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THE DUAL ROLE OF INFLAMMATION IN CANCER

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Abstract Inflammation may have controversial effects, it may be able to eradicate tumor cells and also, when it becomes chronic it can promote tumor growth. Resolution of inflammation and clearance of inflammatory cells remains a process that should to be studied more extensively. Here, we review our results using a lung adenocarcinoma LP07 that develops paraneoplastic syndromes such as hypercalcemia, cachexia and leukocytosis during its evolution. The role and activation of polymorphonuclear neutrophils were studied during tumor development and the cytotoxicity of splenic lymphocytes was determined. The treatment with non steroid anti-inflammatory drugs (NSAIDs) resulted in a reduction of tumor and metastases. The target of NSAIDs by COX-2 antisense transfection of LP07 cells was investigated. The results showed that NFκB was the main target of NSAIDs. The production of IL-1β and IL-6 cytokines and the proteolytic enzyme MMP-9 during tumor growth were also investigated. In this tumor model chronic inflammation induced by the tumor plays an important role in its progression.

Kew words: cancer, inflammation

Resumen ***El rol dual de la inflamación en cáncer.*** La inflamación puede tener efectos duales, por un lado es un componente de la respuesta inmune innata, que elimina agentes extraños, tales como podrían ser las células tumorales, pero si llega a cronicidad puede tener efectos pro tumorigénicos. En este artículo se resumen los resultados obtenidos utilizando un modelo de adenocarcinoma de pulmón LP07, que presenta síndromes paraneoplásicos, como hipercalcemia y leucocitosis, a expensas de polimorfonucleares neutrofilos y caquexia, es decir una respuesta de inflamación sistémica. Se estudió el rol y la actividad de los neutrófilos polimorfonucleares durante la progresión tumoral. Se determinó la capacidad citotóxica de leucocitos esplénicos. Se utilizaron antiinflamatorios no esteroideos (AINEs) para estudiar su efecto sobre la progresión tumoral, y determinó una reducción del crecimiento del tumor primario y sus metástasis. Para evaluar la función de la enzima COX-2 se utilizaron células LP07 transfectadas con el vector COX-2 antisentido. Se determinó que la acción de los AINEs fue más importante sobre el factor de transcripción NFκB que sobre COX-2. Se cuantificaron las citoquinas IL-1β e IL-6 y la actividad de la enzima proteolítica MMP9. En este modelo tumoral la inflamación crónica inducida por el tumor favorece su progresión.

Palabras clave: cáncer, inflamación

Inflammation, like most biological processes, may have dual effects. Under some conditions or environmental factors, it may activate host immune defenses, while under others, it may induce opposite effects. Acute inflammation is the innate immune response that leads to adaptive immunity; but when it becomes chronic it increases the risk to develop several diseases. It is a health promoting response that is normally self-limiting; but chronic inflammation can result from the persistence of an inflammatory stimulus or the dysregulation of endogenous anti-inflammatory mechanisms. The resolution of inflammation is an active by actively coordinated process that requires the production of anti-inflammatory mediators, the termination of proinflammatory pathways, and the appropriate clearance or migration of inflammatory cells. Disruption of any of these events can lead to chronic and persistent inflammation[1].

During acute inflammation, recruitment of polymorphonuclear granulocytes takes place followed by monocytes which differentiate into local macrophages. This response is often initiated by the activation of macrophages through pattern-recognition receptors and it is followed by triggering the sequential release of pro-inflammatory mediators such as cytokines IL-1α, IL-1β, IL-6, TNF-α, COX-2-derived eicosanoids, 5-LOX products[2], proteases such as MMP-9, chemokines IL-8 (CXC8), IL-18, and growth factors such as VEGF, which lead to leukocyte recruitment and activation[1, 3-8]. Some of these molecules may have both protective and pro-inflammatory roles, like

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nitric oxide (NO), or PGE_2, depending on the inflammatory stimulus and on other molecules present in the environment [5]. These mediators can act with synergy and redundancy, and it is thought that their inhibition can have anti-inflammatory effects. Most of the genes that are overex-pressed as a consequence of these mediators, are regulated by the transcription factor NFκB[8, 9]. NFκB is active in most tumors and can be induced by carcinogens, tumor promoters, chemotherapeutic agents, and gamma irradiation[8]. While acute inflammation is a part of the host defense mechanism, chronic inflammation can lead to cancer, diabetes, and pulmonary, cardiovascular and neurological diseases. There are clear links between chronic inflammation and cancer[1, 4, 9]. First, inflammation or persistent infection may create a context of tissue injury that promotes cell transformation through DNA damage. Second, tumor cells may also produce proinflammatory factors that lead to chronic inflammation and thus enhance tumor growth.

