

# A model for cancer-suppressive inflammation

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In oncology, inflammation is generally regarded as a cancer-promoting process only. Here, we argue that this view may represent a misleading oversimplification. We present evidence from our own work and from the literature documenting cancer-suppressive aspects of inflammation. We propose that specific types of inflammation, in particular inflammation driven by tumor-specific Th1 cells, may repress rather than promote cancer. Th1 cells collaborate with tumor-infiltrating M1 macrophages to efficiently recognize and eliminate malignant cells. In a Th1 environment, pro-inflammatory cytokines such as interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6 and tumor-necrosis factor  $\alpha$  (TNF $\alpha$ ) enhance anti-cancer immunity. Inducing Th1-type inflammation may significantly improve immunotherapeutic strategies against cancer.

## Introduction

Inflammation is an immunological process which is essential for the elimination of invading pathogens as well as for wound healing. Inflammation may be considered to be the normal response of the body to harmful stimuli. At cellular and molecular levels, characteristic features of inflammation include increased movement of plasma and leukocytes from the blood into the injured tissue, and production of pro-inflammatory cytokines such as interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6 and tumor-necrosis factor  $\alpha$  (TNF $\alpha$ ).

A number of studies have demonstrated a link between inflammation and cancer development (reviewed in refs. 1–4). For instance, high IL-6 serum levels have been associated with shorter survival in patients affected by multiple myeloma, lymphoma and lung cancer.<sup>5–7</sup> Chronic inflammatory diseases like ulcerative colitis, gastritis and rheumatoid arthritis predispose to colorectal cancer, gastric cancer and lymphoma, respectively.<sup>8–10</sup> Furthermore, circumstantial evidence suggests that individuals taking non-steroidal anti-inflammatory drugs such as aspirin may have a reduced risk for cancer.<sup>11–13</sup> Based on these observations, inflammation is widely considered to be detrimental in terms of cancer occurrence, growth and metastasis. Accordingly, dampening inflammation has been suggested as a novel strategy to fight cancer.<sup>1–4</sup>

Here, we argue that the general view that inflammation promotes cancer may represent a misleading oversimplification. We review published evidence in support of cancer-suppressive properties of inflammatory cells and of the canonical

pro-inflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF $\alpha$ . This leads us to propose a model in which certain types of inflammation, in particular inflammation driven by tumor-specific Th1 cells, may repress rather than promote cancer.

## Th1-Driven Inflammation Protects Mice Against B-Cell Cancer

We investigated the mechanisms of cancer prevention mediated by tumor-specific CD4<sup>+</sup> T cells in mouse models for myeloma and B-cell lymphoma. We used T-cell receptor (TCR) transgenic mice in which all CD4<sup>+</sup> T cells recognize a tumor-specific, immunoglobulin-derived antigen that is secreted by the MOPC315 myeloma as well as by the F9 B-cell lymphoma.<sup>14,15</sup> Tumor-specific TCR-transgenic mice are resistant against subcutaneous challenge with MOPC315 or F9 cells. Protection was shown to be antigen-specific, CD4<sup>+</sup> T-cell mediated and independent of B cells and CD8<sup>+</sup> T cells.<sup>14,15</sup> Successful cancer elimination required antigen secretion by malignant cells, and migration of effector CD4<sup>+</sup> T cells from the draining lymph node to the incipient tumor site.<sup>16–18</sup> To study the mechanisms of cancer rejection by TCR-transgenic mice, we developed a strategy consisting of embedding injected tumor cells in a collagen gel. This method allowed us to analyze infiltrating immune cells and to quantify the cytokines that were secreted locally during a primary antitumor immune response.<sup>16,19</sup> Using this technique, we uncovered a common core of nine cytokines that were consistently present at higher levels at the incipient tumor site during successful cancer immunosurveillance (Fig. 1). Strikingly, this core included both pro-inflammatory (IL-1 $\alpha$ , IL-1 $\beta$  and IL-6) and Th1-associated cytokines such as IL-12 and interferon  $\gamma$  (IFN $\gamma$ ). Cancer eradication was achieved by a collaboration between tumor-specific Th1 cells and tumor-infiltrating, antigen-presenting M1 macrophages (i.e., IFN $\gamma$ -activated macrophages).<sup>20,21</sup> Th1 cells induced secretion of IL-1 $\beta$  and IL-6 by inflammatory M1 macrophages. Th1-derived IFN $\gamma$  was shown to render macrophages directly cytotoxic against cancer cells. Therefore, we concluded that successful immunosurveillance of B-cell cancer in mice consists of an inflammatory reaction driven by tumor-specific Th1 cells<sup>19</sup> (Fig. 1).

