STATE OF THE ART: CONCISE REVIEW

The Role of Tumor-Infiltrating Immune Cells and Chronic Inflammation at the Tumor Site on Cancer Development, Progression, and Prognosis

Emphasis on Non-small Cell Lung Cancer

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Abstract: In addition to malignant neoplastic cells, cancer tissues also include immune cells, fibroblasts, and endothelial cells, including an abundant collection of growth factors, proangiogenic mediators, cytokines, chemokines, and components of the extracellular matrix. The main physiological function of the immune cells is to monitor tissue homeostasis, to protect against invading pathogens, and to eliminate transformed or damaged cells. Between immune cells and malignant cells in the tumor stroma, there is in fact a complex interaction which has significant prognostic relevance as the immune system has both tumor-promoting and -inhibiting roles. In non-small cell lung cancer (NSCLC), there is a marked infiltration of different types of immune cells, and the distribution, tissue localization, and cell types are significantly associated with progression and survival. Cancer immunotherapy has seen a significant progress during the last decade. An increased understanding of the mechanisms by which lung cancer cells escape the immune system, and the recognition of the key tumor antigens and immune system components in tumor ignorance have led to the development of several lung cancer vaccines. As the NSCLC prognosis in general is dismal, one may hope that future immunotherapy may be an effective adjunct to standard therapy, reversing immunologic tolerance in the tumor microenvironment. This review reports on the tumor stroma and in particular tumor-suppressing and -promoting roles of the immune system. Furthermore, it presents recent literature on relevant immune cell-related research in NSCLC.

Until recently, the principal focus in cancer research has primarily been the malignant cell. As cancers are not solely neoplastic cells but harbor tumor microenvironments, which may vary according to cancer type, the lack of research interest in the tumor microenvironment has led to a significant discrepancy between the profound knowledge on cancer cell biology and more limited knowledge on which roles the tumor microenvironment plays.

The predominant host cells recruited to and activated in the tumor microenvironment are immune cells, fibroblasts, and pericytes/endothelial cells. Furthermore, there is an abundant collection of growth factors, proangiogenic mediators, cytokines, chemokines, and components of the extracellular matrix. The microenvironment can exert inhibitory effects on even aggressive malignant cell, but during their progression, tumor cells may circumvent these inhibitory signals and instead exploit immune cells and others for their own benefits, resulting in growth, invasion, and metastasis.[1–3]

Approximately 150 years ago, Virchow postulated that inflammation is a predisposing factor of tumorigenesis. This hypothesis was based on his observation that cancerous tissue often arose at sites of chronic inflammation and that inflammatory cells were present in the resected tumors.[4] In contrast, Burnet[5] proposed, in 1970, the concept of immunological surveillance: the immune system spontaneously identifies and eliminates cancer cells, thus protecting against tumor development. Supporting Virchow's hypothesis, epidemiological studies have shown during the last decades that individuals prone to chronic inflammatory diseases have an increased risk of cancer development[6] and that underlying infections and inflammatory responses have been linked to 15 to 20% of all cancer deaths worldwide.[7]

Although cancer-protective and cancer-promoting features of the immune system have been described, accumulat-

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ing data indicate that tumor-associated immune responses in established malignancies more likely contribute to tumor growth, progression, and immunosuppression than they are to establish an effective antitumor response.[2,4,8] The molecular pathways of cancer-associated inflammation are presently being unraveled, identifying molecular targets, which may lead to improved diagnosis and therapy.[8] This review covers the roles of immune cells in the tumor microenvironment in general and in the non-small cell lung cancer (NSCLC) microenvironment in particular. The possibility of immuno-therapeutic targeting in the NSCLC microenvironment will also be addressed.

IMMUNE CELLS AND THEIR ROLE IN NORMAL TISSUE HOMEOSTASIS AND INFLAMMATION

Our immune system plays critical roles in maintaining tissue homeostasis; facilitating debris scavenging, cell turn-over, and tissue remodeling; and preventing infection and cell transformation. It is composed of two distinct compartments mediating innate and adaptive immune responses (Figure 1). Each compartment has, through a diversity of cells and soluble mediators, advanced communication networks, which enable rapid and effective responses to tissue injury.

The Innate Immune System

It consists of the phagocytes (macrophages, neutro-phils, and dendritic cells [DCs]), mast cells, eosinophils, basophiles, natural killer (NK) cells, and NK T cells. The innate immune system is the first line of defense against foreign pathogens and transformed cells.

There are two major pathways for macrophage activation in vivo, M1 or M2 activation.[10] Normally, macrophages undergo M1 activation yielding potent immunologic effector cells, whereas M2 macrophages suppress immune responses, promote tissue remodeling and angiogenesis, and produce growth and survival factors for tumor cells.[10-12]

In the acute response, macrophages, DCs, and mast cells are primary effectors, as they are posted in the tissue. Macrophages and mast cells immediately release soluble mediators such as cytokines, chemokines, matrix metallopro-teinases, reactive oxygen species, and bioactive mediators (histamine), mediating mobilization of additional leukocytes, angiogenesis, and tissue remodeling.[8,13]

NK cells ($CD56^+CD3^-$) play a major role in the rejection of tumor cells or virus infected cells.[14] NK T cells ($CD56^+CD3^+$) are a subset of T lymphocytes but express a variety of molecular markers typical for NK cells.[15]

The $\gamma\delta$ T cells are distributed peripherally and consti-tute an independent population of circulating lymphocytes with specific functions. These cells are able to sense "patho-gens" and induce DC maturation, functional activation, DC migration, and antigen presentation.[16,17]

The Adaptive Immune System

An innate immune response leads to activation of the adaptive immune system (B and T cells), provided direct interactions with antigen presenting cells and a proinflamma-tory environment. The two major T lymphocyte subsets are (1) T helper cells (Th, $CD4^+$) and (2) cytotoxic T cells (CTL,
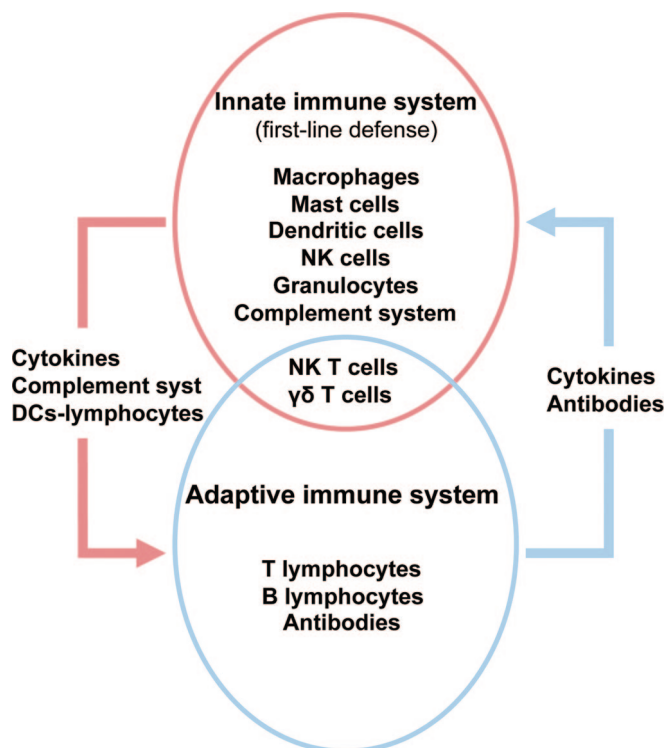

