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INFLAMMATION AND CANCER: THE MACROPHAGE CONNECTION

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Abstract Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes. M1 macrophages which are classically activated by microbial products and interferon-γ, are potent effector cells which kill microorganisms and tumors. In contrast, M2 cells, tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that TAM are a prototypic M2 population. M2 polarization of phagocytes sets these cells in a tissue remodelling, and repair mode. And orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of pro-tumor inflammatory reactions. Rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid. It was recently observed that RET/PTC activates a pro-inflammatory genetic programme in primary human thyrocytes, including in particular chemokines and chemokine receptors. These molecules are also expressed *in vivo* and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement and activation of a pro-inflammatory programme in a human tumor. Therapeutic targeting of cancer promoting inflammatory reactions is in its infancy, and its development is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems. Chemokines are prime targets for interfering with tumor promotion by inflammatory reactions. Ongoing efforts along this line are encouraging.

Key words: cancer, macrophages, inflammation

Resumen ***Inflamación y cáncer: la conexión macrófago.*** Los macrófagos son orquestadores claves de la inflamación crónica. Responden a las señales del microambiente con programas genéticos y funcionales polarizados. Los macrófagos M1, clásicamente activados por productos microbianos e interferon-γ, son potentes células efectoras que destruyen microorganismos y tumores. Al contrario, los macrófagos M2 son responsables tanto de la inflamación como de la inmunidad adaptativa; inducen la proliferación celular produciendo factores de crecimiento y productos de la vía arginasa (ornitina y poliaminas); fagocitan desechos expresando los receptores correspondientes; aumentan la angiogénesis y la reparación tisular. Las células M1 y M2 representan extremos simplificados de un continuo de estados funcionales. Las evidencias sugieren que los macrófagos asociados a tumores (TAM) son del prototipo M2. La polarización de TAM pone en funcionamiento un remodelamiento y reparación de los tejidos asociados a una neoplasia ya establecida. Estudios recientes tratan de explicar la relación entre eventos genéticos que inducen cáncer y la activación de reacciones inflamatorias pro-tumorales. El re-arreglo del oncogen RET (RET/PTC) es un evento frecuente, causal y suficiente en el carcinoma papilar de tiroides. Recientemente se ha observado que RET/PTC activa un programa genético pro-inflamatorio en tiroidocitos primarios humanos, incluyendo en particular a quimoquinas y sus receptores. Estas moléculas se expresan también *in vivo* y más aún en tumores metastásicos. Estos resultados señalan una conexión directa entre un re-arreglo de un oncogen como evento temprano, causal y suficiente junto con una activación de un programa pro-inflamatorio en un tumor humano. Se visualizan tratamientos dirigidos contra las reacciones inflamatorias pro-tumor y su desarrollo depende de manera crucial en definir los mecanismos celulares y moleculares en sistemas relevantes. Las quimoquinas son el primer blanco para interferir contra la progresión tumoral dependiente de reacciones inflamatorias. Los esfuerzos en este sentido son prometedores.

Palabras clave: cáncer, macrófagos, inflamación

Background

Although as early as in the 19th century it was perceived that cancer is linked to inflammation, this perception has waned for a long time. Recent years have seen a reinassance of the inflammation-cancer connection stemming from different lines of work and leading to a generally accepted paradigm[1-4].

Epidemiological studies have revealed that chronic inflammation predisposes to different forms of cancer. The triggers of chronic inflammation which increase cancer

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risk include microbial infections (e.g. *H. pilori* for gastric cancer and mucosal lymphoma), autoimmune diseases (e.g. thyroiditis for papillary carcinoma of the thyroid gland; inflammatory bowel disease for colon cancer), and inflammatory conditions of uncertain origin (e.g. prostatitis for prostate cancer). Usage of non-steroidal antiinflammatory agents is associated with protection against various tumors, a finding which to a large extent mirrors that of inflammation as a risk factor for certain cancers. But the "inflammation-cancer" connection is not restricted to increased risk for a subset of tumors.

An inflammatory component is present in the microenvironment of most neoplastic tissues, including those not causally related to an obvious inflammatory process. Cancer-associated inflammation includes: the infiltration of white blood cells, prominently phagocytic cells called macrophages (TAM)[5]; the presence of polipeptide messengers of inflammation (cytokines such as tumor necrosis factor (TNF) or interleukin-1 (IL-1), chemokines such as CCL2); the occurrence of tissue remodeling and angiogenesis.

Chemokines have emerged as a key component of the tumor microenvironment which shape leukocyte recruitment and function as well as tumor cell biology[6].

