

Smoldering and polarized inflammation in the initiation and promotion of malignant disease

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Introduction

Inflammation is a crucial function of the innate immune system that protects against pathogens and initiates specific immunity. Acute inflammation is a rapid and self-limiting process: chemical mediators are induced in a tightly regulated sequence, and immune cells move in and out of the affected area, destroying infectious agents, repairing damaged tissue, and initiating a specific and long-term response to the pathogen. However, acute inflammation does not always resolve. Many of the diseases of middle and old age may be driven, at least in part, by chronic, "smoldering," and often subclinical inflammation. Here we describe the latest evidence for the involvement of inflammation in cancer, and discuss implications for prevention and therapy. Several lines of evidence, including general or cell-specific gene inactivation and population-based studies, are consistent with the view that inflammation plays an important role in malignant progression. These are listed in Table 1.

Chronic inflammatory diseases predispose to cancer

As summarized in Table 2, diverse agents trigger the inflammation associated with human cancer. These links have been confirmed in a number of murine models, especially in terms of gastric (*H. pylori* infection) (Houghton et al., 2004), liver (cholangitis) (Greten et al., 2004) and colon (colitis) (Pikarsky et al., 2004) cancers. In these and other animal cancer models (e.g., Arnott et al., 2002), the cells and mediators of chronic inflammation act as tumor promoters at distinct phases of malignant progression.

Cancer and the plasticity/polarization of inflammatory reactions

Neoplastic tissue is a Darwinian microenvironment that selects for the type and extent of inflammation most favorable to tumor growth and progression. Plasticity is a hallmark of inflammation.

Table 1. The links between cancer and inflammation

- Chronic inflammation increases risk of cancer, and many cancers arise at sites of chronic inflammation
- The immune cells that mediate chronic inflammation are found in cancers and promote tumor growth in cell transfer experiments
- The chemical mediators that regulate inflammation are produced by cancers
- Deletion or inhibition of inflammatory mediators inhibits development of experimental cancers
- Genetic variations in inflammatory genes alter susceptibility to and severity of cancer
- Long-term use of nonsteroidal anti-inflammatory agents reduces risk of some cancers

Triggering of innate immunity and mounting of an acute response is generally followed by a late phase where regulatory mechanisms, tissue repair, and remodeling prevail. Acute inflammation triggered by exogenous administration of TNF- α , IL-1, and LPS has been known under certain conditions to promote malignancy and metastasis (Balkwill and Mantovani, 2001). However, chronic local inflammatory reactions with little systemic manifestations are more relevant to the pathophysiology of most neoplastic conditions.

The diversity of inflammatory reactions is dictated by the primary stimulus as well as by exogenous and endogenous modifying signals. At one end of the spectrum, type 1 inflammation, typified by granuloma formation, is elicited by intracellular pathogens. At the other extreme, inflammatory reactions to parasites are characterized by eosinophil and mast cell/basophil infiltration and by extensive tissue remodeling (type 2 inflammation).

Macrophages are key cells in chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programs (Gordon, 2003; Mantovani et al., 2002). M1 macrophages are involved in Type 1 reactions and are classically activated by microbial products, killing microorganisms and producing reactive oxygen and nitrogen intermediates. In contrast, M2 cells, involved in Type 2 reactions (Mantovani et al., 2004b; Mantovani et al., 2002), 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, and promote angiogenesis, tissue remodeling, and repair. M1 and M2 cells are extremes in a continuum

Table 2. Inflammatory conditions that predispose to cancer

Malignancy	Inflammatory stimulus
Bladder cancer	Schistosomiasis
Gastric cancer	<i>H. pylori</i> -induced gastritis
MALT lymphoma	<i>H. pylori</i>
Hepatocellular carcinoma	Hepatitis virus (B and C)
Kaposi's sarcoma	HHV8
Bronchial carcinoma	Silica
Mesothelioma	Asbestos
Bronchial carcinoma	Asbestos
Ovarian cancer	Salpingitis/talc/ovulation/endometriosis
Colorectal cancer	Inflammatory bowel disease
Oesophageal cancer	Barrett's metaplasia
Papillary thyroid carcinoma	Thyroiditis
Prostate cancer	Prostatitis

