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IL-1 AND IL-1 REGULATORY PATHWAYS IN CANCER PROGRESSION AND THERAPY

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Abstract

Inflammation is an important component of the tumor microenvironment. IL-1 is an inflammatory cytokine which plays a key role in carcinogenesis and tumor progression. IL-1 is subject to regulation by components of the IL-1 and IL-1 receptor (ILR) families. Negative regulators include a decoy receptor (IL-1R2), receptor antagonists (IL-1Ra), IL-1R8, and anti-inflammatory IL-37. IL-1 acts at different levels in tumor initiation and progression, including driving chronic non-resolving inflammation, tumor angiogenesis, activation of the IL-17 pathway, induction of myeloid-derived suppressor cells (MDSC) and macrophage recruitment, invasion and metastasis. Based on initial clinical results, the translation potential of IL-1 targeting deserves extensive analysis.

Key words: interleukin-1, inflammation, inflammation-associated cancer, therapy

Introduction

Selected chronic inflammatory conditions increase the risk of developing cancer (the extrinsic pathway) (1). These include infection, autoimmunity and autoinflammation. Colitis-associated cancer has served as a typical example of this connection. In the intrinsic pathway, genetic events, which drive carcinogenesis, orchestrate the construction of an inflammatory microenvironment by direct induction of inflammatory genes or through activation of downstream transcription factors (nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3) and hypoxia-inducible factor 1α (HIF-1α)).

Among leukocytes, tumor-associated macrophages (TAM) have served as a paradigm for the connection between inflammation and cancer (2). TAM influence all aspects of tumor growth and progression, including providing a nurturing niche for cancer stem cells, promoting angiogenesis, paving the way to invasion and metastasis, taming adaptive immune responses (3-5). Cytokines play a key role in the tumor promoting functions of TAM. The macrophage cytokine repertoire is vast and includes primary inflammatory mediators (e.g. IL-1 and TNFα), anti-inflammatory cytokines (TGF-β and IL-10) and chemokines, which are differentially expressed depending of the state of activation (2, 6). IL-1 is a prototypic inflammatory cytokine upstream in the cytokine cascade (7, 8). Several lines of evidence indicate that IL-1 and its regulation play a pivotal role in cancer-related inflammation. Here we will review how IL-1 affects various aspects of tumor progression and clinical results highlighting its role as a therapeutic target.

1. An overview of the IL-1 and IL-1 receptor family and of their regulation

IL-1 is the first member of a family of structurally related cytokines. Ligands include two isoforms of IL-1 (α and β), three receptor antagonists (IL-1Ra in three isoforms; IL-36Ra; IL-38), IL-33, IL-18, IL-36 (α and γ) and IL-37 (9). The IL-1 receptor (IL-1R) family is composed of 10 members which assemble as heterodimers and signal via the MyD88-IRAK-NFκB pathway (9).

A key feature of IL-1 is the existence of multiple levels of negative regulation within the system. IL-1R2 is a decoy receptor which in membrane bound or released forms acts as a molecular trap for IL-1β (10). In addition it also traps extracellularly IL-1α in concert with a released isoform of the accessory protein (IL-1R3) and blocks intracellular processing (9). IL-18 binding protein (IL-18BP) is a secreted non-signalling inhibitor of IL-18. Receptor antagonists in the system include IL-1Ra, IL-36γ, □□-38. IL-37 is a member of the family which interacts with IL-1R8 and IL-1R5/IL-1R8α and has anti-inflammatory activity (11, 12). Finally, IL-1R8 is a fringe member of the IL-1R family with distinct structural features and functions as negative regulator. In addition to being part of the receptor complex which recognizes IL-37, IL-1R8 is recruited at signalling receptor complexes of the IL-1 and TLR family and dampens the response by interfering with MyD88 multimer formation (13, 14).

In recent years, IL-1 has emerged as a key cytokine involved in innate and adaptive immunity with new, unexpected vistas. IL-1 is downstream of inflammasome activation and its role in sensing microbial invasion, tissue and cellular damage (9). Moreover, IL-1 is a key driver of innate (ILC3) and adaptive (Th17) lymphocyte differentiation thus playing a role in polarized lymphoid cell-orchestrated responses. These general features of IL-1 and its complex regulation are relevant for its role in carcinogenesis and tumor progression.

