



Review

Cancer-related inflammation: Common themes and therapeutic opportunities

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ABSTRACT

Inflammatory cells and mediators are an essential component of the tumor microenvironment. Inflammatory circuits can differ considerably in different tumors in terms of cellular and cytokine networks and molecular drivers. However, macrophages are a common and fundamental component of cancer promoting inflammation. Drivers of macrophage functional orientation include tumor cells, cancer-associated fibroblasts, T cells and B cells. Dissection of the diversity of cancer-related inflammation is instrumental to the design of therapeutic approaches that target cancer-related inflammation.

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1. Introduction to cancer-related inflammation

Smouldering, non-resolving inflammation is one of the consistent features of the tumor microenvironment. The connection between inflammation and cancer, first perceived in the nineteenth century is now accepted as enabling characteristic of cancer [1,2]. Current estimates suggest that about 25% of cancers are associated with chronic inflammation sustained by infections (e.g. hepatitis) or inflammatory conditions of diverse origin (e.g. prostatitis) [1]. Moreover, tumors that are not epidemiologically related to inflammation are characterized by the presence of inflammatory cells and mediators [1,3].

Inflammation and cancer are connected by two pathways: extrinsic pathways from conditions that cause non-resolving smouldering inflammatory responses, and intrinsic pathways driven by oncogenes or tumor suppressor genes that activate the expression of inflammation-related programmes [1].

In this review we will first focus on some recent advances in cancer-related inflammation (CRI), emphasizing its diversity as well as the common emerging themes. In the past decade our understanding CRI has increased to the point when we can begin to translate our knowledge into new approaches to cancer prevention and treatment. This is the second focus of our review.

Abbreviations: CRI, cancer-related inflammation; EMT, epithelial mesenchymal transition; TAFs, tumor-associated fibroblasts; TAM, tumor-associated macrophages; TSLP, thymic stromal lymphopoietin.

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2. Polarization and diversity of inflammatory cells in the tumor microenvironment

Tumor associated macrophages (TAM) are a common component of CRI and will be used as a paradigm of its complexity. Cells of the monocyte–macrophage lineage exhibit considerable plasticity and diversity [1,4–8]. TAM populations in murine tumors can be quite diverse and hypoxia may be one driver of this [9]. Subsets have been identified between mouse and human monocytes [8]. It remains to be determined whether diversity of TAM reflects their origin from different monocyte precursors or microanatomical differences (e.g. oxygen tension in different part of the cancer tissue).

Molecular pathways driving TAM polarization can differ considerably in tumors arising at different sites. For instance CD4 T cells, B cells, antibodies and Fcγ receptors orchestrate the M2-like phenotype of tumor promoting TAM in a model of human papilloma virus-driven squamous cancer [10]. In contrast, in a mammary carcinoma model, Th2-derived IL-4 is responsible for M2 polarization and promotion of metastasis [11]. Complement can also be a mechanism of myelomonocytic cells recruitment [12]. B cells can skew macrophage function and promote tumor progression, using IL-10 and lymphotoxin (LT) [13–16] and B regulatory cells have been shown to enhance carcinogenesis [14]. Thus, mechanisms of regulation of myelomonocytic cells in tumors can be different organ or tumor contexts but M2-like skewing is a recurrent common denominator. Definition of TAM diversity in different human cancers will be required to translate this recent progress into clinical benefit.

Early studies indicated that in situ proliferation of mature mononuclear phagocytes could contribute to TAM accumulation [17]. Recent evidence suggests that proliferation can indeed contribute to macrophage accumulation, in particular during type II

inflammation [18]. M-CSF and IL-4 may underlie the proliferative capacity of macrophages including TAM [19]. Whether and to what extent TAM proliferate in human tumors remains to be defined.

Tissue repair is an important component of the inflammatory response and fibroblasts are key to this process. Tumors have long been considered ‘wounds that do not heal’ [20]. Tumor-associated fibroblasts (TAFs) are a major component of CRI an important source of tumor promoting cytokines and growth factors, expressing a pro-inflammatory gene signature in skin, breast and pancreatic cancers that is regulated by NF- κ B [21]. TAFs are thought to arise from mesenchymal stem cells [22] but recent data suggest that other bone marrow cells can regulate their activity. In mouse models of breast cancer endocrine signals originating in cancers mobilize Sca+ cKit– granulin-positive bone marrow cells [23]. These cells do not act directly to promote tumor growth but act on local fibroblasts in the tumor microenvironment to switch them to a cancer-promoting phenotype. Genes induced in TAFs by granulin include a range of chemokines, cytokines, and matrix remodeling factors already implicated in tumor-promotion. In human breast cancer high granulin expression correlated with aggressive triple-negative, basal-like tumor subtype and with reduced patient survival [23]. This is another example of cell co-operation in CRI.

