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Pathways connecting inflammation and cancer

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Chronic and persistent inflammation contributes to cancer development and can predispose to carcinogenesis. Infection-driven inflammations are involved in the pathogenesis of approximately 15–20% of human tumors. However, even tumors that are not epidemiologically linked to pathogens are characterized by the presence of an inflammatory component in their microenvironment. Hallmarks of cancer-associated inflammation include the presence of infiltrating leukocytes, cytokines, chemokines, growth factors, lipid messengers, and matrix-degrading enzymes. Schematically, two interrelated pathways link inflammation and cancer: (1) genetic events leading to neoplastic transformation promote the construction of an inflammatory milieu; (2) tumor-infiltrating leukocytes, in particular macrophages, are prime regulators of cancer inflammation. Thus, an intrinsic pathway of inflammation (driven in tumor cells), as well as an extrinsic pathway (in tumor-infiltrating leukocytes) have been described and both contribute to tumor progression.

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Introduction

Recent years have witnessed a renaissance of research into the connection between inflammation and cancer [1••,2••,3–5]. Epidemiological studies revealed that chronic inflammation predisposes to different cancers, colon cancer being a prototype. The triggers of chronic inflammation that increased cancer risk include microbial infections (i.e. *Helicobacter pylori* for gastric cancer and mucosal lymphoma), autoimmune diseases (i.e. inflammatory bowel disease for colon cancer), and cryptogenic inflammatory conditions (i.e. prostatitis for prostate cancer). Accordingly, use of non-steroidal anti-inflammatory agents decreases the incidence of several tumors [6–8].

Cancer-associated inflammation includes leukocyte infiltration, prominently tumor-associated macrophages (TAM) [9]; expression of cytokines such as tumor necrosis factor (TNF) or interleukin (IL)-1, chemokines such as CCL2, tissue remodelling and angiogenesis. Already in 1970, it was found that TAM promote tumor growth *in vitro* and *in vivo*. Accordingly, in most human tumors a high frequency of TAM is associated with poor prognosis [10••]. This pathological finding re-emerged in the post-genomic era: genes associated with leukocyte or macrophage infiltration (i.e. CD68) are part of molecular signatures that herald poor prognosis in lymphomas and breast carcinomas [11,12]. Similarly, functional polymorphisms of master genes of inflammation (TNF and IL-1) are associated with cancer risk or progression [3,13].

Here we will discuss how an intrinsic pathway (oncogene-driven) and an extrinsic pathway (microenvironment-driven) connect inflammatory reactions and neoplastic transformation and progression. In this general context, selected components of the tumor microenvironment (the negative regulator TIR8; tumor-associated macrophages; chemokines) will be emphasized.

The extrinsic pathway

Inflammatory conditions affecting organs such as liver, pancreas, stomach, colon, and prostate are associated with increased risk of cancer. Genetic approaches have proven the role of key components of inflammation in carcinogenesis. These include primary inflammatory cytokines (IL-1, TNF), IL-6, hematopoietic growth factors (M-CSF), and the master transcription factor NF-κB [14••–16••]. In addition to promoting early steps of carcinogenesis, inflammatory conditions can promote tumor invasion and metastasis. Early observations [17,18] have been followed by genetic analysis of cytokine gene polymorphisms in diverse tumors, especially in gastrointestinal malignancy. Individuals with polymorphisms in the IL-1 gene (IL-1B-31*C or-511*T and IL-1RN*2/*2 genotypes) are at increased risk of developing hypochlorhydria and gastric atrophy in response to *H. pylori* infection [13,19]. IL-1 polymorphisms related to greater IL-1 production, have been associated with an increased risk of progression to hepatocellular carcinoma in patients with chronic HCV infection [20] and with shortened survival in pancreatic cancer [21]. In addition to IL-1,