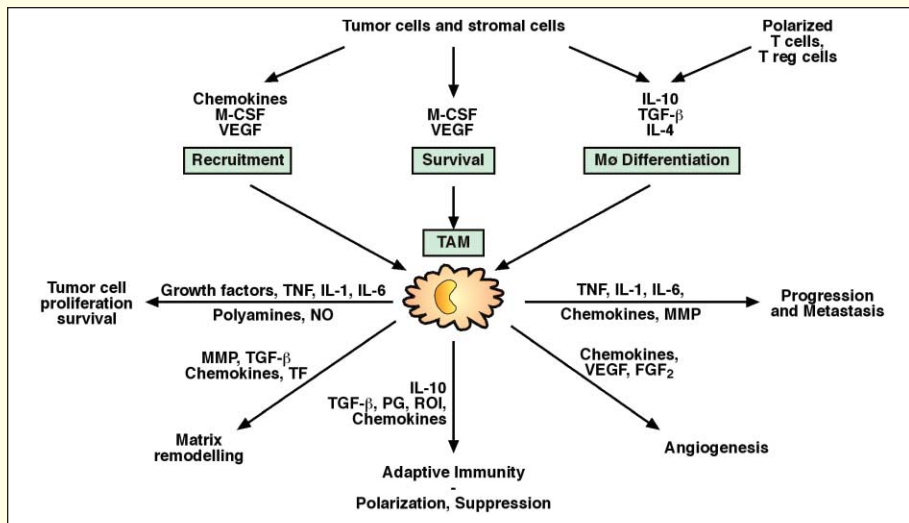


Figure 1. Type II macrophages in the tumor microenvironment.

Mononuclear phagocytes recruited in chimeras polarize in an M2 direction in response to microenvironmental signals. M2 TAMs promote chimera progression, matrix remodeling, and angiogenesis, and divert and tame adaptive immunity.

effector T cells and other inflammatory cells, such as monocytes, via mediators such as TGFβ and IL-10.

T cell-derived TGFβ inhibits an IL-6-dependent signaling cascade that promotes colon cancer carcinogenesis (Becker et al., 2004), and genetic inactivation of the TGFβ axis has long been known to be involved in neoplastic transformation. On the other hand, TGFβ suppresses adaptive immunity and mediates

of functional states. For instance, different forms of M2 cells have been described sharing an IL-12 low/IL-10 high phenotype with variable capacity to produce TNF, IL-1, and IL-6 (Mantovani et al., 2004a). Tumors are diverse, and so are their associated inflammatory reactions. When associated with established neoplasia, inflammation is usually polarized in a type 2 direction.

Tumor-infiltrating leukocytes promote malignant progression

The inflammatory microenvironment of neoplastic tissues is characterized by the presence of host leukocytes both in the supporting stroma and among the tumor cells, with macrophages, dendritic cells, mast cells, and T cells being differentially distributed (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Nakayama et al., 2004).

Tumor-associated macrophages (TAMs) are a major component of this leukocyte infiltrate, accumulating in hypoxic areas of tumors (Bingle et al., 2002; Pollard, 2004) due to HIF-1-dependent upregulation of the chemokine receptor CXCR4 (Schioppa et al., 2003). TAMs are initially recruited by inflammatory CC chemokines (e.g. CCL2); recruitment and survival is sustained by cytokines present in the tumor microenvironment (e.g., CSFs, VEGF-A). In response to cytokines such as TGF-β, IL-10, and M-CSF, TAM acquire M2 properties, promoting tumor proliferation and progression and stroma deposition, and remodeling and inhibiting adaptive immunity (Obermueller et al., 2004; Pollard, 2004; Wyckoff et al., 2004). Stimulation of hemangiogenesis and lymphoangiogenesis by VEGF-A is at least in part indirect, via macrophage recruitment (Cursiefen et al., 2004). This general view of TAM (see Figure 1) is supported by cell transfer or depletion experiments, gene modified mice, and association between high macrophage content and poor prognosis, in many (though not all) human tumors (Bingle et al., 2002; Lin et al., 2001; Robinson et al., 2003).