## A High Density of Immune Cells in Human Primary Tumors Predicts Longer Patient Survival

Infiltration of a tissue by blood-derived leukocytes such as T cells and macrophages, the so-called inflammatory infiltrate, is a typical histological feature of inflammation. It is well known that

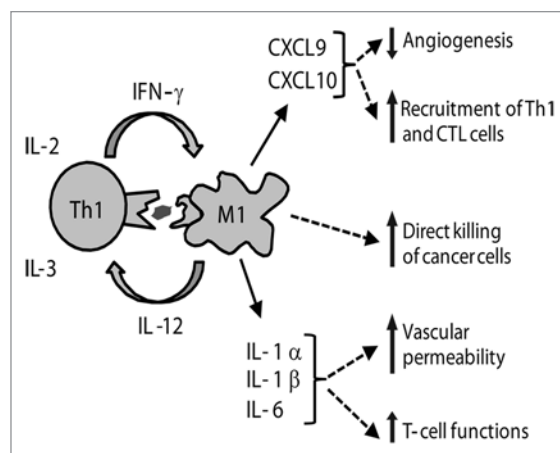
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solid tumors are infiltrated by various numbers of immune cells, which is consistent with an inflammatory reaction taking place within and around tumors. For many types of human cancer, an association has been demonstrated between dense leukocyte infiltrates in primary tumors and longer patient survival. Infiltration of the primary tumor by undefined inflammatory cells predicted longer survival of patients with skin, colorectal, bladder and breast cancer.<sup>22-25</sup> Elevated numbers of intratumoral T cells were shown to correlate with longer survival in patients with ovarian, esophageal, lung, colorectal and breast cancer.<sup>26-30</sup> The presence of both CD8<sup>+</sup> and CD4<sup>+</sup> T cells positively influenced survival, suggesting that both cell types cooperate to exert anticancer effects.<sup>27-30</sup> High numbers of CD4<sup>+</sup> T cells in lymph nodes predicted disease-free survival in breast cancer.<sup>31</sup> Among CD4<sup>+</sup> T cell subsets, Th1 cells seem to be particularly beneficial, as reported for colorectal, liver and breast cancer.<sup>29,32,33</sup> Furthermore, a high density of tumor-infiltrating macrophages was associated with prolonged survival in prostate, colorectal and lung cancer.<sup>23,34-36</sup> Thus, for various types of human cancer, stronger inflammation in the primary tumor, as defined by a high density of infiltrating T cells and macrophages, represents an independent predictor for longer patient survival.

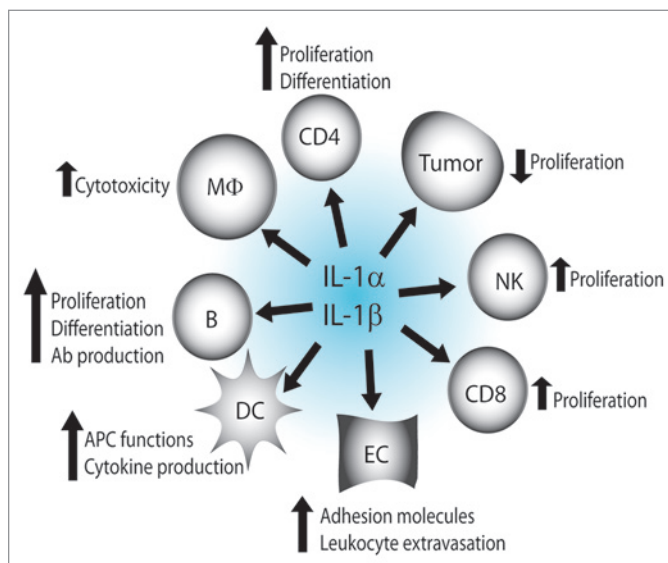
### Cancer-Suppressive Functions of IL-1 $\alpha$ and IL-1 $\beta$

IL-1 $\alpha$  and IL-1 $\beta$  are two canonical pro-inflammatory cytokines that are typically secreted by activated macrophages. IL-1 $\alpha$  and IL-1 $\beta$  have a number of important immunological functions, which are likely to contribute to successful antitumor immunity (Fig. 2). In line with their original name, namely lymphocyte activating factor, both IL-1 $\alpha$  and IL-1 $\beta$  are potent stimulators for all leukocytes. For instance, IL-1 $\alpha$  and IL-1 $\beta$  synergize with IL-2 to stimulate the proliferation of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and natural killer (NK) cells.<sup>37</sup> Both IL-1 $\alpha$  and IL-1 $\beta$  enhance the expansion and differentiation of CD4<sup>+</sup> T cells.<sup>38,39</sup> IL-1 also stimulates B-cell proliferation, generation of plasma cells and antibody production.<sup>40</sup> Furthermore, IL-1 $\alpha$  and IL-1 $\beta$  increase the expression of adhesion molecules on vascular endothelium, which may promote extravasation, i.e., the migration of leukocytes from the bloodstream into the inflamed tissue.<sup>41</sup>

