more sensible to cell death after 72h exposition to 9.6 mM NaOH (mild alkalosis) than normal cells. The aim of this study was to investigate if this alkaline condition also affects cell proliferation. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with 9.6 mM NaOH for 72h. Then, we estimated cell proliferation by BrdU experiments. Our results show at pH 7.4 both RCC derived cell lines proliferate more than normal HK2 cells (% BrdU+ cells: HK2= 31±2; 786-O= 46±3 and Caki-1= 58±3, $p<0.05$ vs HK2 n=10). Normal HK2 cells were not affected by exposure to 9.6mM NaOH for 72hs. However, malignant Caki-1 significantly reduced their proliferation (% BrdU+ cells, pH 7.4= 58±3 vs pH 7.5= 18±3, $p<0.001$ n=8). Previous studies showed that NHE1 isoform of Na^+/H^+ exchanger (NHE1) can favor or inhibit proliferation in some experimental models. Then, we inhibited NHE1 during the 72h exposition to 9.6 mM NaOH. In normal cells, NHE1 inhibition significantly reduced proliferation in alkaline condition (% BrdU+ cells, +NHE1= 37±1 vs -NHE1= 31±1, $p<0.05$ n=8). On the other hand, inhibition of NHE1 in RCC derived 786-O cells rises pro-

liferation in media at pH 7.4. This effect is partially reverted in the presence of alkalosis (Difference in % BrdU+ cells without NHE1 pH 7.4: 14±3 vs pH 7.5: 3±1, p<0.05 n=8). In summary, the combination of alkali plus NHE1 activity reduces tumor proliferation with little effects in normal tissue. Then, this combination of treatment could be an interesting new approach to control RCC cancer.

536. (167) **CD105 IN SPINDLE-SHAPED STROMAL CELLS, A PROGNOSTIC DETERMINANT OF EARLY LUMINAL BREAST CANCER**

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Cancer associated fibroblasts (CAF) play an important role in breast cancer (BC) evolution and could be an interesting target in the development of future therapies. Many of them are α-SMA + ,FSP + , FAP + , CD34 - and CD31 - spindle cells. CAF can originate from bone marrow-mesenchymal stem cells and can be recognized by the expression of CD105 and CD146. Interestingly, 50% of CAF express CD105 in BC-stroma. Previously, we found that the CD34 - spindle-shaped stromal cells from primary tumors of BC patients (BCP) express CD105 but we did not observe its expression in non-malignant breast tissues. Considering that BCP with bone metastasis (BM) have a median overall survival (OS) from BC diagnosis of 8.7 years, it is imperative to find a biomarker that can predict this event. We propose that high CD105 expression could be a prognostic factor of the occurrence of BM in BCP, particularly in the luminal subtype which is known to preferentially develop this type of metastasis. In this retrospective study, we investigate the clinicopathological significance of CD105 in CAF, as prognostic determinant of early BCP. We performed immunohistochemical analysis for CD105 expression in CAF of primary invasive ductal tumors from early BCP (luminal subtype), and analyzed their association with clinicopathological characteristics. Results showed that high CD105 expression in CAF of BCP was significantly associated with a higher risk of metastatic occurrence (p=0.029), in particular BM (p=0.035). Moreover, high CD105 expression was associated with shorter disease-free survival (DFS), metastasis-free survival (MFS), BM free survival (BMFS) and OS (p=0.005, 0.006, 0.013 and 0.008). Finally, CD105 expression was an independent prognostic factor for DFS, MFS, BMFS and OS (p=0.015, 0.034, 0.041 and 0.040). In conclusion, this is the first demonstration that high CD105 expression in CAF could be used as prognostic marker of BM occurrence and lower DFS, MFS, and OS in early luminal BCP.