Many mediators and signaling mechanisms that mediate inflammatory reactions have been identified but not much is known about the factors that mediate resolution of inflammation[1]. It has been demonstrated that resolution is a coordinated process which depends on specific factors; cytokines as IL-10, TGFβ, and lipid mediators such as lipoxins and prostaglandins[1]. It requires apoptosis of leukocytes and their phagocytic clearance by local macrophages. This process is linked to the downregulation of neutrophil and macrophage activation and promotes the release of anti-inflammatory mediators such as TGFβ. Another clearance mechanism is migration of antigen presenting cells (APC), through lymphatics which provide the means to prime T and B cells in local lymph nodes.

During the inflammatory process, myelopoyesis and hematopoyesis may be impaired. An accumulation of myeloid cells that share the $CD11b^+$ and $GR1^+$ markers, with a mixed mature-immature phenotype can be observed, which are responsible for the induction of T cell and dysfunctions. They comprise mature granulocytes, monocytes and varying numbers of immature cells of the myelomonocytic lineage and are called myeloid derived suppressor cells (MDSC). These cells are present in large number during the progression of some tumors and inhibit effector immune mechanisms[10].

Persistence of inflammation and pro-inflammatory molecules can lead to abrogation of activation of antitumor immunity.

LP07 Lung adenocarcinoma

In the Animal Care Laboratory, at the University of Buenos Aires Oncology Institute A.H. Roffo, we have been studying extensively a lung adenocarcinoma P07, which appeared spontaneously in a BALB/c mouse of our inbred colony. Tumor progression is associated with some paraneoplastic symptoms that resemble NSLCC human lung adenocarcinomas, such as leukocytosis, mainly due to polymorphonuclear leukocytes (PMN), hypercalcemia and cachexia[11]. The cell line derived from this tumor, LP07, presents the same characteristics as the parental tumor[11, 12]. Altogether these symptoms reflect a systemic inflammatory response present in the host. We have reported that LP07 tumor cells secrete IL-6, IL-1β, GM-CSF and PThrP (parathyroid hormone related protein) was detected in serum[13].

LP07 tumor was used as a model to study the role of chronic inflammation induced by the tumor on metastases outcome and paraneoplastic syndrome development (Table 1 A and B).

A. PMNs have a central role in innate immunity, and in normal resolution of inflammation they undergo apoptosis. However, in an inflammatory milieu their survival and apoptosis are severely impaired.

TABLE 1.– *Characteristics of LP07 tumor*

A	LP07 Tumor bearing mice	Normal mice
Leukocytes (cells x 10^3 /µl blood)	55 ± 15	6.5 ± 0.58
PMN (%)	70	30
Body weight loss (%)	12	–
Hypercalcemia (Ca^+mM)	6.7 ± 0.2	5.02 ± 0.01
Metastases	26 ± 10	–
Spleen weight (mg)	440 ± 39	113 ± 15
Serum IL-6 (pg/ml)	193 ± 25	62 ± 7
Serum IL-1β (pg/ml)	4.2 ± 1.5	18 ± 2
Plasmatic PTHrP (pM)	11 ± 0.3	0.9 ± 0.3

B	10^6 LP07 cells
IL-1β	8.21 ± 0,86 pg/ml
IL-6	201pg/ml ± 42
GM-CSF	501.8 pg/ml ±40
MMP-9	1.79 AU/mg prot
PGE2	6691 pg/ml

As PMNs are the largest leukocyte population in LP07 peripheral blood, they were used to determine their role in LP07 tumor progression.

A large number of immature PMNs were present in peritoneal exudates of LP07 tumor bearing mice (TBM). PMNs from normal or LP07TBM, when iv co-inoculated with LPO7 tumor cells, proved to be pro-metastatic (150 ± 18 and 140 ± 16 metastasis/lung, respectively), as compared to LP07 alone (25 ± 4 metastasis/lung). Reduction of PMN counts with a specific antigranulocyte antibody (AbM-RB6) together with tumor inoculation, delayed tumor growth, inhibited metastases outcome and diminished cachexia.