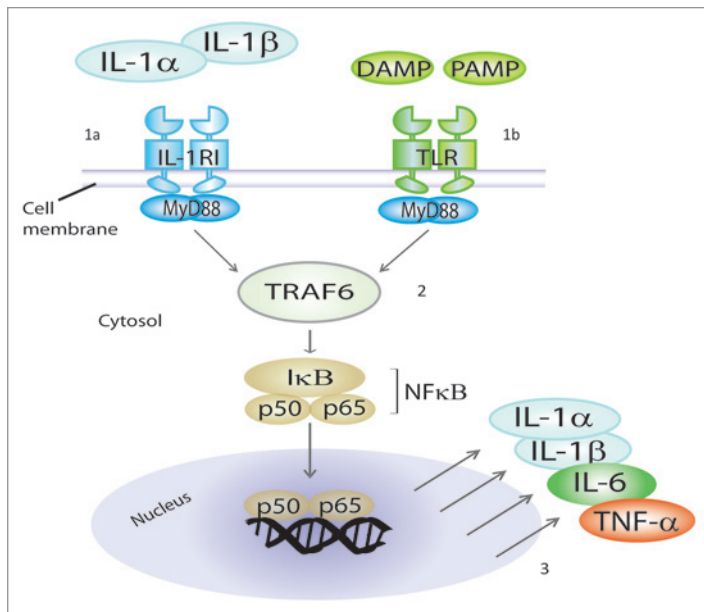
Although tumor-promoting effects of IL-1 $\alpha$  and IL-1 $\beta$  are well documented, in particular during cancer metastasis and tumor angiogenesis,<sup>42,43</sup> a number of studies provide experimental support for cancer-suppressive functions of these two cytokines (Fig. 2). IL-1 was shown to inhibit the proliferation of human A375 melanoma and mouse L929 fibroblast cell lines in vitro.<sup>44</sup> IL-1 rendered human monocytes cytotoxic against a murine fibrosarcoma cell line.<sup>45</sup> Intratumoral injection of IL-1 $\alpha$  successfully cured mice from MethA sarcoma and B16 melanoma.<sup>46</sup> IL-1 $\alpha$  injected intramuscularly was effective in reducing the number of lung metastases in mice with Lewis lung carcinoma.<sup>46</sup> Intraperitoneal injection of IL-1 $\beta$  caused complete regression of subcutaneous SA1 sarcoma and L5178Y lymphoma in mice.<sup>47</sup> Because IL-1 $\beta$  lost its therapeutic effects in T-cell deficient mice, it was concluded that IL-1 $\beta$  functions by stimulating T-cell mediated antitumor immunity.<sup>47</sup> Activated invasive RO1 T-lymphoma cells that displayed short-term IL-1 $\alpha$  expression manifested reduced tumorigenicity and



**Figure 1.** Successful immunosurveillance of B-cell cancer in mice consists of an inflammatory reaction driven by tumor-specific Th1 cells. Eradication of myeloma and lymphoma in mice is achieved through a collaboration between tumor-specific Th1 cells and tumor-infiltrating, antigen-presenting M1 macrophages. During this process, nine cytokines are secreted locally by immune cells, including pro-inflammatory (IL-1 $\alpha$ , IL-1 $\beta$  and IL-6) and Th1-associated cytokines (IL-12 and IFN $\gamma$ ). Th1 cells induce secretion of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, CXCL-9 and CXCL-10 by inflammatory M1 macrophages. Th1-derived IFN $\gamma$  renders macrophages directly cytotoxic to cancer cells. (Figure modified from ref. 19).



**Figure 2.** Cancer-suppressive properties of IL-1 $\alpha$  and IL-1 $\beta$ . IL-1 $\alpha$  and IL-1 $\beta$  synergize with IL-2 to stimulate the proliferation of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and NK cells; enhance the expansion and differentiation of CD4<sup>+</sup> T cells; stimulate B-cell proliferation, generation of plasma cells and antibody production; induce the expression of adhesion molecules on vascular endothelium, which promotes extravasation of leukocytes into the inflamed tissue; directly inhibit the proliferation of specific cancer cells; render human macrophages cytotoxic to some cancer cells; and they amplify antigen-presenting functions and cytokine production by dendritic cells. Ab, antibody; APC, antigen-presenting cell; B, B cell; CD4, CD4<sup>+</sup> T cell; CD8, CD8<sup>+</sup> T cell; DC, dendritic cell; EC, endothelial cell; M $\Phi$ , macrophage; NK, natural killer cell.



**Figure 3.** IL-1RI and TLRs share a common intracellular signaling pathway. Mature IL-1 $\alpha$  and IL-1 $\beta$  mediate their functions by binding to the same IL-1 receptor I (IL-1RI) which is present on the surface of most cells (1a). Pathogen-associated molecular patterns (PAMP) and damage/danger-associated molecular patterns (DAMP) bind to Toll-like receptors (TLRs) (1b). IL-1RI and TLRs share a common intracellular signaling pathway (2), which leads to activation of NF $\kappa$ B and hence to the production of pro-inflammatory cytokines, such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF $\alpha$  (3). Therefore, IL-1 $\alpha$  and IL-1 $\beta$  function within positive feedback loops in inflammation. Because of this common signaling pathway, IL-1 $\alpha$  and IL-1 $\beta$  operate as natural adjuvants, mimicking the detection of microbial products by TLRs. MyD88, myeloid Differentiation primary response gene 88; TRAF6, TNF receptor associated factor 6.

could be used to treat mice with lymphoma.<sup>48</sup> Similarly, fibrosarcoma cells transfected with IL-1 $\alpha$  became strongly immunogenic and failed to generate tumors in mice.<sup>49</sup> Notably, IL-1 $\beta$  secretion is dependent on activation of the NLRP3 inflammasome, a cytosolic molecular complex responsible for generating active IL-1 $\beta$  by cleaving the inactive precursor. The NLRP3 inflammasome has been demonstrated to protect mice against colitis-associated cancer.<sup>50</sup> Furthermore, in mouse models for cancer chemotherapy, activation of the NLRP3 inflammasome in dendritic cells appears to be essential for the induction of IL-1 $\beta$ -dependent adaptive antitumor immunity.<sup>51</sup> In line with these experimental findings, a genetic analysis of multiple myeloma patients revealed that individuals with a polymorphism in the promoter region of the *IL1B* gene, leading to reduced IL-1 $\beta$  production, had significantly shorter survival after chemotherapy.<sup>52</sup> Thus, antitumor effects for both IL-1 $\alpha$  and IL-1 $\beta$  have been demonstrated in various mouse models, and several studies suggest that IL-1 $\alpha$  and IL-1 $\beta$  can significantly enhance T-cell mediated antitumor immunity.