A recent paper highlighted the importance of fibroblasts in the tumor microenvironment. When fibroblast activation protein- α , FAP- α , expressing fibroblasts were depleted in tumor-bearing mice, rapid hypoxic necrosis of tumor and stromal cells occurred accompanied by IFN- γ and TNF- α -mediated CD8+ T cell cytotoxicity [24]. These data suggest that, at least in some cancers, fibroblasts contribute to the inflammatory immunosuppressive milieu.

Other inflammatory cells that have recently been identified as modifiers of some inflammatory tumor microenvironments are neutrophils [25–27] and mast cells, important not only for their release of cytokines and chemokines but also reactive oxygen and proteases [2,28]. Thus, the cellular networks involved differ considerably in different tumors and in tumors originating in different tissues, but common themes emerge and these include skewing and subversion of macrophage function.

3. Cytokines and cancer-related inflammation

TNF- α , IL-6 and IL-1 are among the most studied inflammatory cytokines in the tumor microenvironment and this review will focus on these.

Unlike their normal counterparts, many malignant cells constitutively produce small amounts of TNF- α . There is evidence from animal models that malignant cell-derived TNF- α enhances the growth and spread of syngeneic, xenogeneic, and carcinogen-induced tumors of skin, ovary, pancreas, pleural cavity and bowel (reviewed in Ref. [29]). For instance, in ovarian cancer models, TNF- α is an important component of a malignant cell-autonomous network of inflammatory cytokines, including the chemokines CXCL12 and CCL2, the cytokines IL-6 and macrophage inhibitory factor, MIF as well as vascular endothelial growth factor (VEGF) [30]. This network then acts on the ovarian cancer microenvironment, particularly affecting the leukocyte infiltrate and development of blood vessels in peritoneal tumor deposits. The angiogenic actions of TNF- α may be due, at least in part, to its ability to cause differentiation of myeloid progenitor cells into endothelial cells in the tumor microenvironment [31]. Apart from endothelial cells, other host cells targeted by the paracrine actions of malignant cell-derived TNF- α include tumor-associated macrophages [32] and CD4 cells [33].

It is not only malignant cells that can make TNF- α in the tumor microenvironment. In a genetic model of liver cancer, TNF- α produced by myeloid cells promoted inflammation-associated tumors

[34]; in a model where chemical damage led to liver cancer, Kupffer cell-derived TNF was one of the mitogens driving proliferation of hepatocytes in which DNA damage had already been caused by the carcinogenic agent diethylnitrosamine [35]. In both a chemically induced model of colorectal cancer, and a genetic model of gastric cancer, macrophage TNF- α was implicated in inflammation and subsequent tumor development (reviewed in Ref. [29]).

Whether made by malignant cells or host cells – or both – TNF- α may directly contribute to oncogene activation, DNA damage and stimulate EMT, e.g. [36]. This may partly explain the ability of TNF- α to enhance metastatic activity of tumor cells as first reported in the 1990s and further elucidated by Michael Karin’s group in 2009 [37].

Most of these pro-tumor actions of TNF- α appear to be mediated via TNFR1. This TNF receptor is found on tumor and stromal cells in human cancer biopsies whereas TNFR2 is generally present on the leukocyte infiltrate, although it is also present on malignant cells in renal cell carcinoma [38] where it may allow autocrine growth stimulation acting via Etk-VEGFR2 cross-talk [39].

TNF- α protein is found in many different human cancers, both produced by the malignant cells and or other cells in the microenvironment, and elevated levels are found in plasma (see below) of patients with advanced cancer disease (reviewed in Ref. [29]). High levels of expression of TNF- α made a major contribution to an 11-gene signature of poor prognostic significance in Stage 1 lung cancer [40].

However, it is also clear that high doses of TNF- α can have anti-tumor activity that justify its original naming as a tumor necrosis factor [29]. Recent insight into the apparent paradoxical actions of this cytokine comes from simpler organisms. In *Drosophila* both tumor suppressing and tumor-promoting roles of TNF are conserved, and oncogenic Ras is the ‘switch’ [41]. Flies have just one TNF superfamily member called *dTNF/eiger* (*egr*) and one TNF receptor. Fly larvae mutant for ‘scribble group’ polarity tumor suppressor genes develop imaginal disk tumors [42] and fly TNF acts as a tumor suppressor via the JNK signaling pathway [41]. However, when oncogenic ras (*Ras^{v12}*) is introduced into the model, large invasive tumors develop. In flies lacking *egr/dTNF*, there was no invasion of oncogenic cells, the clones remained at their site of origin and the larvae pupated. This mechanism may have evolved as a tumor suppressor function of the fly innate immune system causing apoptosis of the mutant and potentially tumorigenic cells. However, if the same cells gain a *ras* mutation, they are able to re-direct this immune response to provoke invasive growth in a way that is detrimental to the host.