Inhibition of effective antitumor T cell-dependent immunity occurs in a number of ways, including production of immunosuppressive indoleamine dioxygenase (IDO) metabolites by TAM and immature myeloid cells (Bronte et al., 2003), inhibition of DC maturation by IL-10, TGF-β, and M-CSF, and attraction of T regulatory cells (Treg) to the tumor site (Curiel et al., 2004; Hori et al., 2003; Woo et al., 2002). Treg cells possess a characteristic anergic phenotype and strongly suppress the activity of

the action of Treg cells, and inhibition of TGFβ or IL-10 can under certain conditions promote the activation affecting antitumor-specific immunity (Terabe et al., 2003; Vicari et al., 2002).

Suppression of T cell-mediated antitumor activity by Treg cells is associated with increased tumor growth. In advanced ovarian cancer, an increase in Treg cells present in ascites was predictive of reduced survival (Curiel et al., 2004).

Innate immunity that underlies inflammatory reactions is key to initiation and orientation of specific immunity. Specific IFN-γ-dependent Th1 immunity protects against certain tumors in a process called immunoediting (Dunn et al., 2004). Dendritic cells play an essential role in the activation of specific immunity and infiltrate neoplastic tissues in response to tumor-derived chemokines (Mantovani et al., 2002). However, tumor-associated dendritic cells have an immature phenotype, probably because of inhibitory cytokines in the tumor microenvironment, and promote T cell anergy to tumor antigens and Treg activity. Mast cells are also found in tumors, and there is now direct evidence that they have a protumor function for mast cells (Coussens et al., 1999; Nakayama et al., 2004).

Soluble mediators of inflammation in malignant disease

The microenvironment of many human and murine cancers is rich in cytokines, chemokines, and inflammatory enzymes (Balkwill and Mantovani, 2001; Balkwill, 2004). Of particular interest is tumor necrosis factor α, TNF-α, a pivotal cytokine in inflammatory reactions. Induced by a wide range of pathogenic stimuli, TNF-α induces other inflammatory mediators and proteases that orchestrate inflammatory responses (Balkwill, 2002). True to its name, high doses of locoregional TNF-α cause hemorrhagic necrosis and can stimulate antitumor immunity. However, there is increasing evidence that TNF-α is also produced by cancers and can act as an endogenous tumor promoter (Balkwill, 2002).

General or cell-selective deletion/inhibition of TNF-α reduces the incidence of experimental cancers. For instance, TNF-α^{-/-} and TNFR1^{-/-} mice are resistant to chemically induced carcinogenesis of the skin (Arnott et al., 2004; Moore et al., 1999), and TNFR1^{-/-} mice are resistant to chemical carcinogenesis of the liver (Knight et al., 2000) and development of liver metastasis in experimental colon cancer (Kitakata et al., 2002). TNF-α drives a lymphoproliferative disorder in FasL^{-/-} mice

(Korner et al., 2000), and inhibition of stromal cell TNF- α decreases the incidence of inflammation-induced liver tumors (Pikarsky et al., 2004).

TNF- α is frequently detected in human cancers (produced either by epithelial tumor cells, as in, for instance, ovarian and renal cancer) or stromal cells (as in breast cancer) (Balkwill, 2002). This TNF- α is associated with a poor prognosis, loss of hormone responsiveness, and cachexia/asthenia. An interesting genetic link between TNF- α and malignancy was recently identified in renal cell cancer, where the pVHL tumor suppressor gene is a translational repressor of TNF- α (Galban et al., 2003).