### IL-1 $\alpha$ and IL-1 $\beta$ are Natural Adjuvants for Antitumor Immunity

Although structurally quite different, mature IL-1 $\alpha$  and IL-1 $\beta$  mediate their functions by binding to the same IL-1 receptor

I (IL-1RI) which is present on the surface of most cells. Importantly, IL-1RI share with Toll-like receptors (TLRs) a common intracellular signaling pathway, which leads to activation of the nuclear factor  $\kappa$ B (NF $\kappa$ B) and hence to the transcription of several pro-inflammatory cytokines, such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF $\alpha$  (Fig. 3). Therefore, IL-1 $\alpha$  and IL-1 $\beta$  function within positive feedback loops during inflammation. Because of the common intracellular signaling pathway, IL-1 $\alpha$  and IL-1 $\beta$  operate as natural adjuvants, thus mimicking the detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by TLRs (Fig. 3). Early work by Ralph Steinman and coworkers revealed that IL-1 $\alpha$  can amplify the function of dendritic cells and thereby enhance T-cell dependent immunity.<sup>53</sup> It was also shown that IL-1 $\alpha$  can be used as an adjuvant to trigger the clonal expansion and differentiation of antigen-activated Th cells, as well as Th-mediated antibody production.<sup>54</sup> Several studies investigated the potential of IL-1 $\alpha$  and IL-1 $\beta$  as adjuvants for cancer vaccines. In a mouse model of lung cancer, a vaccine was made by combining irradiated cancer cells with IL-1 $\alpha$  or IL-1 $\beta$ .<sup>55</sup> IL-1 $\beta$  was successfully used as an adjuvant, together with sonicated cancer cells, to induce tumor-specific immunity against MOPC104E plasmacytoma and MethA sarcoma in mice.<sup>56</sup> Finally, an IL-1RI-binding peptide derived from IL-1 $\beta$  was shown to augment antitumor immune responses induced by protein and DNA vaccines against 38C13 mouse B-cell lymphoma.<sup>57</sup> Altogether, these data suggest that IL-1 $\alpha$  and IL-1 $\beta$ , as whole proteins or biologically active fragments, may represent potent adjuvants for cancer vaccines.

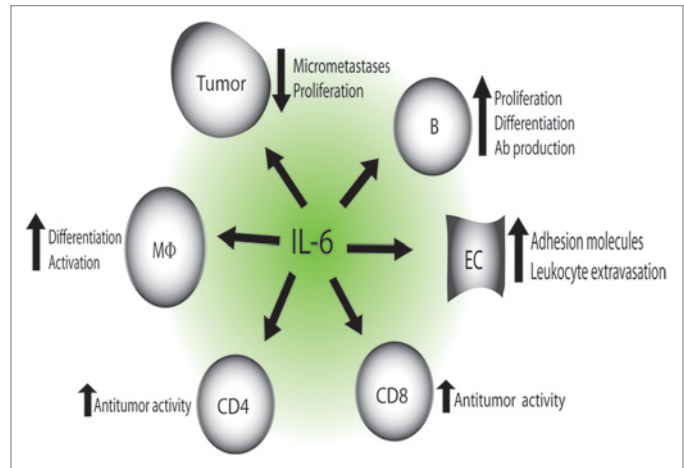
### Cancer-Suppressive Functions of IL-6

The pro-inflammatory cytokine IL-6 exerts multiple functions, including the stimulation of B and T cells as well as the production of acute-phase proteins by hepatocytes. IL-6 plays an essential role in antibacterial and antiviral immunity.<sup>58</sup> IL-6 is a B-cell growth factor, which stimulates proliferation of normal and malignant B cells. In several types of human cancer, such as multiple myeloma, B-cell lymphoma and lung cancer, high IL-6 serum levels have been associated with short patient survival, supporting cancer-promoting effects for IL-6.<sup>57</sup> However, a number of studies documented cancer-suppressive properties of IL-6 (Fig. 4). Treatment of mice with IL-6 induced the regression of established micrometastases in the liver and lungs of sarcoma and colon adenocarcinoma,<sup>59</sup> in a process that required both CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>60</sup> In mice inoculated with acute myeloid leukemia cells, IL-6 injections inhibited tumor development and increased survival.<sup>61</sup> Murine B16 melanoma cells transfected with IL-6 became less tumorigenic, and mice with established melanoma were successfully treated with recombinant IL-6.<sup>62</sup> Combined treatment with IL-6 and cyclophosphamide efficiently cured mice bearing advanced pulmonary metastases from fibrosarcoma.<sup>60</sup> Immunization of mice with IL-6-transfected Lewis lung carcinoma cells induced high levels of tumor-specific cytotoxic T cells and functioned as an efficient