FIGURE 1. Schematic presentation of the interplay be-tween innate and adaptive immunity. NK T cells and $\gamma\delta$ T cells play their roles in the crossroad between the innate and adaptive immune system. The crosstalk between these im-mune systems is mediated by complex interactions between cells of both immune subsets and their soluble factors. The innate immune system, i.e., the first line of immune defense, regulates adaptive immune responses by the production of cytokines, interactions between dendritic cells and lympho-cytes, and activation of the complement system. The adap-tive immune system modulates innate immune responses by cytokine and antibody production. Adapted from *Cancer Im-munol Immunother*.[9] DCs, dendritic cells; NK, natural killer; NT T cells, natural killer T cells.

$CD8^+$). Lymphocytes express somatically generated, diverse antigen-specific receptors allowing a flexible and broad rep-ertoire of responses.[18,19]

Primary adaptive responses are slower than the innate responses, as clonal expansion due to recognition of foreign antigens is required.[20,21]

Combined Innate and Adaptive Immune Responses

There is an interplay between the innate and adaptive immune system through production of cytokines and antibod-ies, interactions between DCs and lymphocytes, and activa-tion of the complement system.[9]

DCs, key players in the interphase between innate and adaptive immunity, take up foreign antigens, migrate to lymphoid organs, and present these antigens to adaptive immune cells. NK cells interact bidirectionally with DCs and macrophages, promoting their maturation and eliminating

immature DCs, thus reciprocally regulating activation of NK cells.[22–24]

THE IMMUNE SYSTEM AND CANCER DEVELOPMENT

When tissue homeostasis is persistently perturbed as in chronic inflammation, interactions between innate and adaptive immune cells, as well as composition of cells and mediators, will change. The roles between these systems can be reversed during chronic inflammation,[2] as adaptive immune responses may cause (1) ongoing and excessive activation of the innate system,[25] (2) antibody deposition in tissues resulting in recruitment of innate immune cells,[26] and (3) T lymphocyte dysfunction instead of activation.[27] The inability to properly regulate the innate and adaptive immune system can result in excessive tissue remodeling, loss of tissue architecture due to destruction, protein alterations and genotoxic DNA damage due to oxidative stress, and subsequently increased cancer risk.[2]

Cancer Inflammation—Tumor Inhibiting or Tumor Promoting?

The developing malignancy is normally associated with inflammation. Tumor-associated inflammation is a chronic process, which is not beneficial, but often unfavorable for the host. Many immune cells of the tumor microenvironment may be associated with the tissue disruption caused by inflammatory agents or be a response to tumor growth. There is, however, no clear association between the presence of any individual innate or adaptive immune cell type and a defined outcome in terms of malignancy or prognosis across various tumors. Even within individual immune cell types, there are opposing functions as CD4$^+$ T cells, CD8$^+$ T cells, macrophages, and NK T cells have either tumor-suppressive or tumor-promoting properties, depending on tissue context and cellular stimuli.[2,3,28,29]

It has been documented that immune escape is a fundamental trait of cancer.[30] The interaction between a tumor and its host immune system is suggested to follow three steps: (1) elimination, in which the immune reaction eliminates nascent tumor cells; (2) equilibrium, in which immune reactions control tumor expansion and metastasis; and (3) escape, when tumor cells have developed resistance to the host's immune system; a process termed "immunoediting"[31] (Figure 2). Among several identified escape mechanisms, a major role is played by changes in the expression and/or function of human leukocyte antigens (HLAs) expressed by tumor cells, because they may markedly affect the tumor cell-host immune system interactions. Nonclassical HLA-G is implicated in such immune escape mechanisms of tumor cells and appears to be one of the most powerful molecules for suppression of the innate and/or adaptive immune response, also toward lung cancer.[32,33]

Immune Reaction—The Seventh Hallmark of Cancer?

Today, the link between inflammation and cancer is well documented.[2,8,34] As signs of "smoldering" inflammation have been observed also in tumors without a causal association to inflammation, inflammatory cells and media-
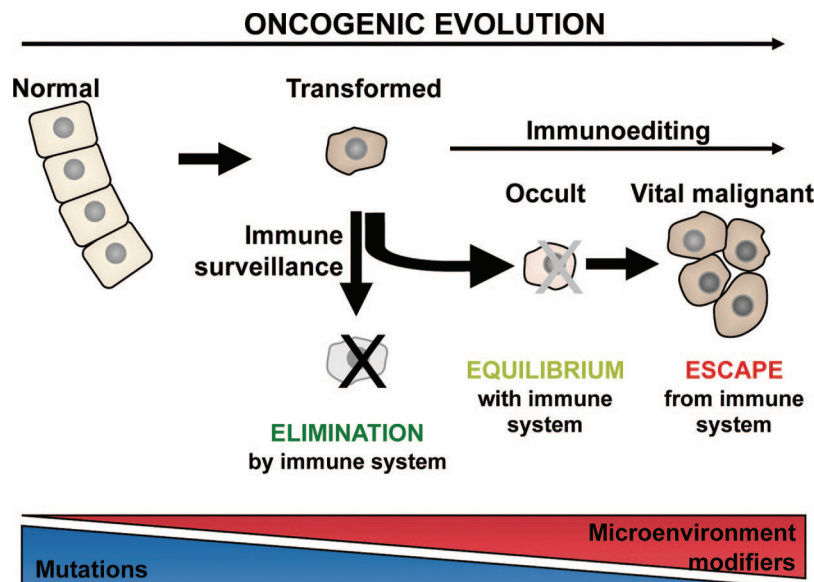

FIGURE 2. Integration of immunoediting and oncogenesis during cancer progression. Oncogenesis leads to transformed cells, which are attacked by immune cells due to neoantigen presentation. This immune surveillance imposes a selection for transformed cells that acquire tactics to escape control. Their genetic instability facilitates evolution of strategies for immune evasion or suppression, which may tilt the tumor microenvironment from hostile to supportive for the transformed cells. At one point, a state of equilibrium may be achieved, corresponding to a clinically occult dormant disease. Further iteration of evasion mechanisms may ultimately drive immune suppression beyond the local microenvironment, accomplishing immune escape and in this manner licensing invasive and metastatic behavior. Adapted from *Oncogene.*[30]

tors appear to be present in the microenvironment of most tumors irrespective of what prompted the development.[6]

Cancer has been defined by six hallmarks acquired during tumor development that are self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion, and metastasis.[35] Immune reaction was proposed as the seventh hallmark of cancer by Zitvogel et al.[36] and has later been supported by others.[37,38]

PARADOXICAL ROLES OF TUMOR-INFILTRATING LEUKOCYTES

Leukocyte infiltration is a cardinal feature of almost all cancers, and the major constituents of these infiltrates include tumor-associated macrophages (TAMs), mast cells, NK and NK T cells, and T and B lymphocytes.