The activation state of PMNs was assessed by their ability to release NO and the activity of myeloperoxidase. In the LP07 model less NO was produced by PMN-TBM than by PMN from normal mice (2.8 ± 1 vs 6.5 ± 2 µM of nitrite, respectively). PMNs of control mice also have greater myeloperoxidase activity than LP07TBM.

In vitro cytotoxic activity of PMNs on LP07 tumor cells was assayed. In spite of their pro-tumoral activity in vivo, an inhibitory effect on LP07 cell viability was detected (40% inhibition) in vitro. This controversial activity possibly can be adscribed to the effect of factors released by PMNs to the tumor stroma, lack of apoptosis of PMNs and hence accumulation of immature leukocytes *in vivo*, that can impair immune effector T lymphocyte activity.

B. Despite the systemic inflammatory status of LP07TBM, their immune functions were not totally impaired. LP07TBM were able to reject an allogeneic tumor when inoculated in the contralateral flank, indicating PBL $CD8^+$ lymphocyte activation. However, in vitro cytotoxicity assay using splenocytes from LP07TBM showed no inhibition of tumor cell proliferation. These observations indicate that although PBL $CD8^+$ cells proved to be immunocompetent, an impaired specific immune response was present.

After LPS injection, LP07TBM showed loss of body weight, as a response to macrophage activation. Peritoneal macrophages were also able to elicit an antitumor response *in vitro* and to produce larger amounts of NO upon LPS stimulation.

C. The inhibitory activity of NSAIDs on tumor growth is mainly due to the inhibition of COX-2 enzyme that is overexpressed in many tumors. LP07 tumors overexpress COX-2 and secrete large amounts of PGE_2. To study if inhibition of inflammation could modulate tumor development we assayed the effect of NSAIDs. The non selective COX inhibitor indomethacin (indo) and the selective COX-2 inhibitor celecoxib (cxb) reduced tumor growth and metastases outcome in sc LP07TBM. Both drugs also inhibited the development of leukocytosis and the weight loss associated to LP07 progression. The serum levels of the inflammatory cytokines IL-1α and IL-6, mediators

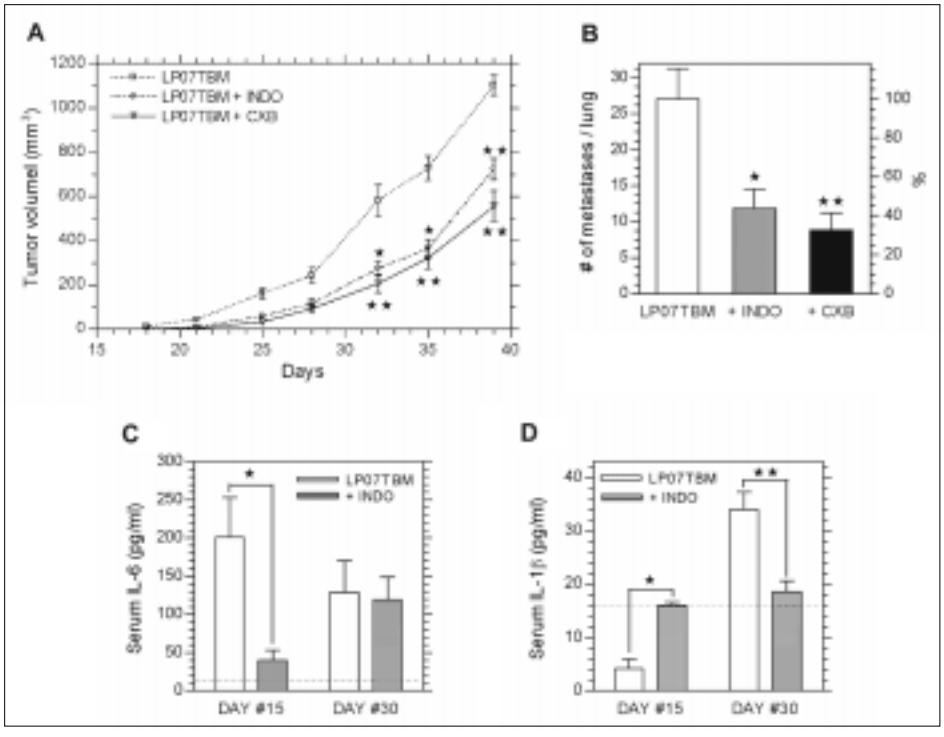

Fig. 1.– A. Primary tumor growth with NSAID treatments. B Percent of metastatic nodules per lung. C and D: IL-6 and IL 1β levels in serum.

of inflammation and cachexia, were modulated by both NSAIDs[14].