Discussion

Strong direct evidence suggests that cancer associated inflammation promotes tumor growth and progression. Already in the late '70 it was found that a major leukocyte population present in tumors, the so called tumor-associated macrophages (TAM), promote tumor growth *in vitro* and *in vivo*. Accordingly, in many but not all human tumors, a high frequency of infiltrating TAM is associated to poor prognosis. Interestingly, this pathological finding has reemerged in the postgenomic era: genes associated to leukocyte or macrophage infiltration (e.g. CD68) are part of molecular signatures which herald poor prognosis in lymphomas and breast carcinomas[7, 8]. Along the same line functional polymorphisms of master genes of inflammation (TNF and IL-1) are associated with cancer risk or progression. The very presence of abundant inflammatory cytokines and chemokines in the darwinian environment of tumors speaks to their role in progression.

Gene modified mice, including some with cell-specific targeted gene inactivation, allowed dissection of molecular pathways of inflammation leading to tumor promotion, as well as the initial analysis of the role of distinct elements of the inflammatory processes in different steps of tumor progression. TNF, IL-1, the macrophage growth and attractant factor CSF-1, CCL2, a chemokine originally described as a tumor-derived macrophage attractant, the prostaglandin producing enzyme cyclooxygenase 2, the master inflammatory transcription factor NFκB, enzymes involved in tissue remodeling, are all essential elements for carcinogenesis and/or for acquisition of a metastatic phenotype in diverse organs including skin, liver, mammary gland, intestine[3, 9-13]. Genetic and cell transfer approaches have also unequivocally proven the role of defined leukocyte populations (mast cells, neutrophils, myeloid cells)[13].

Human papilloma virus (HPV) causes cervical carcinoma in women with a toll of 230,000 deaths per year, almost 80% in developing countries. Transgenic mice carrying the early region genes of HPV16 under the control of the human keratin 14 promoter offer a useful model which recapitulates tumor progression of squamous cell carcinoma from hyperplasia to displasia to overt malignancy. Innate immunity cells, most prominently mast cells and granulocytes infiltrate HPV16 premalignant tissues, followed by macrophages in carcinoma. They drive a chronic inflammatory process which promotes epithelial hyperproliferation, tissue remodeling and angiogenesis, followed by displasia and invasive carcinoma[14]. Coussens and colleagues have now addressed the question of the interplay between innate immunity/inflammation and adaptive responses driven by T and B lymphocytes in this model of tumor progression[15]. By crossing HPV transgenic mice with severly immunodeficient mice (RAG-1 -/-), they found that genetic elimination of T and B lymphocytes blocks recruitment of innate immunity cells, tissue remodeling and angiogenesis, with an arrest of the carcinogenesis process at the stage of epithelial hyperplasia. Dissection of cells and molecules involved revealed that B cells (the antibody producing lymphocyte population), which do not infiltrate the lesions, act as remote orchestrators of the innate immune cells in situ. Cir-

TABLE 1.– *Selected evidence for a protumor function of inflammatory reactions*

- Epidemiological association between inflammation and cancer (eg thyroid, bladder, cervical, ovarian, prostate, oesophageal, gastric, colon, etc).
- Protection against cancer (colon, breast) by NSAIDS and antibiotics.
- Inflammatory cells, cytokines and chemokines present in the darwinian microenvironment of neoplastic tissues.
- Frequent (but not universal) correlation between high macrophage infiltration and poor prognosis.
- Tumor promotion by adoptive transfer of inflammatory cells
- Functional polymorphisms of inflammatory cytokine genes (IL-1, TNF) associated with cancer susceptibility and severity.
- Cytokine gene targeted mice protected against carcinogens, metastases and lymphoproliferative syndrome.

Evidence summarized here is based on ref. [1, 4]

cumstantial evidence suggests that this remote control mechanism of cancer promoting inflammation operates via deposition of immunoglobulins, but this is not formally proven.

The extracellular matrix of tumors can contain peculiar components such splice variants of tenascin or fibronectin[16, 17] leukocytes can mediate remodeling and directly contribute to deposition[17].

Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes[5, 18]. M1 macrophages which are classically activated by microbial products and interferon-γ, are potent effector cells which kill microorganisms and tumors. In contrast, M2 cells[5, 19], tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that TAM are a prototypic M2 population[5, 19]. M2 polarization of phagocytes sets these cells in a tissue remodeling and repair mode and orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia[1]. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of protumor inflammatory reactions[20]. Rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid. It was recently observed that RET/PTC activates a proinflammatory genetic programme in primary human thyrocytes, including in particular chemokines and chemokine receptors. These molecules are also expressed *in vivo* and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement and activation of a proinflam-matory programme in a human tumor.

Future directions

In general, a poorly explored issue is that of the interplay between the innate and adaptive arm of immunity, on the one hand, in surveillance against cancer by T cells (the so called "immunoediting"[21]), and on the other, of promotion of cancer mediated by chronic inflammation. Therapeutic targeting of cancer promoting inflammatory reactions is in its infancy[22], and its development is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems. Chemokines are prime targets for interfering with tumor promotion by inflammatory reactions. Ongoing efforts along this line are encouraging.

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