The presence of immune infiltrates, with varying content between different types of solid tumors and patients, has been long established, but the prognostic value of these components is still controversial. A critical issue will be to understand where and how the immune cells are activated and recruited to the tumor site. Dieu-Nosjean et al.[39] found in many of their NSCLC tumors that immune cells were organized in tertiary lymphoid structures, absent in the nontumoral lung. Using an indirect marker for these structures, the authors found that adaptive immunity in lung cancers can be initiated independent of secondary lymphoid organs. Tertiary lymphoid structures such as bronchus-associated lymphoid

tissues have been described in fetal and infant lung but disappear in the normal lung before adulthood.[40,41] The authors found that these tertiary lymphoid structures they named tumor-induced bronchus-associated lymphoid tissue were highly associated with a favorable outcome.

Moreover, leukocyte infiltration is linked to angiogenesis, and a positive association has been demonstrated between numbers of tumor-infiltrating innate immune cells and numbers of blood vessels.[42,43] In animal studies, angiogenesis and tumor development can be limited by reducing innate immune-cell infiltration.[44–46] Furthermore, vascular endothelial growth factor (VEGF) and related molecules are potent monocyte attractants contributing to the recruitment of monocytes in both primary tumors and metastatic niches.[47–50]

In the following, this chapter will primarily deal with the accumulated evidence from several in vitro and animal in vivo studies, indicating that most of these bone marrow-derived cells can be involved in carcinogenesis and/or tumor invasion and metastasis.[51–54]

The Innate Immune System

TAMs alone constitute a major component of the leukocyte infiltrate in malignant tumors.[55] In several epithelial tumors, the content of TAMs has in general been linked to poor prognosis (Table 1),[56] and the protumoral functions of TAMs are presented in Table 2.[10] Also TAM-related cell populations as TIE2-expressing monocytes, myeloid-derived suppressor cells, myeloid DCs, and mast cells have been

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TABLE 1. Markers and Functions of Immune Cells in the Tumor Microenvironment

Cell Type	Functions in the Tumor Microenvironment	References
TAM	Classically activated macrophages (M1) contribute to tumor rejection, whereas alternatively activated macrophages (M2) promote angiogenesis and tissue remodeling. TAMs, sharing M2 characteristics, are tumor promoting and associated with poor prognosis.	55–57, 64–66
MDSC	Increased in almost all patients with cancer. Suppressive effect with respect to T cells.	28, 57, 58
MSC	Infiltrate different human cancers. In animal models, they increase cancer cell dissemination. Also found to be immunosuppressive, in part through inhibition of T-cell proliferation.	71–74
Mast cell	Important for generating and maintaining innate and adaptive immune responses. Increased numbers of mast cells correlate in some cases with poor prognosis. Have been implicated in angiogenic switch in animal models.	57, 60
TEM	Implicated in angiogenesis in animal models. Have been detected in human tumors and at low frequency in the peripheral blood of cancer patients.	57, 61, 62
Neutrophil	Neutrophil levels are increased in patients with colon, gastric, and lung cancer. Increased neutrophil numbers are associated with poor prognosis in bronchioalveolar carcinoma. Neutrophils have been associated with angiogenesis and metastasis in animal models.	57, 87
NK cell	Effector lymphocytes of the innate immune system. Are cytotoxic to cancer cells. Important role in immunosurveillance of cancer.	13, 80, 81
NK T Cell	T cells cytotoxic to cancer cells and contribute to immunosurveillance of cancer. Type 2 NK T cells have been reported to down-regulate tumor immunosurveillance and suppress antitumor responses.	29, 84, 85
T helper cells	CD4+ T helper cells aid CD8+ T cells in tumor rejection.	2, 28, 87
Cytotoxic T cells	CTLs are effector cells of adaptive immunity and specifically recognize and destroy cancer cells.	2, 87, 90
Regulatory T cells	Treg cells are CD4+ lymphocytes, characterized by presenting the phenotype CD25+CD127-Foxp3+. Treg cells are a subset of T cells with the ability to suppress harmful immunological reactions to self- and foreign antigens and have also been attributed to polarize immunity away from an antitumor response, block CD8+ T cell activation and NK cell killing.	2, 28, 86, 88, 90, 106
B cell	B lymphocytes are essential mediators of the adaptive immune system, but in an animal model of squamous cell carcinoma, it was demonstrated to promote malignancy.	44, 87

CTL, CD8+ cytotoxic T cells; MDSC, myeloid-derived suppressor cells; MSC, mesenchymal stem cells; NK cells, natural killer cells; NK T cells, natural killer T lymphocytes; TAM, tumor-associated macrophage; TEM, TIE2-expressing monocyte; TIE2, angiopoietin receptor; Treg cells, regulatory T cells.

TABLE 2. Protumoral Functions of Tumor-Associated Macrophages (TAMs)

- Production of growth and survival factors of tumor cells
  TGFβ, EGF, IL-6, and IFN-inducible chemokine CXCL8
  IL-4 induced TAM-supplied cathepsin B and S
- Production of angiogenic factors
  VEGF, PDGF, FGF2, IL-8, TNFα, and other ELR-positive CXC chemokines
- Degradation of extracellular matrix and tissue remodeling activity
  Expression/release of MMPs, uPAR, and deposition of fibrin and collagen
  Liberation of matrix sequestered growth factors
- Suppression of adaptive immune responses
  Production of immunosuppressive mediators (IL-10, PGE2, and TGFβ)
  Low/absent immunostimulating cytokines (IL-12)
  Release of chemokines (CCL17, CCL18, and CCL22) recruiting noncytotoxic T cells (e.g., regulatory T cells)

CCLs and CXCLs, chemokines; EGF, epidermal growth factor; ELR, specific amino acid sequence of glutamic acid-leucine-arginine before the first cysteine of the CXC; FGF2, fibroblast growth factor; IFN, interferon; IL, interleukin; MMP, matrix metalloprotein proteases; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; TGFβ, transforming growth factor-β; uPAR, urokinase receptor; VEGF, vascular endothelial growth factor.