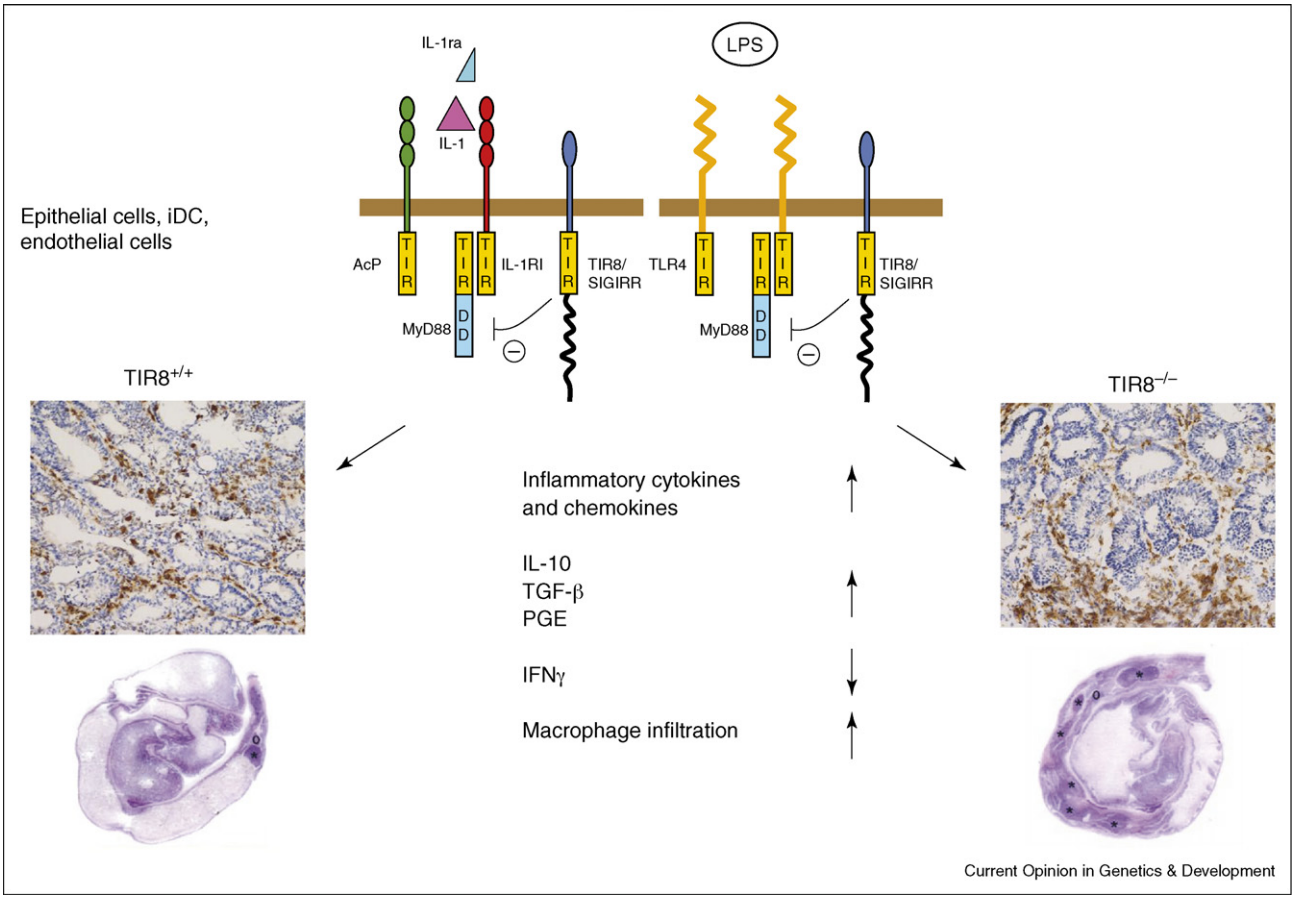
pro-inflammatory genotypes of TNF- and IL-10 have also been identified as risk factors for gastric cancer [21] and colorectal cancer [22]. More recently, a significant association between the *TLR4* Asp299Gly polymorphism and increased risk of gastric cancer has been reported. Interestingly, TLR4 could also be activated by endogenous ligands produced during stress or cell damage, including heat-shock protein 60, ED-A domain of fibronectin, and hyaluronan [23].