2. Promotion of carcinogenesis

Schematically, inflammation and cancer are linked by an intrinsic pathway, whereby genetic events which cause cancer orchestrate the construction of an inflammatory microenvironment, and an extrinsic pathway whereby non-resolving inflammation, drives carcinogenesis (1). IL-1 has been associated to both pathways by genetic studies in mice and man. In a model of epithelial carcinogenesis, IL-1α has been shown to be downstream of Ras activation and to be an essential driver for the activation of NFκB–regulated genes, including cytokines and chemokines required for

the establishment of a protumoral microenvironment. In addition, IL-1α is involved in the suppression of keratinocyte differentiation markers, leading to neoplastic transformation in a cell-autonomous manner (15). In a series of seminal studies Ron Apte and colleagues found that mice genetically deficient in IL-1β or IL-1R1 are protected against methylcholanthrene carcinogenesis (16-18). The role of IL-1α is less evident in this model of carcinogenesis in terms of inflammation and tumorigenicity. However, studies with 3-MCA-induced fibrosarcoma cell lines from IL-1α-deficient mice showed that host-derived IL-1α is involved in cancer immunoediting, by affecting innate and adaptive immunosurveillance mechanisms (19).

Intriguingly, IL-1β deficiency had a more profound impact, in terms of increased susceptibility also to skin carcinogenesis, than genetic inactivation of IL-1α (18). Indeed, IL-1α overexpression in transgenic mice was associated with reduced incidence of DMBA/TPA-induced skin tumors (20). It is tempting to speculate that this difference is related to the "alarmin" function of IL-1α triggering protective immunity.

Chronic non-resolving inflammatory conditions such as inflammatory bowel disease, non alcoholic liver steatohepatitis and obesity increase the propensity of developing tumors (1, 4) and gastrointestinal neoplasias have been invaluable to define the clinical significance and mechanisms of cancer-related inflammation. Genetic polymorphisms at the IL-1 locus in humans have been shown to be strongly associated with susceptibility to gastritis which drives gastric carcinoma (21). Analysis of mice deficient in IL-1 family molecules and MyD88 have phenotypes which are at the interception of tumor promoting inflammation, promoting mucosal integrity and microbial sensing (for review (22)).

3. Promotion of metastasis

In early studies it was observed that IL-1β augments lung metastasis after i.v. injection of tumor cells (23). These early observations were confirmed and extended in models of spontaneous

lung metastasis and carcinogenesis (16, 24-26). Increased secondary localization after treatment with IL-1 was also observed in models of liver metastasis. IL-1- mediated promotion of metastasis was inhibited by IL-1Ra (27, 28). The mechanisms underlying IL-1 mediated stimulation of metastasis are complex and include promotion of angiogenesis and induction of endothelial cell adhesion molecules recognized by tumor cells. For instance, VCAM-1 induced by IL-1 is recognized by VLA4 which is expressed on some melanoma cells, whereas the E-selectin ligand is present on colon cancer cells (17).

4. Mechanisms of IL-1-driven tumor promotion

IL-1 affects multiple aspects of the tumor microenvironment which underlie tumor promotion discussed under point 2 and presumably mediate clinical observations to be discussed below (Fig. 1). IL-1-driven tumor promotion has been associated with promotion of angiogenesis (17, 29, 30). IL-1 has complex effects on the vascular endothelium (9, 31). In general, IL-1 activates endothelial cells in a prothrombotic/proinflammatory direction by inducing procoagulant activity and inducing expression of adhesion molecules and inflammatory cytokines. Moreover, IL-1 induces in endothelial cells and surrounding stromal cells the production of proangiogenic cytokines such as IL-8. In this general perspective, it is interesting that IL-1 is downstream of metabolic rewiring of macrophages with succinate being a key inducing product activating the HIF-1α pathway (32).

Colitis-associated intestinal cancer has served as a paradigm for the connection between inflammation and cancer (1). Signals originating from microbes and dysbiosis are key drivers of gastrointestinal tumorigenesis (33, 34). IL-1 is downstream of microbial sensing by epithelial cells or innate immunity cells. In agreement, mice deficient of IL-1R8/SIGIRR, a negative regulator of the signaling of IL-1R family members and TLR, exhibited a dramatic intestinal inflammation in response to dextran sulfate sodium salt (DSS) administration, with more severe weight loss,

intestinal bleeding, and mortality, and showed increased susceptibility to carcinogenesis in response to azoxymethane and DSS (35, 36).

The differentiation of innate and adaptive lymphoid cells polarized in an ILC3 and Th17 direction, respectively, is sustained by IL-1. The IL-17 pathway has been shown to support carcinogenesis in the gastrointestinal tract (37). There is evidence that neutrophils are an important component of tumor promoting inflammation (38). It remains to be established whether neutrophils are a component of the IL-1-IL-17 pathway of tumor promotion.