linked to a protumor inflammatory microenvironment (Table 1).[28,45,57–63] TAMs and related cell types often have an M2 phenotype oriented toward promoting tumor growth, invasion, angiogenesis, and metastasis and suppressing adaptive immunity.[47,55,57,61,64–68] These cells may actively inhibit antitumor adaptive immunity by inducing T-cell dysfunction through direct cell-cell contact and by production of immunosuppressive mediators.[69–74] Using NSCLC specimen, the prognostic role of general TAM infiltration in the tumors was inconclusive.[75–77] Nevertheless, when differentiating between M1 and M2 phenotypes, M1 conferred a significantly better prognosis, although M2 macrophage infiltration was most prevalent.[78,79]

NK cells and NK T cells, both effector lymphocytes of the innate immune system, have somewhat diverse effects in cancer. NK cells have direct cytotoxic effect on cancer cells, hitherto no known unfavorable effects in cancer development and a favorable effect in immunosurveillance.[14,80,81] In contrast, NK T cells have been reported to have paradoxical effects during cancer development.[82,83] NK T cells have been reported to down-regulate tumor immunosurveillance against transplanted tumors,[84,85] and it has been argued for at least two subsets of NK T cells, where type II NK T cells suppress antitumor immune responses.[29]

The Adaptive Immune System

In clinically overt malignant tumors, effective adaptive immune responses are suppressed through activation of various pathways. Despite the importance of T lymphocytes in immunosurveillance and control of early tumor growth, chronic tumor cell secretion of cytokines and other soluble factors subsequently (1) induce, expand, and recruit Treg cells to tumor sites, (2) stimulate mature and immature DCs to provide antigenic stimulation and immunosuppressive cytokines (interleukin-10, transforming growth factor-β), and

(3) inhibit DC maturation.[2,28,86–92] In fact, Treg cells from patients with lung cancer were found to directly inhibit autologous T-cell proliferation.[93] In such a way, a high percentage of Treg cells in various neoplasms creates an immune-suppressive microenvironment that curb antitumor immunity and promotes tumor growth.[93–95] The favorable effect of eliminating Treg cell functions has been demonstrated in animal models, improving antitumor T-cell responses and inducing regression of experimental tumors.[96,97]

In 2005, de Visser et al.[44] reported that also B lymphocytes may be associated with tumor promotion. They found that genetic elimination of mature B and T lymphocytes in a mouse model limited neoplastic progression, which failed to recruit innate immune cells. Adoptive transfer of B lymphocytes or serum from the HPV16 mice into B- and T-cell-deficient/HPV16 mice restored innate immune cell infiltration into premalignant tissue and reinstated parameters for full malignancy.

THE ROLE OF TUMOR MICROENVIRONMENT IMMUNITY IN NSCLC

In lung cancer, the nontumoral stromal tissue comprises fibroblasts and extracellular matrix components, endothelial cells and angiogenesis, and inflammatory cells, which are largely T cells, macrophages, and mast cells, whereas plasma cells, NK cells, and DCs are relatively rare.[98–100] Two thirds are lymphocytes, of which 80% are T cells,[99] expressing various activation antigens such as CD25 and HLA-DR.[101]

HLA-G has been found to have immunosuppressive and immunomodulatory roles in cancer development. Lately, both tumor cell HLA-G and blood soluble HLA-G of patients with NSCLC have been associated with increased stage, immune suppressive effects on NK-cell-mediated cytolysis and reduced survival.[102,103] Its expression on tumors and how it can be exploited in diagnosis and therapy have already been discussed.[104]

Most translational studies within this field have included relatively few cases, and the stromal component of tumors has often been neglected. Until recently, there was relatively limited knowledge about the specificity of immune cells in the lung cancer stroma.[105] In the following, we will present available research data on in situ immunity in the NSCLC tumor microenvironment.

Innate Immune System and NSCLC
Role of DCs

DCs are the most potent antigen presenting cells for inducing primary immune responses to carcinoma and represent a heterogeneous group of cells, which express different markers.[107] In a large cohort of patients with resected NSCLC, increasing numbers of stromal DCs were significantly associated with increased disease-specific survival (DSS).[75] This corroborates previous studies in NSCLC[39,108,109] and studies in other cancer types.[110–112] In the French study,[39] the density of mature DCs was found to be a better predictor for clinical outcome than the other tested variables.

Low VEGF expression/high DC infiltration and high VEGF expression/low DC infiltration are prognostic extremes, with the latter leading to an appallingly poor NSCLC survival.[108] Moreover, human DCs, in vitro, produce transforming growth factor-β under the influence of lung cancer cells, yielding altered DCs with an increased ability to generate Tregs suppressing T cells.[113,114]

Role of NK Cells

Several studies have demonstrated that NK cells are primarily confined to the tumor stroma.[75,115,116] Based on lung tumors, normal lung tissues, and peripheral blood of patients with untreated NSCLC, Carrega et al.[115] found that the cytotoxic potential of tumor-infiltrating CD56$^+$ NK cells isolated from cancer tissues was lower than NK cells from peripheral blood or normal lung tissue. Increasing numbers of tumor-infiltrating NK cells in resected adeno- and squamous cell carcinomas were associated with a favorable survival prognosis.[117,118] In a large stage I to IIIA NSCLC cohort, Al-Shibli et al.[75] found that increasing numbers of stromal CD56$^+$ NK cells were significantly associated with improved DSS and appeared as a powerful independent prognostic factor (hazard ratio, 0.43).

Role of Macrophages

It has been argued that macrophages may have a potential dual role in lung cancer by supporting both host-defense and tumor progression.[99] In fact, lung cancer metastasis to bone and muscles in an animal model could be inhibited by decreasing the number of monocytes/macrophages in both peripheral blood and tumor stroma.[119]

Assessing the total counts of CD68$^+$ macrophages, several research groups have reported a favorable association between increasing tumor islet macrophages and NSCLC survival.[76,77,109] Dai et al.[109] found the number of macrophages in both the tumor and stromal compartment to be independent prognostic role and higher impact than mature DCs or CTL. In contrast, Al-Shibli et al.[75] did not find CD68$^+$ macrophages in neither tumor nor stromal compartments of 335 NSCLC specimens to correlate with survival. Studying good survival versus poor survival NSCLC groups by identifying the immunological phenotype of TAMs (M1 or M2 type), two independent research groups found that the macrophages infiltrating the tumors were predominantly of the M1 phenotype in patients with extended survival, although type M2 dominated in the tumor tissues (70% versus 30%).[78,79] The M2 macrophage densities were not associated with patients' survival time.