The clinical evidence on the permissive role of chronic inflammation in tumor promotion has received molecular confirmations in inflammation-associated cancer models [14••,15••], which provided *in vivo* evidence for the central role of NF-κB-mediated inflammation in tumorigenesis. NF-κB induces several cellular alterations associated with tumorigenesis and more aggressive phenotypes, including self-sufficiency in growth signals; insensitivity to growth inhibition; resistance to apoptotic signals; immortalization; angiogenesis; tissue invasion; and metastasis [24]. Constitutive NF-κB activation, often observed in cancer cells, may be promoted by either microenvironmental signals, including cytokines, hypoxia, and reactive oxygen intermediates (ROI), or by genetic alterations [25]. In particular, pro-inflammatory cytokines (e.g. IL-1 and TNF), expressed by infiltrating leukocytes, can activate NF-κB in cancer cells and contribute to their proliferation and survival [2••,3,26]. In addition, recent results have highlighted the role of the MYD88 pathway in colon carcinogenesis. Thus, targeting of the MYD88 pathway by sensing microbial moieties or damaged tissues or by the IL-1 amplification pathway leads to NF-κB activation and cancer promotion.

Importance of negative regulation: TIR8/SIGIRR

The signalling by TLRs and IL-1 receptors is under control by negative pathways of regulation [27,28]. There is now evidence that TIR8, an orphan member of the IL-1R family (also known as single immunoglobulin IL-1R-related molecule, SIGIRR) inhibits signalling from the IL-1R/TLR complexes, possibly by trapping IRAK-1 and TRAF-6 [27,29]. TIR8 was identified by searching EST

Figure 1



Regulation of colitis-associated cancer by the negative regulator TIR8. TIR8$^{-/-}$ mice showed increased carcinogenesis of the gastrointestinal tract in a model of colitis. The colon lysates had increased production of prostaglandins, pro-inflammatory cytokines (IL-1, IL-6), and chemokines (KC/CXC, JE/CCL2 and CCL3), while IFNγ was decreased. Tumors arising in TIR8$^{-/-}$ mice were characterized by a more prominent infiltrate of CD68+ macrophages and FoxP3+ cells.

databases for TIR domain containing sequences of yet unknown members of the Toll-like receptor (TLR)/IL-R family [30,31]. Human TIR8 full-length cDNA predicted a 410 amino-acid long protein with unique and interesting characteristics: TIR8 is composed by a single Ig domain in its extracellular region, which does not support ligand binding; a transmembrane domain; an intracellular conserved TIR domain that interestingly lacks two conserved residues (Ser447 and Tyr536) that were shown to be essential for the signalling of IL-1RI; finally, TIR8 has a unique, characteristic 95 amino-acid long tail that differentiates TIR8 from other IL-1/TLR superfamily members; only Drosophila Toll also possesses a 98 amino-acid long tail with inhibitory properties. Tir8 gene transfer experiments have revealed that it reduces NF-κB activation by the IL-1R complex [31], as well as by members of the TLR family such as TLR4 [29]. Recruitment of TIR8 at IL-1R/TLR signalling receptor complexes sequesters key signalling elements such as TRAF6 and IRAK [29].

TIR8 is expressed in several tissues, especially in the epithelial cells of the digestive tract [31]. Accordingly, there is evidence for a non-redundant regulatory role of this molecule in inflammation involving the gastrointestinal mucosa [32]. Colitis-associated cancer is a colorectal illness that arises in patients suffering from chronic inflammatory bowel disease, in particular, ulcerative colitis [33]. In a mouse model of intestinal inflammation in response to dextran sulfate sodium salt (DSS) administration, Tir8-deficient mice exhibited a dramatic susceptibility to inflammation in terms of weight loss, intestinal bleeding and mortality, and showed increased susceptibility to colitis-associated cancer in response to azoxymethane and DSS (Figure 1). Increased susceptibility to colon carcinogenesis was associated to increased permeability and local production of prostaglandin E_2, pro-inflammatory cytokines (IL-1, IL-6) and chemokines (KC/CXC, JE/CCL2 and CCL3) [34,35]. These mediators are downstream of NF-κB and have been shown to promote inflammation-propelled neoplasia [16••,36]. Thus, the lack of a checkpoint (TIR8) of NF-κB activation leads to increased carcinogenesis in the gastrointestinal tract, underlying once more the connection between chronic inflammation and cancer promotion.