Another key inflammatory cytokine, IL-1 β , also increases tumor invasiveness and metastasis, primarily by promoting angiogenic factor production by stromal cells in the tumor microenvironment (Anasagasti et al., 1997; Apte and Voronov, 2002; Song et al., 2003; Voronov et al., 2003).

Blood levels of IL-6 are elevated with age (Harris et al., 1999; Kiecolt-Glaser et al., 2003) due to loss of inhibitory sex steroids. The loss of hormonal regulation of IL-6 is implicated in the pathogenesis of several chronic diseases (Ershler and Keller, 2000), including B cell malignancies, renal cell carcinoma, and prostate, breast, lung, colon, and ovarian cancers (Tripathi et al., 2003). In multiple myeloma, for example, IL-6 promotes the survival and proliferation of cancer cells via activation of STAT3 and ERK pathways (Honemann et al., 2001).

Inflammatory cytokines induce chemokines, which are key components of the plasticity and polarization of inflammatory reactions (Mantovani et al., 2004b). Human and murine cancers possess a complex chemokine network that influences the extent and phenotype of the leukocyte infiltrate, as well as tumor cell and endothelial cell growth and migration, thus regulating the movement of cells both into and out of the tumor (Balkwill, 2004). Inflammatory chemokines produced by neoplastic cells or tumor-associated leukocytes attract preferentially polarized Th2 cells and Treg cells (Balkwill and Mantovani, 2001; Curiel et al., 2004).

A range of inflammatory enzymes, including cyclo-oxygenases (COX), that catalyze the conversion of arachidonic acid to prostaglandins (PG) are also induced by cytokines. An inducible isoform, COX-2, is highly expressed in colorectal, gastric, esophageal, breast, and prostate cancers and non-small cell squamous carcinoma (Choy and Milas, 2003). Increased COX-mediated PGE₂ increases tumor invasion and metastasis with increased production of IL-6, IL-8, VEGF, iNOS, MMP-2, and MMP-9 (Gasparini et al., 2003). Carcinogenesis is inhibited in COX-2 knockout mice, and mice treated with COX-2 selective inhibitors (Jacoby et al., 2000; Peluffo et al., 2004). COX-1 may also provide a similar contribution as COX-2 (Chulada et al., 2000; Kitamura et al., 2004).

Finally, systemic inflammation is characterized by the acute phase response with hepatic production of proteins such as C-reactive protein (CRP) and serum amyloid A protein (SAA). Elevated serum levels of these proteins are associated with increased tumor stage/grade, cachexia, hypercalcemia, anemia, and reduced survival in various malignancies (Bromwich et al., 2004; Mahmoud and Rivera, 2002).

Genetic evidence linking inflammation and cancer

There is a high degree of polymorphism in the cytokine genes. As these polymorphisms are frequently in regions that regulate transcription or posttranscriptional events, they may be functionally significant. The strongest association with cancer comes

from studies of the IL-1 gene cluster. IL-1 β is upregulated during *H. pylori* infection, is important in the inflammatory response of the gastric mucosa, and is a potent inhibitor of gastric acid secretion. Polymorphisms thought to enhance IL-1 β production confer an increased risk of chronic hypochlorhydria and gastric cancer (El-Omar et al., 2000; Furuta et al., 2002). Studies on larger cohorts of patients are now required to confirm these and many of the other associations reported in the literature.

How does chronic inflammation promote cancer?

The mechanisms by which chronic inflammation promotes cancer involve both initiated cells that give rise to the cancer and inflammatory cells in the surrounding stroma. As described above, the absence of the proinflammatory cytokine TNF- α , or its receptor TNFR1, conferred resistance to skin carcinogenesis (Arnott et al., 2004; Moore et al., 1999). TNF- α did not influence the initiation phase of carcinogenesis; DNA adducts and the initiating *h-ras* mutation occurred in its absence. However, epidermal induction of TNF- α , acting primarily, but not exclusively, via TNFR1, was a critical mediator of tumor promotion, acting via a PKC α - and AP-1-dependent intracellular signaling pathway in epithelial cells (Arnott et al., 2002). In the absence of TNF- α , epithelial induction of other cytokines and proteases thought to be important in skin carcinogenesis and tumor-stroma communication was delayed and/or inhibited. Similarly, in a model of chemically induced liver cancer, TNF- α production by hepatocytes was implicated in tumor development (Knight et al., 2000).