Role of Mast Cells

In a previous NSCLC study, the density of mast cells correlated with micro vessel density but not survival.[120] In a larger NSCLC cohort, sparse numbers of CD117$^+$ mast cells were detected, solely in the stromal compartment, and without prognostic relevance.[121] Consistently, two other studies[76,77] reported a neutral prognostic role of mast cell density in stages I to IV NSCLC tumors.

In patients with adenocarcinoma, a higher density of mast cells correlated with improved survival.[122] In contrast, however, Takanami et al.[123] found increased mast cell infiltration in lung adenocarcinomas to be associated with angiogenesis and worse prognosis. Mast cells have been regarded a double-edged sword in cancer immunity, which may explain the variable results with respect to prognostic significance.

Adaptive Immune System and NSCLC

Tumor-infiltrating lymphocytes are considered to indicate level of host immunity toward tumor antigens.[31] Lee et al.[124] observed that a high peritumoral lymphoid index was an independent favorable prognostic factor in patients with stage III NSCLC.

Role of T Helper and Regulatory Lymphocytes

Studying the prognostic role of epithelial and stromal CD4$^+$ helper T cells in patients with resected stages I to IIIA NSCLC, the authors reported that increasing numbers of stromal, but not epithelial, CD4$^+$ cells correlated significantly with improved DSS and that a high density of stromal CD4$^+$ cells was a favorable independent prognostic factor in these patients.[125] Similar CD4$^+$ cell results have been reported by others.[126] Corroborating the previous study by Hiraoka et al.,[127] the Norwegian study[125] reported an augmented positive prognostic effect at simultaneously high stromal density of CD8$^+$ cells, confirming the immunological cooperation between these cell types. In two somewhat smaller studies, Pelletier et al.[128] found peritumoral B and not T cells to yield a better NSCLC survival, whereas Nakamura et al.[129] concluded that neither the total number of intraepithelial T cells nor its helper or cytotoxic subtypes had any prognostic relevance in NSCLC.

The CD4$^+$ category of cells is heterogeneous as it, besides effector cells, contains Treg cells (CD4$^+$, CD25$^+$, and FOXP3+), which mediate immune tolerance and suppression of T-cell effects. The amount of Treg cells are higher in NSCLC tumors than normal lung tissues,[100] and in a murine model, Treg cells appeared as a prerequisite for *k-ras* driven lung tumorigenesis.[130] In resected NSCLC tumors, infiltrating Treg cells correlated positively with intratumoral COX-2 expression and were associated with recurrence.[131] Moreover, patients with stage I NSCLC who had a higher proportion of tumor Treg cells relative to CTL had a significantly higher risk of recurrence.[132] Nevertheless, COX-2/PGE2 inhibition suppresses Treg cell activity and enhances antitumor responses in lung cancer.[133]

Role of Cytotoxic T Lymphocytes

In 335 patients with resected stages I to IIIA NSCLC, Al-Shibli et al.[125] found that increasing numbers of both epithelial and stromal CD8$^+$ CTL correlated with an improved DSS. Actually, an independent significant impact was observed only in the stroma, not in the tumor epithelium. In the same cohort, Donnem et al.[134] detected that high tumor cell VEGF-A/VEGF receptor-2 combined with low density of CD4$^+$ and CD8$^+$ cells in the tumor stroma gave a massive increase in risk of death (hazard ratio, 9.2). The favorable prognostic significance of CD8$^+$ cells has been confirmed by others.[76,109] Although Hiraoka et al.[127] reported that a tumor infiltration of CD8$^+$ cells alone had no prognostic significance, the concurrent infiltration of both CD8$^+$ and CD4$^+$ cells had an independent favorable effect.

A high number of CD3$^+$ T cells (pan T cell marker including CD4$^+$, CD8$^+$, subset of CD3$^+$CD56$^+$ NK T cells, and CD4$^-$CD8$^-$ T cells) have been associated with increased apoptosis in patients with NSCLC.[135] Kataki et al.[99] concluded that CD3$^+$ cells in lung carcinoma result from recruitment of circulating precursors rather than from local replication, which is important when considering the possible therapeutic relevance of these cells. From a recent study, Al-Shibli et al.[121] reported that an increasing number of stromal and epithelial CD3$^+$ T cells was associated with a better DSS and, further, that a high stromal density of CD3$^+$ T cells was an independent indicator for survival, highlighting the significance of the stromal compartment in cancer progression and survival. These findings are supported by a previous study in 710 patients with primary lung cancer.[136]

The CD8$^+$ cell-related findings suggested that these immune cells can modify the tumor stroma and epithelium in ways which reduces disease progression and metastasis. In the Norwegian study,[125] there was a significant association between increased stromal CD8$^+$ cells and low prevalence of angiolymphatic invasion. This may indicate a protective role of CD8$^+$ cells in preventing vascular infiltration of tumor cells and thus inhibiting progression and metastasis. Anyway, the beneficial combined effect of CD8$^+$ and CD4$^+$ cells may be mediated primarily by the tumor stroma.

Role of B Lymphocytes

Increasing numbers of epithelial and stromal CD20$^+$ B lymphocytes correlate with DSS in patients with stages I to III NSCLC[125] but without an independent prognostic impact. Based on these finding, it can be hypothesized that humoral immunity play a role in NSCLC survival. Consistently, Pelletier et al.[128] observed that the presence of peritumoral CD20$^+$ cells was a positive prognosticator in NSCLC. Mechanisms that may explain the prognostic effect of peritumoral B cells are local containment of tumor cells, reducing the incidence of occult micrometastases and thereby prolonging survival by limiting further tumor dissemination.[128] Besides, antibodies produced by B cells are important mediators of tumor cell killing by NK and other inflammatory cells through antibody-dependent cell-mediated cytotoxicity (ADCC). A functional deficiency of B lymphocytes in lung cancer is, on the other hand, considered to be caused by inadequate T-cell help and excessive suppression by T and non-T cells.[137]

THERAPEUTIC APPROACHES BASED ON IMMUNE RESPONSE

Nuclear factor κ-light-chain-enhancer of activated B cells seems to play an important role in determining the balance between protumor and antitumor properties of macrophages,[138,139] hence nuclear factor κ-light-chain-enhancer of activated B cells may be targeted to "re-educate" M2 macrophages toward an antitumor modality. The concept that stimulation of innate immunity can promote protective responses to cancer is not new. In 1972, Ruckdeschel et al.[140] reported that immune stimulation, associated with the postoperative complication of empyema, was associated with a decreased risk of recurrence of resected NSCLC. In addition, in vivo animal studies supported the concept that nonspecific immune stimulation with BCG had effect on lung tumors.[141,142] The larger clinical studies did not, however, show a benefit. Later, nonspecific immunostimulatory interventions with cytokines as interleukin-2, interferon α, and tumor necrosis factor α also proved ineffective in NSCLC.[143]

In 1998, Kerr et al.[144] reported a comparison of 28 possibly regressing lung cancer tumors with 67 minimally to highly inflamed control cases. The possibly regressing tumors showed a significantly higher number of CD3$^+$ T cells, CD68$^+$ macrophages, and CD57$^+$ NK cells, indicating an immunophenotype resembling that seen in regressing malignant melanomas. During the last decade, because of advances in immunology and molecular biology, the interest in and development of therapeutic tumor vaccines have increased.[143,145] This potential treatment modality in NSCLC has moved from using nonspecific immunomodulatory agents to lung cancer-specific tumor antigens and tumor-cell-derived vaccines.[146] In randomized NSCLC phase II trials, encouraging trends toward better survival has been limited to the melanoma-associated antigen 3 and the antigen mucin 1.[147–149] These positive results have prompted the launch of randomized phase III trials.