Connecting hormones and inflammation

Recent results have highlighted unexpected connections between inflammation and sex steroid hormones in the promotion of cancer, in particular, in prostate cancer, a classic hormone-stimulated tumor [37,38••]. Zhu *et al.* [38••] found that the macrophages produce IL-1, which converts selective androgen-receptor modulators (SARMs) from their intended function of inhibitors of androgen-receptor-induced gene expression to activators of expression. The mechanism involves a protein, TAB2, which acts as a sensor for inflammatory signals. TAB2 is a component of a repressor complex N-COR/HDAC: inflammation-dependent phosphorylation of TAB2 causes the complex to detach and unleash gene transcription. Hepatocellular carcinoma, the most common type of liver cancer, is a frequent outcome of chronic inflammation triggered by hepatitis virus infection [1••], with males being more susceptible than females. Naugler *et al.* [39] have explored the mechanisms underlying this difference. They started from the observation that in response to the carcinogen diethylnitrosamine, male mice exhibited higher levels of IL-6, a marker as well a mediator of chronic inflammation. The sex difference in carcinogenesis was abolished in mice deficient in IL-6 and in the adaptor protein MYD88. Thus, carcinogen-induced tissue damage results in MyD88-dependent activation of Kupffer cells (specialized liver macrophages). Tissue damage probably acts via TLRs, though this has not been formally proven. The Kupffer cells produce IL-6 that promotes liver injury, inflammation, compensatory cell proliferation, and carcinogenesis. In female, however, oestrogen steroid hormones inhibit IL-6 production by Kupffer cells and so protect female mice from cancer.

These results provide firm evidence for an unexpected feature of the connection between inflammation and cancer [1••,2••,3], a bidirectional interaction between inflammatory mediators and sex steroid hormones.

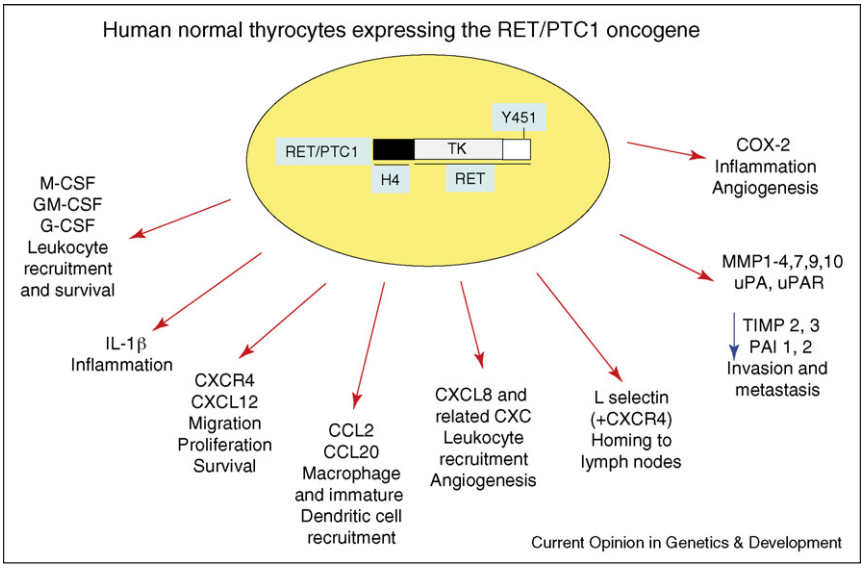
The intrinsic pathway: connecting inflammation and oncogenes

The occurrence of an inflammatory microenvironment in tumors that are not epidemiologically related to inflammation raised the question whether genetic events causing neoplasia are responsible for the construction of an inflammatory milieu.