However, in an inflammation-induced liver cancer model, stromal cell TNF- α was tumor-promoting (Pikarsky et al., 2004). In this system, and in inflammation-induced colon cancer (Greten et al., 2004), NF- κ B was a critical molecular link. Selective deletion of NF- κ B in hepatocytes, or inhibition of TNF- α produced by neighboring parenchymal cells, induced programmed cell death of transformed hepatocytes and subsequently reduced the incidence of liver tumors (Pikarsky et al., 2004). Activation of NF- κ B was not important in the early stages of liver tumor development, but it was crucial for malignant conversion. In the colitis model, selective deletion of IKK β —a key intermediary of NF- κ B—in intestinal epithelial cells did not decrease intestinal inflammation, but it did reduce subsequent development of intestinal tumors, due to increased death of initiated epithelial cells. However, when IKK β was selectively deleted in inflammatory cells that infiltrate premalignant and malignant intestinal tumors, mRNA for several proinflammatory cytokines decreased, as did subsequent tumor development (Greten et al., 2004).

Hence, the NF- κ B pathway has dual actions in tumor promotion: first by preventing death of cells with malignant potential, and second by stimulating production of proinflammatory cytokines in cells of infiltrating myeloid and lymphoid cells. The proinflammatory cytokines signal to initiated and/or otherwise damaged epithelial cells to promote neoplastic cell proliferation and enhance cell survival. However, the tumor-promoting role of NF- κ B may not always predominate. In some cases, especially early cancers, activation of this pathway may be tumor suppressive (Perkins, 2004). Inhibiting NF- κ B in keratinocytes promotes squamous cell carcinogenesis by reducing growth arrest and terminal differentiation of initiated keratinocytes (Seitz et al., 1998).

Can chronic inflammation also cause initiating mutations?

In epithelial cancer, the initiating genetic event is thought to occur in a tissue stem cell. However, in a mouse model of

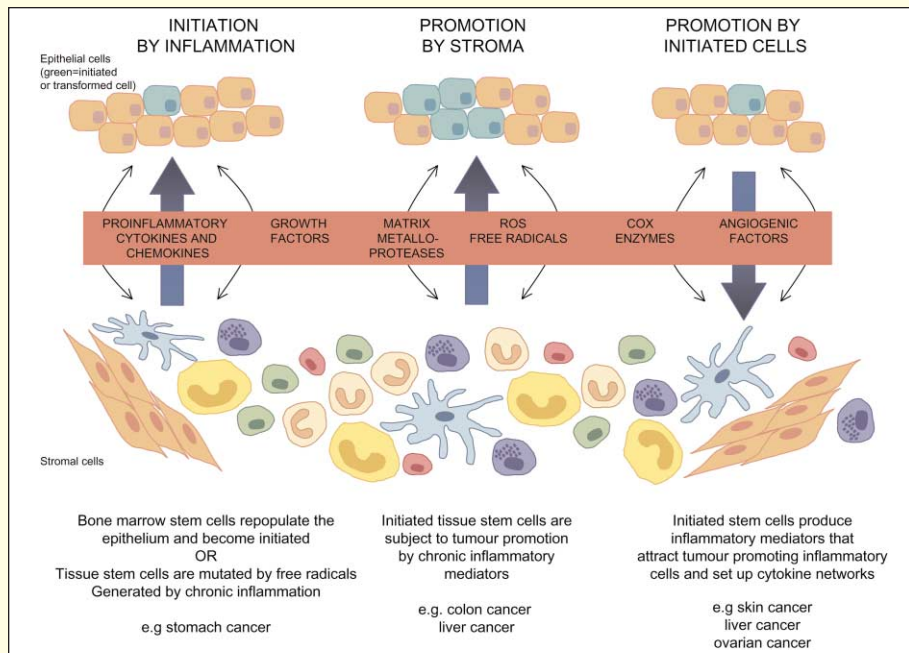


Figure 2. Mechanisms of inflammation-induced cancer

Wide blue arrows indicate the primary source of the inflammatory stimulus.