The results suggest that acquisition of Ras mutations may help tumors evade host immune responses, and this is supported by the fact that *ras^{v12}* is a common mutation in human cancers. The *Drosophila* data may also help us understand why it is advantageous to have cytokines such as TNF produced early on in the process of carcinogenesis. A common feature of tumor progression is the deregulation of epithelial cell polarity and adhesion.

IL-6 is another major mediator of acute inflammation and as with other major cytokines, dysregulation of IL-6 signaling contributes to many inflammatory diseases [43]. IL-6 also has tumor-promoting actions on both malignant and stromal cells in a range of experimental cancer models [43–45], is a downstream effector of oncogenic *ras* [46] and has been implicated in several human cancers including multiple myeloma [47] and hepatocellular carcinoma [48].

IL-6 can be an autocrine or paracrine growth factor in some malignancies, especially those of hematologic origin, it blocks apoptosis and signals through STAT3 that is activated in many cancers [43]. Via STAT3, genes that promote cell proliferation and angiogenesis are induced in the tumor microenvironment by IL-6 [43].

IL-6 is a critical tumor promoter in colitis-associated cancer. Produced by lamina propria myeloid cells, IL-6 enhances proliferation of tumor-initiating cells and protects normal and malignant intestinal epithelial cells from apoptosis in a STAT3-dependent manner [44,45]. Continuous treatment with recombinant IL-6 increased tumor size in colitis-induced cancer models.

IL-6/gp130/STAT3 signaling also provides autocrine and paracrine amplification loop in lung adenocarcinoma [49] and ras-transformed cancer cells [46]. Bone marrow-derived IL-6 contributes to the formation of a bone marrow microenvironment that favors progression of neuroblastoma and increases survival of neuroblastoma cells [50].

In ovarian cancer, there is pre-clinical evidence that IL-6 enhances tumor cell survival and increases resistance to chemotherapy via JAK/STAT signaling in tumor cells [51] and IL-6 receptor alpha transsignaling on tumor endothelial cells [52]. In addition, IL-6 has pro-angiogenic properties [53], as well as regulating immune cell infiltration, stromal reaction and the tumor-promoting actions of Th17 lymphocytes [54]. In patients with advanced disease, high plasma levels of IL-6 correlate with poor prognosis [55,56], and elevated levels are also present in malignant ascites [57]. Some ovarian cancer cell lines constitutively secrete IL-6, and its production is enhanced when these cells are co-cultured with other cells from the ovarian cancer microenvironment [30,58,59]. We have found that this IL-6 is part of a malignant cell autocrine cytokine network in ovarian cancer cells [30,60]. This network involves co-regulation of the cytokines TNF- α and IL-1 β , CCL2, CXCL12 and VEGF and has paracrine actions on angiogenesis in the tumor microenvironment.

There is strong renewed interest in IL-1 in relation to CRI. For instance, IL-1 is involved in the generation of IL-17 producing CD4⁺ T cells and the IL-23/IL-17 axis has been shown to promote skin carcinogenesis [61]. Direct evidence for a role of IL-1 in human cancer has been obtained in multiple myeloma. Proteasome inhibitors and cytokine production inhibitors thalidomide and lenalidomide are credited to act by disrupting this axis. IL-1 β released by myeloma cells induces IL-6 production by bone marrow stromal cells, and this cytokine is in turn a growth factor for myeloma cells.

4. Chemokines and cancer-related inflammation

Chemokines and their receptors are a key component of cancer-related inflammation affecting several pathways of tumor progression including leukocyte recruitment and function, cell senescence, tumor cell proliferation and survival, invasion and metastases [62].

CCL2 (MCP-1) and inflammatory CC chemokines have long associated to recruitment of TAM in tumors, in particular TAM [63–65]. Unequivocal genetic evidence for a non-redundant role of inflammatory CC chemokines in carcinogenesis has now been obtained [63,66]. M2 polarization and survival of TAM are also promoted by CC chemokines. CCL2 and its cognate receptor CCR2 have been investigated in mouse tumors in particular in prostate cancer [67].