Today there are several drugs that can target cancer-related inflammation, such as chemokine-receptor antagonists, cytokine-receptor antagonists, and COX-inhibitors. There are ongoing early phase trials of antagonists of interleukin-6, interleukin-6 receptor, and different chemokines for various epithelial malignancies.[8] Especially as tumor cells in general are genetically instable and frequently become therapy resistant, an optimal future treatment should be a combined attack on both tumor cells and the tumor microenvironment (inflammatory cells), possibly yielding better and more durable efficacy. The future challenge will be to tip the balance from cancer promoting to cancer-inhibiting inflammatory responses in established malignancies.

CONCLUSION

In the NSCLC microenvironment, there is a complex interaction between immune cells and tumor cells as well as other stromal cells types and tissue constituents. A marked infiltration in tumor of different types of immune cells has been reported. The distribution of these cells and the expression of different inflammatory molecules throughout the tumor microenvironment are, to various extents, related to tumor progression and survival. This interaction has a significant unfavorable prognostic relevance as it may facilitate tumor-promoting processes such as evasion from immunosurveillance, tumor growth, invasion, angiogenesis, and metastasis. Increasing evidence shows that the density of various immune cells in the tumor stromal compartment, in particular, has significant prognostic relevance for NSCLC survival.

Advances in immunology and molecular biology have led to a rapid progress in the development of cancer immunotherapy, and positive immunotherapy studies in NSCLC have led to ongoing randomized phase III trials. The potential for reversing tumor-supporting inflammation may be the start of an exciting new era for anticancer therapies. For this, we need more knowl-

edge and a deeper understanding of the processes involved in the cancer-related chronic inflammation.

REFERENCES

1. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006;6: 392–401.
2. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 2006;6: 24–37.
3. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009;9:239–252.
4. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–545.
5. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res* 1970;13:1–27.
6. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005;7:211–217.
7. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006;118:3030–3044.
8. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. *Nature* 2008;454:436–444.
9. de Visser KE, Coussens LM. The interplay between innate and adaptive immunity regulates cancer development. *Cancer Immunol Immunother* 2005;54:1143–1152.
10. Allavena P, Sica A, Solinas G, et al. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* 2008;66:1–9.
11. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. *Immunity* 2005;23:344–346.
12. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005;5:953–964.
13. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004;4:499–511.
14. Walzer T, Jaeger S, Chaix J, et al. Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses. *Curr Opin Immunol* 2007;19:365–372.
15. Vivier E, Anfossi N. Inhibitory NK-cell receptors on T cells: witness of the past, actors of the future. *Nat Rev Immunol* 2004;4:190–198.
16. Casetti R, Martino A. The plasticity of gamma delta T cells: innate immunity, antigen presentation and new immunotherapy. *Cell Mol Immunol* 2008;5:161–170.
17. Munz C, Steinman RM, Fujii S. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J Exp Med* 2005;202:203–207.
18. Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature* 1999;402:255–262.
19. Tonegawa S. Somatic generation of antibody diversity. *Nature* 1983; 302:575–581.
20. McHeyzer-Williams MG. B cells as effectors. *Curr Opin Immunol* 2003;15:354–361.
21. Sprent J, Surh CD. T cell memory. *Annu Rev Immunol* 2002;20:551–579.
22. Degli-Esposti MA, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat Rev Immunol* 2005;5:112–124.
23. Hamerman JA, Ogasawara K, Lanier LL. NK cells in innate immunity. *Curr Opin Immunol* 2005;17:29–35.
24. Raulet DH. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol* 2004;5:996–1002.
25. Hoebe K, Janssen E, Beutler B. The interface between innate and adaptive immunity. *Nat Immunol* 2004;5:971–974.
26. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; 423:356–361.
27. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* 2001;61:4756–4760.
28. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. *Curr Opin Genet Dev* 2008;18:11–18.
29. Terabe M, Berzofsky JA. NKT cells in immunoregulation of tumor immunity: a new immunoregulatory axis. *Trends Immunol* 2007;28: 491–496.
30. Prendergast GC. Immune escape as a fundamental trait of cancer: focus on IDO. *Oncogene* 2008;27:3889–3900.
31. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002;3:991–998.
32. Carosella ED, Favier B, Rouas-Freiss N, et al. Beyond the increasing complexity of the immunomodulatory HLA-G molecule. *Blood* 2008; 111:4862–4870.
33. Yie SM, Yang H, Ye SR, et al. Expression of human leucocyte antigen G (HLA-G) is associated with prognosis in non-small cell lung cancer. *Lung Cancer* 2007;58:267–274.
34. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420: 860–867.
35. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100: 57–70.
36. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol* 2006;6:715–727.
37. Mantovani A. Cancer: inflaming metastasis. *Nature* 2009;457:36–37.
38. Pages F, Galon J, eu-Nosjean MC, et al. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene* 2010;29:1093–1102.
39. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol* 2008;26:4410–4417.
40. Gould SJ, Isaacson PG. Bronchus-associated lymphoid tissue (BALT) in human fetal and infant lung. *J Pathol* 1993;169:229–234.
41. Tschernig T, Pabst R. Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases. *Pathobiology* 2000;68:1–8.
42. Esposito I, Menicagli M, Funel N, et al. Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma. *J Clin Pathol* 2004;57:630–636.
43. Yano H, Kinuta M, Tateishi H, et al. Mast cell infiltration around gastric cancer cells correlates with tumor angiogenesis and metastasis. *Gastric Cancer* 1999;2:26–32.
44. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 2005;7:411–423.
45. Coussens LM, Raymond WW, Bergers G, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999;13:1382–1397.
46. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* 2004;6: 447–458.
47. Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest* 2007;117:1155–1166.
48. Fischer C, Jonckx B, Mazzone M, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* 2007;131:463–475.
49. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 2005;438:820–827.
50. Shojaei F, Wu X, Zhong C, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* 2007;450:825–831.
51. Bunt SK, Yang L, Sinha P, et al. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res* 2007;67: 10019–10026.
52. Coussens LM, Tinkle CL, Hanahan D, et al. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 2000; 103:481–490.
53. Lin EY, Nguyen AV, Russell RG, et al. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001;193:727–740.
54. Mantovani A, Bottazzi B, Colotta F, et al. The origin and function of tumor-associated macrophages. *Immunol Today* 1992;13:265–270.
55. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. *Cancer Metastasis Rev* 2007;26:373–400.
56. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated mac-

rophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002;196:254–265.