IL-1 promotes recruitment of myeloid cells in tumors and their tumor promoting function. Chemokines such as CCL2 and endothelial cell expression of adhesion molecules mediate leukocyte recruitment at sites of inflammation. In a recent study, the IL-1R-MyD88 pathway was shown to upregulate expression of ten-eleven-2 (tet2) in TAM in mouse models of melanoma and in melanoma patients. Tet2 is a DNA methylcytosine dioxigenase which was found responsible for the immunosuppressive program of TAM (39). Genetic inactivation of tet2 shifted macrophage polarization, with recruitment of effector T cells and antitumor activity. In early studies IL-1 was found to stimulate haematopoiesis (hemopoietin-1). Expansion of myeloid cells in cancer results in the appearance in peripheral blood and lymphoid tissues of a heterogeneous population of immature elements endowed with immunosuppressive activity, operationally defined myeloid derived suppressor cells (MDSC) (40). IL-1 sustains MDSCs generation and immunosuppressive function (41-43). Thus, IL-1 by promoting MDSCs and sustaining the immunosuppressive activity of TAM contributes to suppression of effective adaptive antitumor immune responses.

Clinical perspective

Human polymorphisms at the IL-1 locus have shown that an imbalance between proinflammatory IL-1 and anti-inflammatory IL-1Ra is a major risk factor for gastritis, ulcer, and

hence gastric carcinoma (21). In addition polymorphisms in the IL-1Ra and IL-1 gene are associated with lung cancer risk (44-47), a consistent finding possibly relevant to anti-IL-1β mediated protection against this tumor, as discussed below.

At present clinically available anti-IL-1strategies include IL-1Ra (Anakinra), anti-IL-1β mAb and anti-IL-1α mAb. Anakinra has been studied in patients with smouldering myeloma with evidence suggesting inhibition of progression to frank neoplasia (48), although this observation has not been followed up.

In a phase I study in patients with end-stage cancers of various origins, a naturally occurring human neutralizing anti-IL-1α mAb has shown activity in reducing cancer cachexia (i.e. in increasing lean body mass and decreasing fatigue, pain, and appetite loss, as well as circulating IL-6 levels) (49). Since IL-1α is barely detectable in plasma, the mAb was supposed to target IL-1α expressed on the surface of platelets, $CD14^+$ and $CD16^+$ monocytes, and in particular malignant cells, which contain IL-1 precursor. Neutralization of tumor-associated IL-1α as well as platelet or monocyte-associated IL-1α would reduce local and systemic inflammatory processes, leading to correction of a metabolic defect and increased lean body mass, as well as potentially reducing IL-1-dependent angiogenesis and immune suppression.

Atherosclerosis is a manifestation of vessel wall inflammation and IL-1 has long been known to drive atherosclerosis and its complications. On this basis a large prospective study was conducted using anti-IL-1β (Canakinumab) in high risk atherosclerosis patients (50). In the same study involving 10,061 patients, a dramatic (>50%) reduction of the incidence and mortality from lung cancer was observed (51). These impressive results have broad implications and are thought provoking. Given the relatively short follow up (3.7 years) and natural timeframe of carcinogenesis in humans, it is likely that mechanisms other than blocking of IL-1-driven cancer progression are involved. As discussed above, IL-1 drives pathways of myeloid cell-mediated suppression of specific antitumor immunity. It is therefore tempting to speculate that IL-1β blockade interferes

with MDSCs and TAM-mediated immune suppression and that the tumor protection observed is a reflection of unleashed T cell mediated responses.

Concluding remarks

IL-1 is a key mediator of innate and adaptive immunity at the crossroad of diverse pathways of microbial recognition and activation and orientation of lymphoid cell function. IL-1 has emerged as a key component of tumor promoting inflammation by shaping different constituents of the tumor microenvironment including tumor infiltrating myeloid cell recruitment, angiogenesis, and skewing and suppression of anti-tumor immunity. Available information suggests that IL-1 targeting in the clinic may affect cancer-associated cachexia (49) and incidence and mortality from cancer, based on a large cohort (10,061) of patients at risk of atherosclerosis related complications (51). These impressive results need confirmation and extension. As they stand, they raise the issue of anti-IL-1 strategies as a form of immunotherapy. Preclinical results summarized here and available clinical observations raise the issue of combining IL-1 inhibition with checkpoint blockade.

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Figure legend

Figure 1. IL-1 in cancer related inflammation. Key mechanisms by which IL-1 promotes carcinogenesis, progression and metastasis are summarized in a schematic form.