Inhibition of in vitro LP07 cell migration and invasion, and a reduction in angiogenesis were attained when cells were treated with either indo or cxb. Activity of the proteolytic enzyme MMP-9 was higher in conditioned media of LP07 cells than in the media from LP07 cells treated with either drug (Fig 1).

In order to study COX-2 function in the inhibition of inflammation by NSAIDs, LP07 tumor cells were stably transfected with an antisense vector for COX-2 mRNA (LP07AS). *In vitro*, LP07AS cells were more sensitive to cxb than control cells. Proteolytic enzymes such as µPA and MMP-9 in conditioned media were diminished in LP07AS cells with respect to control-transfected LP07cells. Surprisingly, when injected sc in mice, LP07AS tumor grew larger than in mice inoculated with control LP07 cells, being the growth of both tumors inhibited by cxb. In spite of the reduction of proteases *in vitro*, COX-2 appears not to be the only target of NSAIDs for reduced tumor growth.

NFκB activation was shown to be an important link between inflammation and cancer[9]. In our model, the reduction of activation of NFκB by treatment with cxb independently of COX-2, suggests that this transcription factor may modulate the effect of cxb on tumor cell viability.

D. Some of the mechanisms involved in the effectiveness of the association of acetate medroxyprogesterone (MPA) and the indo in LP07TBM were analyzed. Association of indo plus MPA significantly inhibited tumor growth and lung metastatic nodules. This effect was not observed by MPA alone. Paraneoplastic syndromes, leukocytosis and cachexia were abolished by treatment of MPA alone and with the association of both drugs. Circulating cytokines, such as IL-1β and IL-6, which may regulate cachexia and inflammation, were decreased by combined treatments or either drug alone. The combination of indo and MPA proved to be effective in inhibiting tumor and metastatic growth and diminishing paraneoplastic symptoms as well as the systemic inflammatory response[15].

Conclusion

Understanding the interaction of the innate and adaptive arm of the immune system, and why and how chronic inflammation is maintained is crucial for limiting the role of inflammation in cancer.

It has been shown that primary tumors may influence the environment in the lungs before metastases appear by the secretion of pro-inflammatory molecules and the presence of bone marrow hematopoietic cells that express VEGFR1[16].

In our lung adenocarcinoma system, PMNs seem to play an important role in enhancing tumor progression. Thus, limiting chronic inflammation with NSAIDs decreased tumor progression and paraneoplastic syndrome development.

In spite of COX-2 having a role in tumor progression, NFκB seems to be as important in LP07 tumor development. Although NSAIDs treatment was not able to resolve inflammation, it still may inhibit the production of several factors that can limit this response.

Inflammation is a double edge sword. In the first steps of tumor development, inflammatory responses can limit proliferation of arising tumor cells by driving an effective immune response. An example of the biology determining immune-mediated tumor rejection was recently described by Panelli et al.[7] Following the events leading to regression of basal cell carcinoma in response to the treatment with a Toll-7 like receptor agonist imiquimod, a strong activation, of genes associated with innate and adaptive immune effector mechanisms was observed. This observation may reconcile the ambiguous role attributed to inflammation in cancer progression[17]. However, if inflammation becomes chronic, it may favor tumor progression. Thus, it is important to find a balance between these two facets of inflammation through the different stages of cancer.

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The cultural traditions which have originated in the Third World, whatever their strengths and weaknesses, did inculcate respect for learning and scholarship. Because of this, and also because of the fact of your smaller number, it gives you a position of prominence in your own societies often greater than what your peers from developed nations enjoy in theirs.

Las tradiciones culturales originadas en el Tercer Mundo, cualquiera sean sus fuerzas o debilidades, inculcaron el respeto por la educación y el estudio. Debido a eso, y debido también a que ustedes son pocos, es que tienen una posición prominente en sus propias sociedades, a menudo más importante que la que ocupan sus pares en países desarrollados.

Abdus Salam (1926-1996)

Premio Nobel de Física, 1979