57. Murdoch C, Muthana M, Coffelt SB, et al. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer* 2008;8:618–631.

58. Youn JI, Nagaraj S, Collazo M, et al. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol* 2008;181:5791–5802.

59. Soucek L, Lawlor ER, Soto D, et al. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat Med* 2007;13:1211–1218.

60. Ribatti D, Crivellato E, Roccaro AM, et al. Mast cell contribution to angiogenesis related to tumour progression. *Clin Exp Allergy* 2004;34:1660–1664.

61. De Palma M, Venneri MA, Galli R, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005;8:211–226.

62. Venneri MA, De PM, Ponzoni M, et al. Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. *Blood* 2007;109:5276–5285.

63. Mantovani A, Sozzani S, Locati M, et al. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002;23:549–555.

64. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;3:23–35.

65. Mantovani A, Sica A, Sozzani S, et al. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25:677–686.

66. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–969.

67. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004;4:71–78.

68. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006;124:263–266.

69. Serafini P, De SC, Marigo I, et al. Derangement of immune responses by myeloid suppressor cells. *Cancer Immunol Immunother* 2004;53:64–72.

70. Gabrilovich DI, Velders MP, Sotomayor EM, et al. Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol* 2001;166:5398–5406.

71. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8:726–736.

72. Spaeth E, Klopp A, Dembinski J, et al. Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther* 2008;15:730–738.

73. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007;449:557–563.

74. Lodie TA, Blickarz CE, Devarakonda TJ, et al. Systematic analysis of reportedly distinct populations of multipotent bone marrow-derived stem cells reveals a lack of distinction. *Tissue Eng* 2002;8:739–751.

75. Al-Shibli K, Al-Saad S, Donnem T, et al. The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma. *Histopathology* 2009;55:301–312.

76. Kawai O, Ishii G, Kubota K, et al. Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. *Cancer* 2008;113:1387–1395.

77. Welsh TJ, Green RH, Richardson D, et al. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. *J Clin Oncol* 2005;23:8959–8967.

78. Ma J, Liu L, Che G, et al. The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. *BMC Cancer* 2010;10:112.

79. Ohri CM, Shikotra A, Green RH, et al. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. *Eur Respir J* 2009;33:118–126.

80. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. *Oncogene* 2008;27:5932–5943.

81. Vivier E, Tomasello E, Baratin M, et al. Functions of natural killer cells. *Nat Immunol* 2008;9:503–510.

82. Smyth MJ, Godfrey DI. NKT cells and tumor immunity—a double-edged sword. *Nat Immunol* 2000;1:459–460.

83. Papamichail M, Perez SA, Gritzapis AD, et al. Natural killer lymphocytes: biology, development, and function. *Cancer Immunol Immunother* 2004;53:176–186.

84. Terabe M, Matsui S, Noben-Trauth N, et al. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol* 2000;1:515–520.

85. Moodycliffe AM, Nghiem D, Clydesdale G, et al. Immune suppression and skin cancer development: regulation by NKT cells. *Nat Immunol* 2000;1:521–525.

86. Wang RF. CD8+ regulatory T cells, their suppressive mechanisms, and regulation in cancer. *Hum Immunol* 2008;69:811–814.

87. Janeway C, Travers P, Walport M, et al. Immunobiology—the immune system in health and disease. New York: Garland Science Publishing, 2005.

88. Jarnicki AG, Lysaght J, Todryk S, et al. Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. *J Immunol* 2006;177:896–904.

89. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005;5:263–274.

90. Kiniwa Y, Miyahara Y, Wang HY, et al. CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer. *Clin Cancer Res* 2007;13:6947–6958.

91. Gilliet M, Liu YJ. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med* 2002;195:695–704.

92. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;10:942–949.

93. Woo EY, Yeh H, Chu CS, et al. Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol* 2002;168:4272–4276.

94. Bates GJ, Fox SB, Han C, et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol* 2006;24:5373–5380.

95. Gao Q, Qiu SJ, Fan J, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007;25:2586–2593.

96. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999;163:5211–5218.

97. Onizuka S, Tawara I, Shimizu J, et al. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. *Cancer Res* 1999;59:3128–3133.

98. Salgaller ML. The development of immunotherapies for non-small cell lung cancer. *Expert Opin Biol Ther* 2002;2:265–278.

99. Kataki A, Scheid P, Piet M, et al. Tumor infiltrating lymphocytes and macrophages have a potential dual role in lung cancer by supporting both host-defense and tumor progression. *J Lab Clin Med* 2002;140:320–328.

100. Ishibashi Y, Tanaka S, Tajima K, et al. Expression of Foxp3 in non-small cell lung cancer patients is significantly higher in tumor tissues than in normal tissues, especially in tumors smaller than 30 mm. *Oncol Rep* 2006;15:1315–1319.

101. Kuo SH, Chang DB, Lee YC, et al. Tumour-infiltrating lymphocytes in non-small cell lung cancer are activated T lymphocytes. *Respirology* 1998;3:55–59.

102. Schutt P, Schutt B, Switala M, et al. Prognostic relevance of soluble human leukocyte antigen-G and total human leukocyte antigen class I molecules in lung cancer patients. *Hum Immunol* 2010;71:489–495.

103. Lin A, Zhu CC, Chen HX, et al. Clinical relevance and functional implications for human leukocyte antigen-G expression in non-small-cell lung cancer. *J Cell Mol Med* 2010;14:2318–2329.

104. Tripathi P, Agrawal S. Non-classical HLA-G antigen and its role in the cancer progression. *Cancer Invest* 2006;24:178–186.

105. Mami-Chouaib F, Echchakir H, Dorothee G, et al. Antitumor cytotoxic T-lymphocyte response in human lung carcinoma: identification of a tumor-associated antigen. *Immunol Rev* 2002;188:114–121.

106. Gobert M, Treilleux I, driss-Vermare N, et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid

infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res* 2009;69:2000–2009.

107. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991;9:271–296.

108. Inoshima N, Nakanishi Y, Minami T, et al. The influence of dendritic cell infiltration and vascular endothelial growth factor expression on the prognosis of non-small cell lung cancer. *Clin Cancer Res* 2002;8: 3480–3486.