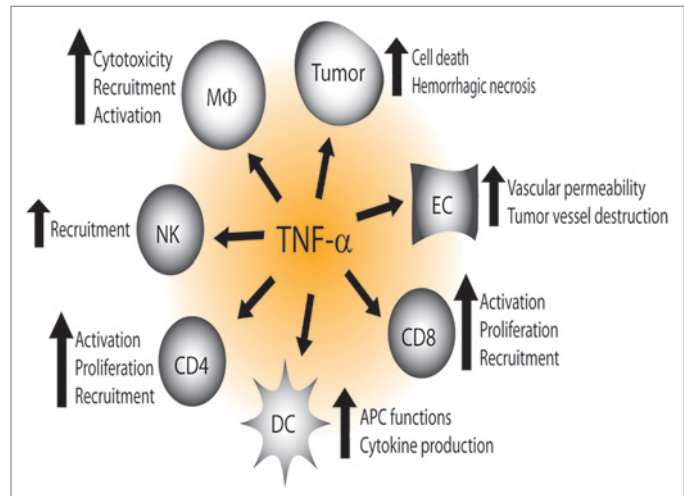
prophylactic and therapeutic cancer vaccine.<sup>63</sup> Fibrosarcoma cells transduced with IL-6 exhibited reduced tumorigenicity, increased immunogenicity and decreased metastatic potential.<sup>64</sup> Similar findings were obtained in rats, in which IL-6-transduced glioma cells showed attenuated tumorigenicity and functioned as an efficient vaccine against intracranial glioma.<sup>65</sup> In mice bearing B16F10 melanoma treated with the synthetic triacylated lipopeptide Pam<sub>3</sub>CSK<sub>4</sub> (a TLR2 agonist), mast cell-derived IL-6 was essential for the inhibition of tumor growth.<sup>66</sup> Endogenous IL-6 reportedly increases CD8<sup>+</sup> T cell trafficking to the tumors in mice with B16 melanoma treated with systemic thermal therapy combined with adoptive T cell transfer.<sup>67</sup> In humans, IL-6 has been shown to suppress the proliferation of acute myeloid leukemia and B-chronic lymphocytic leukemia cells, revealing an inhibitory effect of IL-6 on some human B-cell malignancies.<sup>61,68</sup> Moreover, *in vitro* studies on human melanoma demonstrated that exposure to IL-6 inhibits the growth of early-stage but not advanced stage melanoma cells.<sup>69</sup> Thus, IL-6 has been shown to inhibit *in vitro* proliferation of several types of human cancer cells, and cancer-suppressive effects of IL-6 have been demonstrated *in vivo*, in several animal models.

### Cancer-Suppressive Functions of TNF $\alpha$

TNF $\alpha$  is a pleiotropic pro-inflammatory cytokine typically produced by activated macrophages. The effects of TNF $\alpha$  are mediated by two distinct receptors: TNF $\alpha$  receptor 1 (TNFR1) and TNFR2. TNFR1 is present on most cell types and accounts for the majority of pro-inflammatory (through induction of NF $\kappa$ B) and apoptotic effects of TNF $\alpha$ .<sup>70</sup> TNFR2 is present on the surface of lymphocytes and promotes activation and proliferation. TNF $\alpha$  has an impact on all aspects of T-cell biology including T-cell proliferation, co-stimulation and survival (reviewed in ref. 71). A number of studies documented tumor-promoting effects of TNF $\alpha$  (reviewed in ref. 72). For example, TNF $\alpha$ -deficient mice were less susceptible to carcinogen-induced papilloma, a benign epithelial tumor, suggesting that TNF $\alpha$  may promote skin cancer development.<sup>73</sup> In chronic lymphocytic leukemia, patients with high plasma levels of TNF $\alpha$  had a significantly shorter survival rate.<sup>74</sup> However, a large body of literature has revealed the cancer-suppressive properties of TNF $\alpha$  (Fig. 5). TNF $\alpha$  was first identified as an endotoxin-induced serum factor produced by macrophages that caused hemorrhagic necrosis of MethA sarcoma and other solid tumors.<sup>75</sup> The hemorrhagic reaction apparently resulted from the destruction of tumor blood vessels.<sup>76</sup> Experiments with T-cell deficient mice revealed that TNF $\alpha$  does not solely damage tumor blood vessels, but it also stimulates tumor-specific T cells.<sup>76</sup> Furthermore, *in vitro* studies demonstrated that TNF $\alpha$  is directly cytotoxic for various human cancer cell lines such as melanoma, breast, cervix and colon carcinoma.<sup>77,78</sup> TNF $\alpha$  rendered human monocytes cytotoxic against a murine fibrosarcoma cell line *in vitro*.<sup>45</sup> Synergistic *in vivo* antitumor effects against the murine MCA-106 sarcoma were reported for the combined administration of TNF $\alpha$  and IL-2.<sup>79</sup> J558L myeloma cells transfected with TNF $\alpha$  were efficiently eliminated in mice, and it was proposed that TNF $\alpha$



**Figure 4.** Cancer-suppressive properties of IL-6. IL-6 stimulates B-cell proliferation, differentiation as well as antibody production and it influences macrophage differentiation and activation. Treatment of mice with IL-6 may induce regression of established micrometastases in liver and lungs in a process which requires both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Immunization of mice with IL-6-transfected carcinoma cells may generate high levels of tumor-specific CD8<sup>+</sup> T cells and functions as an efficient prophylactic and therapeutic cancer vaccine. Fibrosarcoma cells transduced with IL-6 exhibit reduced tumorigenicity, increased immunogenicity and decreased metastatic potential. IL-6 may increase CD8<sup>+</sup> T cell trafficking to tumors by affecting endothelial cells and it inhibits proliferation of human acute myeloid leukemia, B-chronic lymphocytic leukemia and early-stage melanoma cells.