Many cancer microenvironment papers focus on the tumor-promoting or tumor-inhibiting roles of a particular immune, fibroblast or endothelial cell but, as discussed above, the reality is complex and dynamic interactions between the many different cells that comprise a malignant tumor. One recent study showed a role for the chemokines CCL17 and 22 in such interactions. Th2 lymphocytes are abundant in the fibrotic stroma that is characteristic of human pancreatic cancer. De Monte et al. [68] hypothesized that tumor-resident dendritic cells, conditioned by local factors, may be able to favor differentiation of tumor-specific Th2 cells in the draining lymph nodes. They also had an idea that an IL-17-like cytokine, thymic stromal lymphopoietin (TSLP) might be involved. In work

conducted entirely with human cells and tissues the authors found a significant association between tumor Th2 cell infiltrates and poor prognosis with the ratio between Th2/Th1 cells in tumor biopsies being independently predictive of patient survival. Using biopsies, isolated cells and short-term primary cultures, they found that inflammatory cytokines produced by the malignant cells (probably downstream of oncogenic mutations) stimulate fibroblasts to make TSLP. TSLP activates/matures resident tumor dendritic cells that migrate to draining lymph nodes where they in turn activate CD4⁺ T cells to a Th2 phenotype. Th2-attracting chemokines CCL17 and 22 bring the CD4⁺ cells back to the tumor where they have a major promoting influence.

A final example of cell co-operation and chemokines involves CCL28. Hypoxia, immune evasion and the formation of new blood vessels are key enabling characteristics of a progressing tumor microenvironment—and recent work [69] shows that, at least in intraperitoneal ovarian cancer, CCL28 might link this ‘vicious triangle’.

CCL28 was frequently and strongly upregulated when human ovarian cancer cells were exposed to hypoxia. CCL28 is normally associated with mucosal immunity but it also recruits immunosuppressive T regulatory, Treg cells, during liver inflammation. This malignant cell-produced CCL28 recruited FoxP3 positive T regulatory cells that also expressed chemokine receptor CCR10—a receptor for CCL28. And the Treg cells did not only contribute to immune tolerance—they also produced the angiogenic factor VEGFA.

Hypoxia can induce a type of cell death that can trigger immune rejection of tumors—the induction of CCL28 is a mechanism to counter this via recruitment of immune suppressive and angiogenic T regulatory cells. It will be interesting to see if different chemokines have similar actions in tumors at other sites in the body or whether CCL28 has a more universal role.

5. Therapeutic opportunities

Our understanding CRI has increased to the point when we can begin to translate our knowledge into new approaches to cancer prevention and treatment. This section will review some recent pre-clinical and clinical studies relating to the cells and soluble mediators of CRI.

5.1. Targeting TAM

There is evidence from experimental cancer models that it may be possible to ‘re-educate’ tumor-promoting TAM to reject malignant cells, e.g. [70]. There is one promising recent example of a clinical study that involved modulating the function of TAM. Starting with the hypothesis that activation of the TNF receptor family member CD40 may reverse the immunosuppressive tumor microenvironment, Beatty et al. conducted a small Phase II clinical trial of the fully human CD40 agonist antibody CP-870,893 in combination with gemcitabine chemotherapy, in twenty-one patients with advanced pancreatic cancer [71]. Four patients had partial responses (i.e. a greater than 50% reduction in the size of a tumor mass) and eleven patients had a period of disease stabilization. This response rate was greater than the 5% expected response rate to gemcitabine alone and the progression-free survival was increased from a historic rate of 2.3 months to 5.6 months [71]. Intriguing hints to mechanism of action were found in two biopsies from treated patients—a prominent cellular infiltrate that was devoid of lymphocytes. To investigate this further, clinical trial was modeled in the KPC mouse genetic model of pancreatic cancer using a mouse CD40 agonist. Within 18 h of administration, the CD40 agonist had bound to tumor-associated macrophages, TAM that

had up-regulated MHC Class II and the co-stimulatory molecule CD86. These cells were then able to lyse the pancreatic tumor cells in vitro and in vivo destruction of the tumor stroma was observed. Macrophage depletion prevented these actions of the CD40 agonist, but the therapy was still active in mice depleted of CD4+ or CD8+ T cells. This means that cancer immune surveillance does not necessarily depend on stimulating T cells, but that 're-educating' the abundant macrophages in the tumor microenvironment may be just as effective—or more. These results, while not dramatic in terms of clinical response, can be seen as encouraging and certainly warrant further clinical investigation of CD40 agonists.