REPRODUCCIÓN

537. (143) **AQP9 MEDIATES LACTATE TRANSPORT IN HUMAN PLACENTA AS AN ALTERNATIVE ENERGY SUBSTRATE**

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Emerging evidence shows that placental aquaporin-9 (AQP9) is not involved in the transfer of water between the mother and the fetus. However, its role in human placenta is still unknown. AQP9 is an aquaglyceroporin that also permeates other solutes such as lactate. In brain, AQP9 may transport lactate as an alternative energy substrate.
OBJECTIVE: Our aim was to evaluate the participation of AQP9 in the lactate transfer across the human placenta.
METHODS: This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were cultured in low glucose with or without L-lactate, and in presence and absence of AQP9 inhibitors (0.3 mM HgCl2, a general blocker of AQPs, or 0.5mM Phloretin, to block AQP9). Normal glucose medium was used as control. Cell viability was assessed by MTT assay and LDH release. Apoptosis indexes were analyzed by Bax/Bcl-2 protein expression ratio and TUNEL assay.
RESULTS: In low glucose medium, MTT decreased while LDH release did not change compared to controls, suggesting that cell death is not due to necrosis. Moreover, Bax/Bcl-2 ratio and apoptotic nuclei increased (n=5, p <0.02) and the blocking of AQP9 did not abrogate apoptosis. However, when explants were cultured in low glucose medium supplemented with L-lactate, explant viability and apoptotic indexes were similar to controls indicating that L-lactate could be replacing glucose as an energy substrate. In this case, the blocking of AQP9 resulted in an increase in cell death (n=4, p <0.05), proposing that this protein has a role in lactate transport.
CONCLUSION: Our results show that placental AQP9 may have a key role in lactate transport as an alternative energy substrate. Thus, the blocking of lactate transport mediated by AQP9 negatively affects the survival of trophoblast cells.

538. (232) **ASSESSMENT OF FERTILITY IN MALE MICE CHRONICALLY EXPOSED TO VARIABLE DIETARY OMEGA 3 FATTY ACID LEVELS**

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Background: dietary levels of omega-3 polyunsaturated fatty acids (ω3 PUFA) are reflected in tissue PUFA composition and can influence several processes of mammalian reproductive function. In a previous mouse study, we observed that chronic dietary ω3 PUFA deficiency produced an increase of bending immature sperm forms, which in turn can be harmful to sperm migration and oocyte fertilization. Objectives: to assess the effect of dietary ω3 PUFA level on male reproductive capacity in vivo and the oxidative stress in epididymal sperm. Material and methods: albino Swiss mice were exposed to ω3 PUFA Control (soybean oil, 7%), Deficient (sunflower oil, 7%), or Excess (blend oil; 4.2% cod-liver+2.8% soybean) diet before conception until adulthood. Five males per treatment were individually mated to two adult females fed with commercial pellet. On gestational day 18, dams were euthanized and their uteri and ovaries dissected to assess: number of live and atrophied fetuses, litter weight, number of corpora lutea and embryo loss. Other eight males per treatment were euthanized, and their epididymides dissected to evaluate the sperm production of reactive oxygen species (ROS) by chemiluminescence. Statistics: ANOVA and LSD post hoc test. Results: no significant differences in the assayed reproductive parameters were found among groups: number of fetuses ($F(2,26)= 0.63$, $P=0.53$), atrophied fetuses ($F(2,26)=0.08$, $P=0.92$), litter weight ($F(2,26)=1.17$, $P=0.32$), corpora lutea ($F(2,26)= 0.06$, $P=0.93$) and percentage of embryo loss ($F(2,26)= 1.53$, $P=0.23$). Regarding sperm ROS production, no significant differences among

experimental groups were found (F(2,21)=0.18, P=0.83). Conclusions: these results indicate that bending sperm immaturity in PUFA ω3 deficient mice is not related to higher ROS production and does not affect in vivo fertilization capability. Further investigations are needed to better understand the relevance of dietary PUFA ω3 in male fertility.

539. (278) **HYPEROSMOLARITY INDUCES CAVEOLAE INTERNALIZATION IMPAIRING EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION**

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During placentation, human extravillous trophoblast (EVT) cells need to proliferate, migrate, and differentiate correctly to ensure proper placental development. Previously, we reported that caveolae are required for the proper migration and endovascular differentiation of EVT. Recently, we found that hyperosmolarity alters EVT cell migration and invasion. However, up to now, the molecular mechanism is unknown. We hypothesized that hyperosmolarity increases caveolae endocytosis and caveolin-1 (Cav-1) degradation, altering EVT cell differentiation.

Objectives: Our aim was to explore the effect of hyperosmolarity on caveolae microdomains and the impact on the EVT cell differentiation.

Methods: The human EVT Swan-71 cell line was cultured in complete DMEM/F-12. 100 mM of sucrose was added to generate the hyperosmolar condition. Cell viability was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The localization of caveolae was analyzed by Transmission Electron Microscopy (TEM). Cav-1 expression was determined by WB in different conditions (isoosmolarity or control and hyperosmolarity, with or without MG-132- a proteasome inhibitor- and NH_4Cl- a lysosomal inhibitor). Endovascular differentiation was analyzed by the formation of tube-like structures in plates coated with Matrigel®.