**Figure 5.** Cancer-suppressive properties of TNF $\alpha$ . TNF $\alpha$  stimulates T-cell activation, proliferation and recruitment; causes hemorrhagic necrosis of solid tumors through destruction of tumor blood vessels; is directly cytotoxic to several human cancer cell lines; and it renders human macrophages cytotoxic to some cancer cells. TNF $\alpha$ -transfected myeloma cells are eliminated in mice through recruitment and activation of macrophages. TNF $\alpha$  mediates recruitment of NK cells to the peritoneum to eliminate MHC Class I-negative cancer cells; is important for priming, proliferation and recruitment of tumor-specific CD8<sup>+</sup> T cells; stimulates antigen-presenting cell functions and cytokine production; and it increases vascular permeability leading to improved penetration of chemotherapeutics in the tumor tissue.

would function by recruiting and activating macrophages.<sup>80</sup> In a follow-up study, rejection of TNF $\alpha$ -transfected J558L myeloma cells was shown to be partly dependent on CD8<sup>+</sup> T cells.<sup>81</sup> In a mouse model for T-cell lymphoma, an opposing effect of TNF $\alpha$  on tumor growth was reported: TNF $\alpha$  induced the rejection of solid tumors but promoted the formation of hepatic metastases.<sup>82</sup> TNF $\alpha$  was essential for recruitment of NK cells to the peritoneum to eliminate MHC Class I-negative RMA-S lymphoma cells.<sup>83</sup> TNF $\alpha$ -deficient mice were more susceptible than wild-type mice to sarcoma induction with the carcinogen methylcholanthrene.<sup>84</sup> TNF $\alpha$  was critical for the prevention of pancreatic tumors in a mouse model of cancer immunosurveillance mediated by tumor-specific CD8<sup>+</sup> T cells.<sup>85</sup> In this model, TNF $\alpha$  was important for the priming, proliferation and recruitment of tumor-specific CD8<sup>+</sup> T cells.<sup>85</sup> Importantly, this study identified the critical roles of TNFR1 on antigen-presenting cells and TNFR2 on T cells for successful antitumor immunity.<sup>85</sup>

### Using TNF $\alpha$ for Cancer Immunotherapy in Humans

On the basis of the experimental evidence described above, TNF $\alpha$  is currently being used for human cancer immunotherapy (reviewed in ref. 86). A main limitation for clinical application of TNF $\alpha$  has been the toxicity that is associated with systemic administration. To circumvent this problem, isolated limb perfusion was performed and successful treatment of advanced melanoma and soft tissue sarcoma was achieved.<sup>87,88</sup> During isolated limb perfusion, TNF $\alpha$  has a double effect on the tumor-associated vasculature: (1) TNF $\alpha$  increases vascular permeability leading to improved penetration of chemotherapy within the tumor tissue, and (2) TNF $\alpha$  selectively kills angiogenic endothelial cells resulting in tumor vessel destruction.<sup>86</sup> Several strategies are being developed to allow for the systemic administration of TNF $\alpha$  while limiting toxicity to the patient, in particular by that targeted delivery of TNF $\alpha$  to tumors.<sup>89-91</sup>

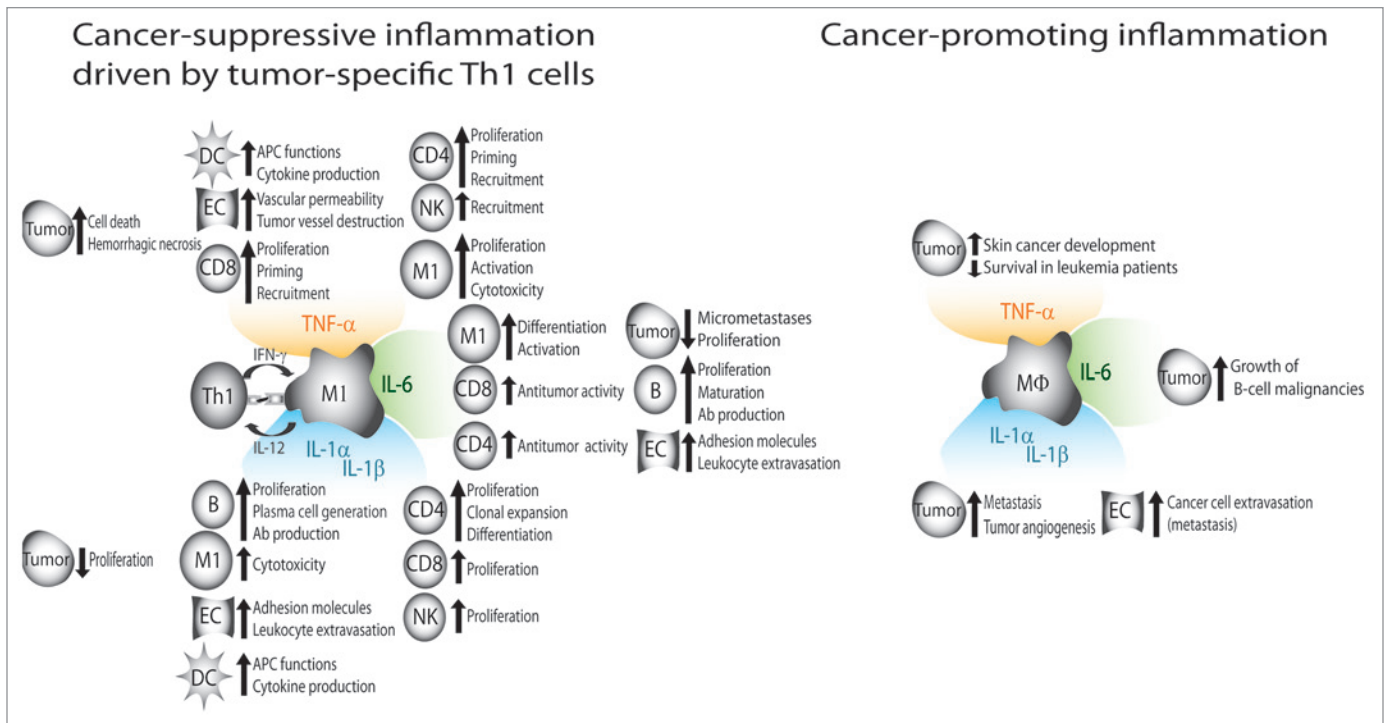
### Pro-Inflammatory Cytokines Synergize With Other Cytokines to Fight Cancer