A number of reports have indicated that tumoral pathways leading to inflammation include, for instance, the von Hippel Lindau (vHL)/hypoxia-inducible factor (HIF); oncogene-driven pathways (e.g. Ras), and the activation of the master transcription factor NF-κB.

Advantage was taken of papillary carcinoma of the thyroid (PTC), a tumor characterized by the presence of chemokine-guided macrophage and dendritic cell infiltrate. Rearrangements of the tyrosine kinases are involved in the pathogenesis of this tumor. In particular, the RET/PTC rearrangement represents an early, causative, and sufficient genetic event in the pathogenesis of PTC. In an appropriate cellular context, provided by primary human thyrocytes, RET/PTC activates a genetic program related to inflammation [40••] (Figure 2). In particular, the RET/PTC1 oncogene activated transcriptome profile includes CSFs, which promote leukocyte recruitment and survival; IL-1, a primary inflammatory cytokine; the inflammatory mediator COX2; chemokines attracting monocytes and

Figure 2



The RET/PTC oncogene causes the neoplastic transformation of normal human thyrocytes and activates an inflammatory program. Activated genes include chemokines and receptors, interleukin-1, colony-stimulating factors, L-selectin, matrix proteases, and COX-2.

dendritic cells (CCL2, CCL20 [41]; angiogenic chemokines; coordinate induction and inhibition of matrix degrading enzymes and inhibitors; L-selectin; and CXCR4.

Key elements of the RET/PTC-activated inflammatory program were found in biopsy specimens. Interestingly, patients with lymph node metastasis showed higher levels of inflammatory molecules. These results show that an early, causative genetic event (RET/PTC) involved in the pathogenesis of a human tumor directly promotes the build up of an inflammatory microenvironment to its direct advantage.

Transfer of activated ras oncogene into a cervical carcinoma line (HeLa) induces IL-8/CXCL8 production that is sufficient to promote angiogenesis and tumor progression [42]. The chemokine receptor CXCR4 that is frequently expressed on malignant cells and implicated in cell survival and metastasis lies downstream of the von-Hippel Lindau/hypoxia inducible factor (HIF) axis, as does the inflammatory cytokine TNFa (reviewed in reference [3]). In non-small cell lung cancer (NSCLC) mutation of the tumor suppressor gene PTEN results in upregulation of HIF-1 activity and in HIF-1-dependent transcription of the CXCR4 gene that promotes metastasis formation [43]. Braf, frequently activated in malignant melanoma, induces cytokine production that contributes to pro-tumor milieu [44]. Alpha catenin is more than a tumor suppressor sequestering beta-catenin. Its ablation results in NF-κB activation, induction of genes involved in inflammation, cell proliferation, wound healing, and ultimately squamous cell carcinoma [45]. Myc oncoprotein instructs and maintains a complex inflammatory program and Myc-driven recruiting of mast cells has been recently demonstrated instrumental for the tumorigenicity of pancreatic islets [46]. It seems that genetic events causing cancer impinge on the 'intrinsic pathway' of innate immunity and inflammation to promote cell autonomous survival and increased invasiveness, as well as on extrinsic tumor promoting inflammatory reactions.

TAM as key components of the inflammatory microenvironment

TAM represent the major inflammatory component of the stroma of many tumors, able to affect different aspects of the neoplastic tissue. Many observations indicate that TAM express several characteristics of M2-polarized macrophages [9] and display several pro-tumoral functions, including promotion of angiogenesis, matrix remodelling, and suppression of adaptive immunity. The pro-tumoral role of TAM in cancer is supported by many clinical studies that found – in most tumors – a correlation between the high macrophage content and poor patient prognosis [10••]. In turn, genetic studies in mice have shown decreased rates of tumor growth and metastasis to be associated with decreased TAM number [47,48].