Results: Cell viability was not affected by the experimental conditions. TEM showed that hyperosmolarity induced the internalization of caveolae. In addition, hyperosmolarity also increased Cav-1 protein degradation by lysosomal proteolysis ($p<0.05$, n=3) and significantly reduced the formation of tube-like structures compared to control ($p<0.05$, n=4).

Conclusion: Our results show that hyperosmolarity leads to the internalization of caveolae and the degradation of Cav-1, impairing endovascular differentiation of EVT cells.

540. (362) **THE ROLE OF AQP3 IN AMNION CELLS EXPOSED TO AN OSMOTIC STRESS**

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INTRODUCTION: AQPs in fetal membranes have been proposed to regulate the amniotic fluid volume. Altered expression of these proteins might be associated with oligo and polyhydramnios syndromes. However, we recently observed that the blocking of AQP3 did not prevent cell swelling in amnion cells. In addition, under osmotic stress the pattern expression of amnion AQP3 was different from other AQPs, suggesting a different role for this protein.

OBJETIVE: To study the regulation of AQP3 and its role in the amnion.

METHODS: Amnion-derived WISH cells were cultured in hypo (150 mOsm) and hyperosmolar (400 mOsm) conditions. Levels of phosphorylated ERK (pERK), JUNK (pJNK) and p38 (p-p38) were studied. Nf-κB and tonEBP expressions were assessed in nuclear and cytosolic fractions. AQP3 expression was analyzed after the inhibition of Nf-κB and tonEBP pathways with Sodium Salicylate and Cyclosporine-A, respectively. Cell viability was studied by MTT assay. Apoptosis was studied by TUNEL assay and Bax/Bcl-2 ratio after the inhibition of AQP3 using $CuSO_4$ or the specific siRNA.

RESULTS: pERK levels increased in hyperosmolarity and did not change in hypoosmolarity ($p<0.001$; n=6). No significant differences were observed in p-p38 and pJNK (ns; n=6). Nf-κB and tonEBP expressions increased in nuclear fraction only in hyperosmolarity ($p<0.05$; n=5; $p<0.01$; n=5). In this condition, the blocking of Nf-κB pathway increased AQP3 expression ($p<0.001$; n=5) compared to controls, while the inhibition of tonEBP pathway did not modify its expression. Regarding cell viability in hiperosmolarity, the blocking of AQP3 decreased MTT incorporation ($p<0.01$; n=8). Moreover, Bax/Bcl-2 ratio and the number of apoptotic nuclei increased after $CuSO_4$ treatment ($p<0.001$; n=5; $p<0.001$; n=9) and AQP3 silencing ($p<0.05$; n=5; $p<0.01$; n=10).

CONCLUSION: Our findings suggest that AQP3 may have an important role in the survival of the amniotic cells and its expression may be regulated by ERK, Nf-κB and tonEBP pathways.

TOXICOLOGÍA

541. (303) **GLYPHOSATE NEUROTOXICITY IN ADULT RATS: ANALYSIS OF NEURO-MOTOR AND BEHAVIORAL PARAMETERS**

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The neurotoxicity caused by glyphosate (Glyph) exposures has long been described in several studies; however, there are few studies that extensively evaluate an important variable such as motor activity. Dysfunctions on motor activity induced by pesticides can be determined with relatively simple techniques and may be used as a component of the FOB (Functional Observational Battery). The aim of this experiment was to determine the neuro-motor and behavioral changes caused by sub-acute administration of Glyph. Male Wistar rats 80-90 days old were treated subcutaneously with a Glyph solution (Sigma, without adjuvants) in saline phosphate buffer (PBS). One dose of 70 mg/kg was tested every 48 hours for 14 days (n=12). A group of rats was used as control (n=12) that was injected with the vehicle. Body mass was recorded daily. Two tests were carried out in control and treated animals to determine the locomotor activity: open field and elevated plus maze, according to established protocols. The test recording was done through video camera, and then the data and statistical analysis were carried out by t student method. We found that Glyph exposed rats showed a decrease in motor activity time ($p<0.05$; p=0.0194) and distance traveled ($p<0.05$; p=0.0167) by the open field test. Regarding elevate plus maze test, rats exhibited a decrease in motor activity time ($p<0.05$; p=0.0246). On the other hand, Glyph exposed animals showed a significant decrease on body weight throughout the treatment (p=0.0001). In conclusion, these findings suggest that sublethal doses of Glyph exposure alter nervous system functionality impairing motor and behavior parameters.