The biological effects of a given cytokine are strongly dependent on the cytokine milieu. In cancer immunology, it is well documented that pro-inflammatory cytokines may synergize with other cytokines against neoplastic cells. For example, TNF $\alpha$  and IFN $\gamma$  acted in synergy to block the *in vitro* proliferation of murine Lewis lung carcinoma and B16 melanoma cells.<sup>92</sup> Similarly, treatment of mice bearing B16 melanoma with both TNF $\alpha$  and IFN $\gamma$  was more efficient than the administration of either cytokine alone.<sup>92</sup> Combined treatment with IL-1 $\beta$  and IL-2 generated a synergistic T-cell dependent antitumor response against highly metastatic Friend leukemia in mice.<sup>93</sup> Mice with established subcutaneous MCA-106 sarcoma tumors were cured by the combinatorial treatment with TNF $\alpha$  and IL-6, revealing a synergistic antitumor effect of these two pro-inflammatory cytokines.<sup>99</sup> *In vitro* studies demonstrated that a combination of IFN $\gamma$  and IL-1 $\beta$  induced necrosis of murine L929 fibrosarcoma cells.<sup>94</sup> Combined treatment of human papillary thyroid

carcinoma cell lines with IL-1 $\beta$  and IFN $\gamma$  efficiently inhibited proliferation and invasiveness.<sup>95</sup> TNF $\alpha$  and IFN $\gamma$  synergized to mediate *in vitro* cytotoxicity against a large number of human cell lines including breast, cervix and colon carcinoma cells.<sup>78</sup> Finally, TNF $\alpha$  and IFN $\gamma$  were shown to synergize to induce the tumoricidal activity of murine macrophages.<sup>96</sup> Thus, although proinflammatory cytokines may be directly cytotoxic to cancer cells, synergistic interactions with other cytokines are likely to be very important for successful antitumor immunity. Importantly, the combination of pro-inflammatory cytokines with Th1 cytokines like IL-2 and IFN $\gamma$  seems to be particularly beneficial for cancer therapy.

### A Model for How Inflammation May Either Suppress or Promote Cancer

From the literature overviewed above, it is apparent that inflammatory cells and pro-inflammatory cytokines not only can promote but also may suppress cancer in various settings. Therefore, cancer-related inflammation cannot be generalized as solely detrimental or beneficial. To reconcile conflicting data, we propose that, among all the existing types of inflammation, certain types are cancer-suppressive while others may be cancer-promoting. Based on our own findings and evidence from the literature, we suggest that inflammation driven by tumor-specific Th1 cells may be particularly beneficial against cancer (Fig. 6, left). During Th1-driven cancer-suppressive inflammation, tumor-specific Th1 cells collaborate with tumor-infiltrating M1 macrophages to efficiently recognize and eliminate malignant cells. M1 macrophages function as efficient antigen-presenting cells that process and present cancer antigens to tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the tumor. Th1 cells are characterized by the production of IFN $\gamma$ <sup>97</sup> and experiments with gene-targeted mice demonstrated that IFN $\gamma$  is critical for cancer immunosurveillance.<sup>19,98,99</sup> IFN $\gamma$  is a potent macrophage-activating factor, stimulating their tumoricidal activity and the acquisition of a M1 phenotype.<sup>100-102</sup> M1 macrophages are cancer-suppressive *in vitro*<sup>100-102</sup> and *in vivo*,<sup>16,19</sup> and produce pro-inflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ ), the Th1-polarizing cytokine IL-12, as well as IFN $\gamma$ -induced chemokines (CXCL9/MIG, CXCL10/IP-10 and CXCL11/I-TAC).<sup>21</sup> In a Th1 environment, pro-inflammatory cytokines may play essential roles in cancer elimination, e.g., by recruiting T cells and macrophages from the circulation and by stimulating leukocyte tumoricidal functions (Fig. 6, left). Pro-inflammatory cytokines synergize with Th1-derived cytokines (IL-2 and IFN $\gamma$ ) to fight cancer.<sup>78,92-96</sup> CXCL9, CXCL10 and CXCL11 may participate in antitumor immunity by blocking tumor angiogenesis<sup>99,103-105</sup> and by recruiting Th1 cells, CD8<sup>+</sup> T cells and NK cells to the tumor site.<sup>106,107</sup> In contrast to Th1-driven cancer-suppressive inflammation, other types of inflammation may instead promote cancer development (reviewed in refs. 1-4). In the absence of sufficient numbers of tumor-specific Th1 cells, pro-inflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ ) may participate in cancer development, progression and metastasis, for instance by stimulating angiogenesis and cancer cell growth, and by increasing vascular permeability<sup>1-4</sup> (Fig. 6, right).