Tumor angiogenesis is often activated during the early preneoplastic stages of tumor development [46,49,50] and is controlled by a number of positive or negative regulators produced by cancer cells and tumor-associated leukocytes. Macrophages can exert a dual influence on blood vessel formation and function. On the one hand, they produce molecules that are proangiogenic and, on the

other, they can express anti-angiogenic molecules and damage the integrity of blood vessels. In general, as for interaction with neoplastic cells, the pro-angiogenic functions of TAM prevail. Indeed, several studies, in human cancer, have shown that TAM accumulation is associated with increased angiogenesis and with the production of angiogenic factors such as VEGF and platelet-derived endothelial cell growth factor [1••]. TAM accumulate in hypoxic regions of tumors, and hypoxia triggers a pro-angiogenic program in these cells. A number of molecules with possible impact on angiogenesis have been shown to be expressed by macrophages in low oxygen conditions, such as VEGF, TNF-α, bFGF, and CXCL8 [51]. Therefore, macrophages recruited *in situ* represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumor cells. Strikingly, it was reported that the HIF-1-dependent chemokine CXCL12 [52] acts as a potent chemoattractant for endothelial cells of different origins bearing CXCR4 and is a participant in angiogenesis that is regulated at the receptor level by VEGF and bFGF. In agreement with these observations, our data suggest that the angiogenic program established by hypoxia relays also on the increased expression of CXCR4 by endothelial cells [53].

Lymphoangiogenesis is mediated by the action of VEGF-C and VEGF-D acting on the receptor VEGFR3. More recently VEGF-A, a chemotactic factor for monocytes, was shown to increase lymphoangiogenesis via the recruitment of circulating monocytes [54]. In human cervical cancer, VEGF-C production by TAM was proposed to play a role in peritumoral lymphoangiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis [55]. In addition, TAM participate in the proangiogenic process by producing the angiogenic factor thymidine phosphorylase (TP), which promotes endothelial cell migration *in vitro* and whose levels of expression are associated with tumor neovascularization [56].

The contribution of chemokines to angiogenesis has been the object of intensive investigation. A variety of chemokines, including CCL2, CXCL12, CXCL8, CXCL1, CXCL13, CCL5, CCL17, and CCL22 have been detected in neoplastic tissues as products of either tumor cells or stromal elements [57]. CXCL1 and related molecules (CXCL2, CXCL3, CXCL8, or IL-8) have an important role in melanoma progression by stimulating neoplastic growth, promoting inflammation, and inducing angiogenesis [58]. Strong evidence demonstrates that levels of CCL2 are associated with TAM accumulation [9] and that CCL2 may play an important role in the regulation of angiogenesis [59].

TAM contribute to tumor progression and invasion also by expressing molecules that directly affect tumor cell proliferation and dissolution of connective tissues. These include epidermal growth factor (EGF), members of the FGF family, TGFβ, VEGF, chemokines, and cytokines. It is well established that IL-1β augments metastasis [12,17]. Genetic ablation of IL-1β in mice resulted in absence of metastasis development, either with melanoma cells or with mammary and prostate tumors, suggesting the importance of IL-1β in the tumor milieu. Both IL-1β, and to a minor extent IL-1α, were required for *in vivo* angiogenesis and invasiveness of tumors *in vivo* [60••]. In co-culture experiments with tumor cells, macrophages lead to an enhanced invasiveness of the malignant cells by a TNF-α-dependent MMP induction in the macrophages [61]. In lung cancer, TAM may favor tumor progression by contributing to stroma formation and angiogenesis through their release of PDGF, in conjunction with TGF-β1 production by cancer cells [62]. Ahmed *et al.* described a method to observe the orientation of individual tumor cells as they enter blood vessels, in real time and in a living animal [63]. They found that tumor cells seem to be attracted to macrophages, which line the outside of the vessels. Goswami *et al.* [64] described a paracrine signalling loop between tumor cells and macrophages, in which tumor cells secrete M-CSF that, in turn, causes macrophages to secrete epidermal growth factor, a chemoattractant for the tumor cells. Interrupting either of these signals results in decreased tumor-cell motility. Accordingly, The intercross of transgenic mice susceptible to mammary cancer (PyMT) with mice containing a recessive null mutation in the M-CSF gene (Csf^{op}) [47] demonstrated that TAM recruitment is an absolute requirement for productive metastatic growth [65].