carcinoma, or patients genetically predisposed to the development of adenomas and colorectal cancer. Aspirin use may also protect against cancer of the esophagus, lung, stomach, and ovary, as well as Hodgkin's lymphoma, and preventative trials show that aspirin may delay progression in Barrett's esophagus (e.g., Chang et al., 2004; Garber, 2004; Ness and Cottreau, 1999; Wang et al., 2003). However, some data suggest that aspirin use may increase risk of pancreatic cancer and non-Hodgkin's lymphoma (Cerhan et al., 2003; Schernhammer et al., 2004).

At this moment in time, there does not seem a strong case for general use of aspirin or other NSAIDs for the sole reason of reducing risk of sporadic cancer,

Helicobacter-induced gastric cancer, the chronic inflammatory environment of the gastric mucosa induced repopulation of the stomach with bone marrow-derived stem cells (Houghton et al., 2004), and it was these stem cells that underwent malignant progression to give rise to gastric tumors. Further experiments will be needed to assess whether chronic inflammation might be initiating in other malignancies where there is a large amount of tissue destruction. The liver and colon cancer experiments, where inflammatory signals were inhibited in either initiated epithelial cells or stromal cells, do not suggest any evidence for a bone marrow stem cell origin of the tumors (Gretten et al., 2004; Pikarsky et al., 2004); neither do the skin carcinogenesis studies, where a single topical application of DMBA results in *h-ras* mutation in the epidermal stem cells (Arnott et al., 2002). However, this is an attractive explanation for the development of tumors at sites of long-term chronic inflammation with considerable destruction of the epithelial compartment. Another way in which chronic inflammation may be an initiating factor in malignancy is through the generation of free radicals and subsequent DNA damage. During chronic inflammation and in malignant tumor tissue, there is excessive demand for oxygen by resident and infiltrating inflammatory cells, which leads to the release of increased concentrations of free radicals (Hussain et al., 2003). One of the enzymes involved in free radical generation is nitric oxide synthetase, and its inducible form, iNOS, is frequently expressed in premalignant lesions and tumor tissues (Jaiswal et al., 2000; Jaiswal et al., 2001; Ohshima et al., 2003). The ways in which chronic inflammation may give rise to epithelial cancers are summarized in Figure 2.

Implications for cancer prevention strategies

COX-2, a major target of nonsteroidal anti-inflammatory drugs, NSAIDs, is strongly upregulated in some cancers. Population-based studies show that chronic use of NSAIDs such as aspirin reduce overall risk of colorectal cancer by 40%–50%. (Koehne and Dubois, 2004). Aspirin, and more selective COX-2 inhibitors such as celecoxib, delay the development of premalignant adenomas in patients who have been cured of sporadic colorectal

when potential adverse effects are taken into account. But these epidemiological studies strengthen the case for a link between cancer and inflammation, and again implicate COX enzymes and their inflammatory cytokine inducers.

Cytokine antagonist therapies are effective treatments in some inflammatory diseases. Inhibitors of TNF- α , in particular, have been used successfully in several hundred thousand patients worldwide (Feldmann, 2002). As described above, TNF- α , either of stromal or tumor origin, can act as an endogenous tumor promoter, but increased susceptibility to opportunistic infections such as *M. tuberculosis* in patients taking TNF- α antagonists (Keane et al., 2001; Wolfe et al., 2004) would preclude wide-scale use of these drugs as preventative agents against sporadic cancer. However, ongoing monitoring of large cohorts of patients with inflammatory conditions will show if TNF- α antagonists have a positive or negative impact on cancer risk. An increased incidence of lymphoma has been noted in rheumatoid arthritis patients treated with anti-TNF agents compared to the general population, as well as placebo-treated patients, although the followup time for the placebo patients has been shorter than for the anti-TNF-treated patients. At the moment, it is not possible to determine whether the increased incidence of lymphoma is due to the medication, the underlying disease, or a combination of both factors, because rheumatoid arthritis is associated with an increased risk of this malignancy (Wolfe and Michaud, 2004).