**Figure 6.** A model for how inflammation may either suppress or promote cancer. (Left) Cancer-suppressive inflammation driven by tumor-specific Th1 cells. Tumor-specific Th1 cells collaborate with tumor-infiltrating M1 macrophages to efficiently recognize and eliminate malignant cells. M1 macrophages function as efficient antigen-presenting cells that process and present cancer antigens to tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the tumor. Th1 cells are characterized by the production of IFN $\gamma$ , which is a potent macrophage-activating factor, inducing their tumoricidal activity. IFN $\gamma$ -induced M1 macrophages are cancer-suppressive *in vitro* and *in vivo*, and produce pro-inflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ ) as well as the Th1-polarizing cytokine IL-12. In a Th1 environment, pro-inflammatory cytokines play essential roles in cancer elimination by stimulating various aspects of the antitumor immune response. (Right) Cancer-promoting inflammation. In the absence of sufficient numbers of tumor-specific Th1 cells, tumor-infiltrating macrophages do not differentiate into a cancer-suppressive M1 phenotype. In this setting, pro-inflammatory cytokines may contribute to cancer development, progression and metastasis, for instance by stimulating angiogenesis and cancer cell growth and/or by increasing vascular permeability.

### Inducing Inflammation to Treat Cancer

On the basis of the model described above, we propose that immunotherapy protocols that induce or promote an inflammatory reaction driven by tumor-specific Th1 cells may be particularly efficient at eradicating cancer. PAMPs tend to induce Th1-type inflammatory immune responses upon binding to receptors such as TLRs on immune cells. Several reports document successful cancer immunotherapy protocols based on inducing inflammation with PAMPs. Early studies in mice revealed that treatment with double-stranded RNA or lipopolysaccharide induced lymphocyte-mediated rejection of L5178Y lymphoma and FS6 fibrosarcoma.<sup>108</sup> More recently, mice with C26 colon carcinoma and B16F0 melanoma have been successfully treated with a combination of anti-IL-10-receptor antibodies and the intratumoral injection with CpG, a TLR-9 ligand.<sup>109</sup> This treatment has been shown to stimulate the production of IL-12 by tumor-infiltrating dendritic cells, thereby enhancing T-cell mediated antitumor immunity.<sup>109</sup> In humans, the intravesical application of the bacterium *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) is an efficient and widely-used treatment for superficial bladder cancer (reviewed in ref. 110). Moreover, imiquimod, an imidazoquinoline compound which activates immune cells via TLR7,<sup>111</sup>

is an effective treatment for several human malignancies such as superficial basal cell carcinoma, actinic keratinosis and vulvar intraepithelial neoplasia.<sup>112-114</sup> Collectively, these data suggest that inducing Th1-type inflammation may represent an efficient strategy to achieve successful cancer immunotherapy.

### Concluding Remarks

**Treating cancer with anti-inflammatory drugs.** This new therapeutic approach is based on the well-documented cancer-promoting aspects of inflammation.<sup>1-4</sup> The model that we present here suggests that caution should be taken. Anti-inflammatory drugs that dampen ongoing cancer-suppressive Th1 inflammation in patients may potentially exacerbate, rather than cure, established malignancies.

**Anti-inflammatory drugs and cancer risk.** Novel anti-inflammatory drugs that specifically block key inflammatory molecules, e.g., pro-inflammatory cytokines, have been developed. These drugs have revolutionized the treatment of several chronic inflammatory diseases such as rheumatoid arthritis. Moreover, evidence suggests that some non-steroidal anti-inflammatory drugs, e.g., aspirin, may lead to a reduced risk for cancer.<sup>11-13</sup> However, anti-inflammatory drugs may potentially weaken

cancer immunosurveillance. Therefore, it is advisable that patients receiving long-term treatment with novel anti-inflammatory drugs should be followed up for cancer development.

**Acute vs. chronic inflammation.** It has been suggested that acute inflammation protects against cancer, whereas chronic inflammation promotes cancer. This hypothesis is based on the observation that acute inflammation may cure cancer,<sup>108-110,112-114</sup> whereas several chronic inflammatory diseases predispose to cancer.<sup>8-10</sup> However, there are indications that chronic inflammation may not always be detrimental. For several types of human cancer, a strong inflammatory response in the primary tumor, as defined by a high density of infiltrating T cells and macrophages, has been shown to represent an independent predictor for longer patient survival.<sup>22-30,32,34-36</sup> Because a detectable tumor takes a long time to develop, tumor-associated inflammation, unless treatment-induced, should arguably be defined as chronic inflammation. This implies that a strong chronic inflammation in tumors may repress rather than promote cancer, therefore representing an example of beneficial chronic inflammation against cancer.

**Synergy between chemotherapy and immunotherapy.** Although chemotherapy is generally considered

immunosuppressive, several reports revealed that it may improve cancer immunotherapy. For instance, treatment of mice with AB1 mesothelioma with the cytidine analog gemcitabine significantly enhanced immunotherapy with a viral cancer vaccine or with anti-CD40 monoclonal antibodies.<sup>115,116</sup> In humans, chemotherapy of melanoma patients with the DNA alkylating agent dacarbazine was shown to improve the CD8<sup>+</sup> T-cell response to a cancer vaccine.<sup>117</sup> Mouse studies revealed that inflammatory mediators induced by chemotherapy may stimulate antitumor immunity.<sup>51,118</sup> Thus, induction of a strong inflammation in tumors is likely to be a main component of the observed synergy between chemotherapy and immunotherapy.

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