Macrophages can produce enzymes and inhibitors that regulate the digestion of the extracellular matrix, thus favoring tumor invasion. TAM produce several matrix metalloproteases (e.g. MMP2, MMP9) and activators of MMPs, such as chemokines. TAM also produce factors, such as TGFβ, PDGF, IL-6, urokinase plasminogen activator, and tissue-type plasminogen activator (t-PA) that may cause matrix degradation [66]. Direct evidence has been presented that MMP-9 derived from hematopoietic cells of host origin contributes to skin carcinogenesis [65]. Chemokines have been shown to induce gene expression of various MMPs and, in particular, MMP-9 production, along with the uPA receptor [68••]. Evidence suggests that MMP-9 has complex effects beyond matrix degradation, including promotion of the angiogenesis switch and release of growth factors [67••]. In addition to innate immunity, humoral immunity, in the form of antibodies, can act as a remote control system to activate inflammatory reactions that promote cancer progression [68••].

Another major pro-tumoral function of TAM in established tumors is the suppression of adaptive anti-tumor immune responses. TAM are characterized by an $IL\text{-}12^{low}$ $IL\text{-}10^{high}$ phenotype and also produce prostaglandins, TGFβ, and

indoleamine dioxigenase (IDO) metabolites [9]. In addition, various cytokines (M-CSF, IL-6, IL-10) present in the tumor microenvironment contribute to blocking DC maturation in tumors. Immature myeloid cells are expanded in chronic infections and cancer and act as potent suppressors of T cell dependent anti-tumor immunity via unbalanced iNOS and arginase-1 activity [67••,69]. Thus, tumor-associated myelomonocytic cells favor progression by taming and skewing anti-tumor T cell responses.

Indeed, a part of the immunosuppressive activity of TAM is exerted indirectly by their release of chemokines that preferentially attract T cell subsets devoid of cytotoxic functions. CCL18 has been identified as the most abundant chemokine in the ascitic fluid of human ovarian carcinoma [70]. When the source of CCL18 was investigated, it was tracked to TAM, with no production by ovarian carcinoma cells. In normal macrophages CCL18 is inducible by Th2 cytokines: IL-4, IL-13, and IL-10 and recruit naive T cells by interacting with an unidentified receptor [71]. Attraction of naive T cells in a peripheral microenvironment dominated by M2 macrophages and immature DC is likely to induce T cell anergy. Two other chemokines, CCL17 and CCL22, are abundantly expressed by TAM [9,72]. These chemokines interact with the CCR4 receptor, expressed mostly by Th2 cells and by Treg [73], two T cell subsets lacking anti-tumor functions.

Concluding remarks

Cancer-associated inflammation may result from persistent inflammation due to non-resolved pathogen infections; however, several lines of evidence indicate that even in tumors not directly linked to pathogens, the microenvironment is characterized by the presence of a smouldering inflammation, fuelled primarily by stromal leukocytes and by hypoxic conditions. Genetic alterations occurring in tumor cells may also activate an inflammatory program that profoundly impacts on cancer development. In this context, the transcription factor NF-κB has emerged as a 'master and commander' acting as an endogenous tumor promoter. Interestingly the characterization of negative regulatory pathways (e.g. TIR8), acting as checkpoints of NF-κB activation, has recently attracted attention, and their relevance in cancer is being tested in mouse model of carcinogenesis. Tumor-associated macrophages are a major player in the inflammatory response and produce a host of growth factors for epithelial and endothelial cells, as well as inflammatory cytokines and chemokines that contribute to tumor survival, proliferation, and invasion. In addition, immunosuppressive mediators released by local inflammatory or tumor cells extinguish host-mediated anti-tumor responses and facilitate tumor progression. In this scenario it is reasonable to believe that anti-inflammatory therapies – which showed effectiveness in reducing tumor incidence in epidemiological studies – will find utility to complement more conventional pharmacological treatments.

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