5.2. Targeting key cytokines in the tumor microenvironment

If TNF- α was involved in growth of experimental tumors, then anti-TNF- α antibodies or other TNF antagonists should have therapeutic activity in similar mouse models. This is indeed the case as reported in experiments involving carcinogen-induced, transplantable, xenograft and genetic models of common epithelial cancers (reviewed in Ref. [29]). This raised the possibility that it might be beneficial to neutralize TNF- α activity in cancer patients. This has been tested in early Phase clinical trials of TNF antagonists as single agents, with some evidence of clinical activity (reviewed in Ref. [29]). For instance, in a Phase I study using the anti-TNF antibody infliximab, stabilization of disease was observed in 7 of 41 patients with previously progressing advanced cancer; in a Phase II study in ovarian cancer, 6 of 30 progressing patients also showed stable disease after treatment with the TNF- α antagonist etanercept (a soluble TNFR2 fusion protein that binds and neutralizes TNF- α) and in renal cell cancer 14 of 39 patients achieved stable disease with 3 of 39 obtaining partial responses after infliximab treatment. Clinical benefit of TNF- α antagonists has also been seen in the pre-malignant condition of myelodysplasia.

There is as yet no clear idea of mechanisms of action of anti-TNF- α in cancer patients but nearly twenty years experience in patients with chronic inflammatory disease shows that TNF- α antagonists inhibit cytokine and chemokine production, recruitment of inflammatory cells, angiogenesis and extra-cellular matrix degradation [72]—all actions that could be useful in a cancer treatment. In addition, binding of TNF antagonists to transmembrane TNF- α may have direct effects on TNF-producing cells, stimulating a number of cytotoxic pathways. Two specific actions of TNF- α antagonists on the immune system in patients with inflammatory disease are of particular interest in terms of cancer treatment; modulation of the function of T regulatory cells and a reduction in Th17 inflammatory responses, both of which are implicated in tumor promotion.

The therapeutic anti-IL-6 antibody siltuximab (CNO328) has been evaluated in Phase II trials of Castleman's disease [73] and castration-resistant prostate cancer [74]. In Castleman's disease, in which IL-6 is a key pathogenic driver, the objective response rate was 52%; by contrast, in prostate cancer, the response rate was 3.2%.

We combined pre-clinical and in silico experiments with a Phase II clinical trial of siltuximab in patients with platinum-resistant ovarian cancer [60]. Automated immunohistochemistry on tissue microarrays from 221 ovarian cancer cases demonstrated that intensity of IL-6 staining in malignant cells significantly associated with poor prognosis. Treatment of ovarian cancer cells with siltuximab reduced constitutive cytokine and chemokine production and also inhibited IL-6 signaling, tumor growth, the tumor-associated macrophage infiltrate and angiogenesis in IL-6-producing intraperitoneal ovarian cancer xenografts. In the clinical trial, one patient of eighteen evaluable had a partial response, while seven others had periods of disease stabilization. In patients treated for six months, there was a significant decline in plasma levels of IL-6-regulated CCL2, CXCL12 and VEGF. Gene expression

levels of factors that were reduced by siltuximab treatment in the patients significantly correlated with high IL-6 pathway gene expression and macrophage markers in microarray analyses of ovarian cancer biopsies. Hence we concluded that IL-6 stimulates inflammatory cytokine production, tumor angiogenesis and the tumor macrophage infiltrate in ovarian cancer and these actions can be inhibited by a neutralizing anti-IL-6 antibody in pre-clinical and clinical studies [60].

5.3. Targeting key chemokines and their receptors

Antibodies against CCL2 or its cognate receptor CCR2 have been investigated in preclinical models and a strong case for anti CCL2 therapy has been made for prostate cancer [67]. Administration of antibodies to CCL2 in mice bearing prostate cancer resulted in decreased tumor burden and bone resorption, with lower CCL2-induced VEGF release. Combination studies with anti-CCL2 and chemotherapy have also yielded improved survival in pre-clinical settings [75]. Anti-CCL2 antibodies are currently being evaluated in humans in prostate and ovarian cancer [76].

An extensive study of CCL2 in breast cancer metastases has shown that CCL2 synthesized by metastatic tumor cells and by stroma at sites of metastases, is critical for continual recruitment of CCR2-positive monocytes that enhance the extravasation of tumor cells. Blockade of CCL2 with neutralizing antibodies inhibited monocyte recruitment, reduced metastases and prolonged survival of tumor-bearing mice [64]. Such anti-CCL2 antibodies are undergoing clinical evaluation in prostate and ovarian cancer [76].

Cutaneous T cell lymphoma and T-cell acute lymphoblastic leukemia express the chemokine receptor CCR4. A humanized defucosylated antibody to CCR4 has anti-tumor activity in lymphoma-bearing mice [77]. Anti-CCR4 antibody therapy was associated with increased numbers of tumor-infiltrating CD56⁺ NK cells mediating ADCC, and reduced the number of FOXP3⁺ Treg cells. Clinical trials are now underway with this antibody.