Implications for cancer therapy

Activation of the NF- κ B pathway is observed in many human and experimental cancers. Over 100 agents, such as antioxidants, proteasome inhibitors, NSAIDs, and immunosuppressive agents are NF- κ B inhibitors. None of these is entirely specific, but the cell possesses a range of endogenous inhibitors of NF- κ B, and these may provide opportunities for the development of novel strategies (Chen, 2004). An alternative is to target downstream effectors in the NF- κ B pathway, such as inflammatory cytokines. In clinical studies in rheumatoid arthritis patients, TNF- α antagonists inhibit inflammatory cytokines, MMPs,

angiogenic activity, and leukocyte trafficking to sites of inflammation (Feldmann, 2002), all actions that could be useful in a cancer drug. On the basis of these results and data described above, a number of clinical trials of TNF- α antagonists alone, and in combination with other therapies, are currently underway in cancer patients. There are suggestions of activity against advanced disease in advanced ovarian and renal cell cancer (Madusudhan et al., submitted; Maisey et al., submitted). Anti-IL-6 therapies are also being trialed in IL-6-producing cancers (Triakha et al., 2003). Treatment with anti-IL-6 monoclonal antibodies in myeloma patients showed effective blockade of myeloma cell proliferation, reduction in fever, and acute phase response, and some disease stabilization (Bataille et al., 1995; van Zaanen et al., 1998).

Multimodal therapy studies with various combinations of celecoxib, chemotherapy, and radiotherapy are underway (Gasparini et al., 2003). Preliminary results of a combination of exemestane and celecoxib in patients with postmenopausal hormone-sensitive breast cancer have been encouraging (Chow et al., 2003). Results from clinical trials of radiotherapy plus celecoxib have also shown increased therapeutic benefit, because inhibition of COX-2 enhances radiosensitivity of malignant cells as well as decreasing the radiation-dependent effects of increased angiogenesis and normal cell toxicity (Choy and Milas, 2003).

Targeting TAMs or molecules that attract them and mediate their function is another therapeutic strategy. For instance, a bisphosphonate compound, zoledronic acid, suppressed MMP-9 expression by TAMs, inhibiting tumor metalloprotease activity, and reduced the association of VEGF with its receptor on endothelial cells (Giraud et al., 2004). In another experimental model, in which the chemokine CCL5 was key to recruitment of TAMs, an antagonist of the CCL5 receptor reduced the infiltrate and slowed tumor growth (Robinson et al., 2003). Hence, therapeutic targeting of cancer-associated inflammation is in its infancy, but initial clinical results are encouraging, and may complement more conventional treatments.

Finally, it is important to remember that cancer-associated inflammation can also influence the pharmacokinetics of anti-cancer drugs. Much of the interpatient variability in the clearance of chemotherapy is due to differences in levels of drug metabolizing enzymes, especially cytochrome P450 (CYP) 3A4 (Kivisto et al., 1995). Patients with advanced cancer have significantly reduced hepatic CYP3A4 activity associated with increased plasma concentrations of inflammatory mediators (Rivory et al., 2002). The relationship between inflammation and CYP activity has been extensively studied in various animal models of acute inflammation and cancer (Slaviero et al., 2003). The inflammatory response leads to reduced CYP levels, decreased microsomal metabolism, and CYP-mediated drug clearance (Assenat et al., 2004; Morgan et al., 2002; Pascucci et al., 2000).

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