CXCR4 is the most commonly over-expressed chemokine receptor in human cancer and affects tumor cell proliferation, survival, invasion and metastasis. It is upregulated by inflammatory cytokines such as TNF- α and induced by hypoxia. CXCR4 has been targeted by a number of small antagonists, including the bicyclam AMD3100 and analogs and peptides designed to the amino-terminal region of the chemokine such as T22, TN14003, and CTCE-9908. CXCR4 antagonists inhibited the primary tumor and metastasis in animal models of melanoma, osteosarcoma, breast, and prostate tumors, e.g. [78,79].

The bicyclam AMD3100 was first developed as an anti-HIV agent. Unexpectedly, AMD3100 was found to mobilize CD34⁺ stem cells from the bone marrow [80]. At present AMD3100 is clinical use for hematopoietic stem cell mobilization [81]. By mobilizing malignant cells from the bone marrow niche, AMD3100 enhances the sensitivity of multiple myeloma or acute myeloid leukemia blasts to the cytotoxic effect of chemotherapy in preclinical models [81].

6. Stimulating 'good' inflammation

Inflammation may not always be 'bad' in the context of malignant disease; the cancer cytokine network can also contribute to therapeutic response. It is a question of balance of individual members of the cytokine network. The actions of the cells and cytokines of inflammation in the tumor microenvironment are very context-dependent and one approach to cancer therapy is to attempt to switch the tumor-promoting immune suppressive microenvironment to one that kills tumor cells, is anti-angiogenic and promotes adaptive immune responses as described above with the anti-CD40 antibody [71].

At the turn of the 19th century a New York surgeon appeared to achieve impressive clinical results by using inflammation-stimulating bacterial extracts to treat patients with intractable cancers reviewed in Ref. [29]. We now realize that Coley's mixed toxins must have been powerful stimulants of Toll-like receptors, TLRs, inducing a range of inflammatory mediators. The closest recent approximation to Coley's work is probably the successful local treatment of bladder cancer with bacillus Calmette–Guerin, BCG. Current thinking is that both BCG and Coley's toxins trigger a 'good' inflammatory response, via TLRs, that not only stimulates macrophages to kill tumor cells but also promotes development of sustained and effective adaptive immunity to the tumor [70].

Stressed and dying tumor cells may emit a particular pattern of 'danger signals' that are either expressed on the cell surface or secreted into the microenvironment influencing the switch from non-inflammatory 'silent' removal of dead cells to a 'good' inflammatory reaction that can stimulate rapid invasion of $\gamma\delta$ IL-17-producing T cells and generate tumor-specific IFN- γ -producing CD8+ T cell adaptive immune responses that are important to the outcomes of some chemotherapies and radiotherapy [82,83]. In mouse models, dying tumor cells were able to cross-present antigen to dendritic cells in a TLR4-dependent manner and breast cancer patients with a mutation in TLR4 had an increased frequency of metastasis [84]. Inflammasome activation and IL-1 β production can also underlie activation of protective immunity after cytotoxic chemotherapy. Associations of TLR4 and PrX7 polymorphisms with clinical outcome are consistent with the relevance of these pathways [85].

Another paper on TLR signaling and cancer may also give some clues about 'good' immune responses and TNF [86]. The TLR-receptor associated signaling adaptor MyD88 plays a critical role in TLR and inflammatory cytokine signaling. As might be expected MyD88-/- mice were resistant to DMBA/TPA skin carcinogenesis, but they were also resistant to MCA-induced sarcomas—tumors that are very susceptible to immune-surveillance. However, while searching for downstream effectors of MyD88-induced tumor promotion, Mark Smyth's group found that TNF knockout mice were actually more susceptible to MCA-induced sarcoma. One explanation could be that these sarcomas, like tumor cells dying after chemotherapy, are inherently immunogenic and under such circumstances the inflammatory microenvironment stimulated by TNF- α actually protects against tumor development [86].

The exact mechanisms whereby a 'good' inflammatory response can be reliably triggered during cancer therapy are not clear, but even before Coley's time there was evidence for cancer regression after some bacterial infections. The priority is to find the best stimuli to change the cytokine network of a tumor-promoting microenvironment to a tumor-inhibiting state and to understand the signaling mechanisms involved.

7. Combining therapies

Over the past decade a number of exciting and more targeted cancer treatments have entered the clinic, a direct result of twenty-five years research into the genetic basis of malignant disease. Even these treatments, however, are liable to generate clones of cancer cells that overcome this specific receptor or signaling pathway blockade. Now that the importance of the inflammatory tumor microenvironment is recognized and early clinical trials are underway, the next steps are to devise and test therapeutic strategies that combine targeting the malignant cells with targeting CRI maybe alongside, or in sequence with anti-angiogenic therapies and immune checkpoint blockade. It is also likely that combination of chemotherapy with immune checkpoint blockade with CTLA4 will provide useful treatments for cancers and block

immune escape. As described above, some chemotherapies may create a microenvironment that can stimulate 'good' inflammation and promote useful adaptive immune responses.

TAM may also have a profound influence on a tumor's response to chemotherapy [87] because common cytotoxic drugs may recruit tumor-promoting monocytes/macrophages into the tumor microenvironment. In a genetic mouse model of breast cancer, paclitaxel chemotherapy up-regulated the macrophage chemotactic factors CSF1, CCL8 and IL-34 which led to an increase in CSF1 receptor-expressing macrophages in the tumor microenvironment. Blockade of macrophage recruitment with inhibitors of CSFR1 in combination with chemotherapy, enhanced therapeutic activity, inhibited metastases. The late-stage carcinomas that did develop during this combination therapy contained large areas of necrosis, paralleled by reduced vessel density in the treated tumors. Inhibition of the macrophage infiltrate also increased T cells in the tumors and mRNA for a number of cytotoxic effector molecules such as granzyme A and B and perforin-1. Depleting CD8+ cytotoxic effector T cells in the mice abrogated the positive effects of macrophage depletion [87].

The extent of the CD8+ T cell infiltrate positively correlates with good prognosis in many cancers and macrophage infiltrates usually show a negative correlation in the same cancers. This work gives us important mechanistic insights into the role of the tumor's immune profile in the response to chemotherapy and hence the outcome for an individual patient. The association found by DeNardo et al. seems to be true for different breast cancer subtypes suggesting that targeting leukocytes in the tumor microenvironment during other treatments is an option that will be widely applicable, especially in those patients who have an unfavorable immune profile at presentation.

Some recent pre-clinical studies suggest that it may also be of interest to target TAM during radiotherapy, as macrophages, in a manner that required intact TNF- α signaling and VEGF production, contributed to radioresistance [88].

8. Prevention strategies

Everything we have learnt so far about cancer-related inflammation leads to the hypothesis that anti-inflammatory agents may have potential as cancer preventative agents. In terms of non-steroidal anti-inflammatory agents, there are several observational studies suggesting that aspirin reduces risk of certain cancers, e.g. [89]. A recent study of particular interest was an analysis of deaths due to cancer in a cohort of patients randomized to receive aspirin or placebo in trials originally planned to study prevention of vascular disease [90]. In eight trials with over 25,000 individuals, aspirin significantly reduced deaths from solid cancers with the benefit increasing with duration of treatment. The latent period before an effect on deaths was about 5 years for esophageal, pancreatic, brain and lung cancer but was more delayed for stomach, colorectal and prostate cancer. The overall effect on 20-year risk of cancer death was greatest for adenocarcinomas and was independent of smoking history, sex, or a dose of aspirin above 75 mg, but increased with age [90].

One potential mechanism for the cancer-preventative actions of NSAIDs has recently been reported. Chronic pancreatitis is a highly significant risk factor for PDAC but a majority of patients who develop the disease have no history of this condition. Maybe sub-acute pancreatic inflammation drives the majority of human cases of PDAC? Guerra et al. [91] induced sub-acute asymptomatic pancreatitis in the PDAC mouse model. Providing the pancreatic ductal cells harbored a K-Ras mutation typical of the human disease, this inflammatory stimulus, which induced atrophy, fibrosis and a persistent infiltrate of macrophages and T lymphocytes, was sufficient

to induce pre-malignant and malignant lesions. The development of the malignant lesions was dependent on the extent of tissue damage and the inflammatory response: inflammation abrogated the senescence barrier characteristic of low-grade pre-malignant lesions. Remarkably, malignant changes happened even if the *K-Ras* mutation was switched on after inflammation-induced damage, but treatment of mice with a non-steroidal anti-inflammatory drug attenuated development of premalignant lesions and progression to PDACs. Relating the mouse results to human pancreas biopsies, premalignant lesions in patients with chronic pancreatitis who had received anti-inflammatory drugs had high expression of the senescence marker P16INK4A and no evidence of cell proliferation; the opposite was seen in biopsies from patients not treated with anti-inflammatory drugs. These data also suggest that anti-inflammatory treatments in people diagnosed with pancreatitis may reduce their risk of developing PDAC.

The effects of NSAIDs such as aspirin are likely to be, at least in part, due to inhibition of the inflammatory enzyme COX2. A more specific inhibitor of COX-2, celecoxib, was evaluated in a randomized trial of patients with extensive actinic keratoses, the pre-malignant precursor of nonmelanoma skin cancers. Eleven months after start of the study, the incidence of basal and squamous cell carcinomas was significantly decreased in the group receiving celecoxib [92].

The cancer-preventative potential of more specific antagonists of cytokines such as TNF- α or IL-6 is more theoretical. Certainly mouse model experiments suggest a role for TNF- α in promotion of early cancers (e.g. [93]). Both herbal medicines and tea polyphenols inhibit TNF release, e.g. [94] but given the role of TNF- α in regulating innate immunity, increased risk of infection would preclude wider use of current TNF- α antagonists. However, tens of thousands of people with rheumatoid arthritis and other chronic inflammatory disease are being monitored for cancer incidence during TNF- α antagonist treatment. Analyses are complicated by underlying immune system dysfunction in these patients, prior treatment with immunosuppressive and mutagenic drugs, and the small number of malignancies so far recorded. In one meta-analysis of nine double blind placebo controlled trials of anti-TNF- α antibodies in patients with rheumatoid arthritis an increased risk of cancer was recorded [95]. However, in a later review the same authors concluded that with over fifty trials of anti-TNF- α in inflammatory disease now published there was no clear evidence for overall increase in cancer risk [96]. The current view is that caution may be necessary when considering treatment of patients with past or concurrent cancer or premalignant lesions and there seems to be an increase in rare $\gamma\delta$ T cell lymphomas in patients with juvenile Crohn's disease [95,96]. There is also no evidence of an increase in overall cancer incidence in patients receiving with anti-TNF- α therapies as compared to a matched cohort of the general public.

The administration of the IL-1 receptor antagonist (anakinra) to patients with indolent, smouldering myeloma has been reported to inhibit progression to aggressive disease [97]. More potent IL-1 inhibitors have now entered clinical evaluation for autoinflammatory diseases and may provide better tools to interfere with CRI.

Finally, on a more general note, many drugs currently used in long-term treatment of common diseases such as statins, histone deacetylase inhibitors and PPAR agonists, have anti-cytokine effects that may give them a cancer-preventative role [98].

9. Biomarkers of CRI

Plasma or serum levels of inflammatory cytokines and chemokines, and in some cases their soluble receptors, are elevated in patients with a range of advanced cancers and this is generally a poor prognostic sign. For example, raised blood TNF- α levels

generally associated with poor prognosis (reviewed in Ref. [99]). To take the example of prostate cancer, blood TNF- α concentrations are elevated in those patients with advanced, cachectic disease and TNF- α levels correlate positively with extent of disease, e.g. [100]. Plasma or serum levels of IL-6, which increase with age anyway, are also elevated in many solid and hematological cancers. For instance in ovarian cancer patients, a number of studies have shown that high IL-6 levels correlate with stage of disease, poor prognosis, and reduced survival (e.g. [56]). More recent studies have shown that high plasma IL-6, dysregulated cortisol and vegetative depression are all linked in patients at diagnosis of ovarian cancer [55]. However, IL-6 levels do not seem to be as useful a biomarker as CA125 in this disease.

In prostate cancer high pre-diagnostic IL-6 was significantly associated with time to prostate cancer progression/death in healthy weight prostate cancer cases [101].

In acute myelogenous leukemia, AML, and myelodysplasia a range of cytokines and chemokines were consistently elevated or decreased compared with normal controls with the patterns having prognostic significance [102]. There also seemed to be signatures associated with the distinct cytogenetic abnormalities of AML subtypes, possibly more evidence that cytokine and chemokine production in cancer may be driven by oncogenic changes.

While inflammatory cytokines are not as yet seen as useful markers of prognosis and response to treatment, we can now study a range of different cytokines and chemokines in small volumes of plasma or serum using multiplexing techniques as shown by the work of [60,102]. It is possible that response to some treatments (e.g. cytokine antagonists described above) may be assessed by such assays and some insight may be gained on mechanisms of action. A recent study, for example, used longitudinal profiling of cytokine levels during intensity-modulated radiotherapy, IMRT, for prostate cancer. Both IFN- γ and IL-6 were raised during treatment and increasing levels of IL-2 and IL-1 were associated with gastrointestinal and genitourinary toxicity respectively [103]. Stable disease induced by dendritic cell-based vaccination in patients with colorectal cancer, was associated with increased Th1 cell cytokines in plasma [104]. In a Phase II trial of an anti-IL-6 antibody in ovarian cancer patients, those patients who had stable disease for six months or longer showed significant declines in plasma CCL2, CXCL12 and VEGF—all factors that can be induced by IL-6 [60].

If sufficient care is taken in preparation and storage of blood samples that are taken at a standard time of day (because of diurnal rhythms in cytokine levels) and we have better knowledge of the stability of cytokines in plasma and serum during storage, it is possible that these mediators may be useful biomarkers of prognosis and/or response to treatment, especially if we consider the tumor to be the major source of elevated cytokines in blood of cancer patients.

Conflict of interest

None declared.

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