109. Dai F, Liu L, Che G, et al. The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. *BMC Cancer* 2010;10:220.

110. Yuan A, Steigen SE, Goll R, et al. Dendritic cell infiltration pattern along the colorectal adenoma-carcinoma sequence. *APMIS* 2008;116: 445–456.

111. Cai XY, Gao Q, Qiu SJ, et al. Dendritic cell infiltration and prognosis of human hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2006; 132:293–301.

112. Coventry BJ, Morton J. CD1a-positive infiltrating-dendritic cell density and 5-year survival from human breast cancer. *Br J Cancer* 2003;89: 533–538.

113. Dumitriu IE, Dunbar DR, Howie SE, et al. Human dendritic cells produce TGF-beta 1 under the influence of lung carcinoma cells and prime the differentiation of CD4+CD25+Foxp3+ regulatory T cells. *J Immunol* 2009;182:2795–2807.

114. Li Q, Guo Z, Xu X, et al. Pulmonary stromal cells induce the generation of regulatory DC attenuating T-cell-mediated lung inflammation. *Eur J Immunol* 2008;38:2751–2761.

115. Carrega P, Morandi B, Costa R, et al. Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. *Cancer* 2008;112:863–875.

116. Esendagli G, Bruderek K, Goldmann T, et al. Malignant and non-malignant lung tissue areas are differentially populated by natural killer cells and regulatory T cells in non-small cell lung cancer. *Lung Cancer* 2008;59:32–40.

117. Villegas FR, Coca S, Villarrubia VG, et al. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. *Lung Cancer* 2002;35:23–28.

118. Takanami I, Takeuchi K, Giga M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. *J Thorac Cardiovasc Surg* 2001;121:1058–1063.

119. Hiraoka K, Zenmyo M, Watari K, et al. Inhibition of bone and muscle metastases of lung cancer cells by a decrease in the number of monocytes/macrophages. *Cancer Sci* 2008;99:1595–1602.

120. Dundar E, Oner U, Peker BC, et al. The significance and relationship between mast cells and tumour angiogenesis in non-small cell lung carcinoma. *J Int Med Res* 2008;36:88–95.

121. Al-Shibli K, Al-Saad S, Andersen S, et al. The prognostic value of intraepithelial and stromal CD3-, CD117- and CD138-positive cells in non-small cell lung carcinoma. *APMIS* 2010;118:371–382.

122. Tomita M, Matsuzaki Y, Onitsuka T. Correlation between mast cells and survival rates in patients with pulmonary adenocarcinoma. *Lung Cancer* 1999;26:103–108.

123. Takanami I, Takeuchi K, Naruke M. Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. *Cancer* 2000;88:2686–2692.

124. Lee TK, Horner RD, Silverman JF, et al. Morphometric and morphologic evaluations in stage III non-small cell lung cancers. Prognostic significance of quantitative assessment of infiltrating lymphoid cells. *Cancer* 1989;63:309–316.

125. Al-Shibli KI, Donnem T, Al-Saad S, et al. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clin Cancer Res* 2008;14:5220–5227.

126. Wakabayashi O, Yamazaki K, Oizumi S, et al. CD4+ T cells in cancer stroma, not CD8+ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. *Cancer Sci* 2003;94:1003–1009.

127. Hiraoka K, Miyamoto M, Cho Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. *Br J Cancer* 2006;94:275–280.

128. Pelletier MP, Edwardes MD, Michel RP, et al. Prognostic markers in

resectable non-small cell lung cancer: a multivariate analysis. *Can J Surg* 2001;44:180–188.

129. Nakamura H, Saji H, Ogata A, et al. Immunologic parameters as significant prognostic factors in lung cancer. *Lung Cancer* 2002;37: 161–169.

130. Granville CA, Memmott RM, Balogh A, et al. A central role for Foxp3+ regulatory T cells in K-Ras-driven lung tumorigenesis. *PLoS One* 2009;4:e5061.

131. Shimizu K, Nakata M, Hirami Y, et al. Tumor-infiltrating Foxp3+ regulatory T cells are correlated with cyclooxygenase-2 expression and are associated with recurrence in resected non-small cell lung cancer. *J Thorac Oncol* 2010;5:585–590.

132. Petersen RP, Campa MJ, Sperlazza J, et al. Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. *Cancer* 2006;107:2866–2872.

133. Sharma S, Yang SC, Zhu L, et al. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. *Cancer Res* 2005; 65:5211–5220.

134. Donnem T, Al-Shibli K, Andersen S, et al. Combination of low vascular endothelial growth factor A (VEGF-A)/VEGF receptor 2 expression and high lymphocyte infiltration is a strong and independent favorable prognostic factor in patients with nonsmall cell lung cancer. *Cancer* 2010;116:4318–4325.

135. Tormanen-Napankangas U, Soini Y, Paakko P. High number of tumour-infiltrating lymphocytes is associated with apoptosis in non-small cell lung carcinoma. *APMIS* 2001;109:525–532.

136. Johnson SK, Kerr KM, Chapman AD, et al. Immune cell infiltrates and prognosis in primary carcinoma of the lung. *Lung Cancer* 2000;27:27–35.

137. Venkataraman M, Rao DS, Iyer BS, et al. The functional deficiency of B lymphocytes in patients with lung cancer is due to inadequate T-cell help and excessive suppression by T and non-T cells. *Cancer Invest* 1989;7:7–16.

138. Saccani A, Schioppa T, Porta C, et al. p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. *Cancer Res* 2006; 66:11432–11440.

139. Hagemann T, Lawrence T, McNeish I, et al. "Re-educating" tumor-associated macrophages by targeting NF-kappaB. *J Exp Med* 2008;205: 1261–1268.

140. Ruckdeschel JC, Codish SD, Stranahan A, et al. Postoperative empyema improves survival in lung cancer. Documentation and analysis of a natural experiment. *N Engl J Med* 1972;287:1013–1017.

141. Baldwin RW, Pimm MV. BCG immunotherapy of pulmonary growths from intravenously transferred rat tumour cells. *Br J Cancer* 1973;27: 48–54.

142. McKneally MF, Maver C, Kausel HW. Regional immunotherapy of lung cancer with intrapleural B.C.G. *Lancet* 1976;1:377–379.

143. Van den Heuvel MM, Burgers SA, van ZN. Immunotherapy in non-small-cell lung carcinoma: from inflammation to vaccination. *Clin Lung Cancer* 2009;10:99–105.

144. Kerr KM, Johnson SK, King G, et al. Partial regression in primary carcinoma of the lung: does it occur? *Histopathology* 1998;33:55–63.

145. Choudhury A, Palma M, Mellstedt H. The future of cancer vaccines for non-small-cell lung cancer: ongoing trials. *Clin Lung Cancer* 2008; 9(Suppl 1):S37–S44.

146. Ho C, Ochsenbein AF, Gautschi O, et al. Early clinical trial experience with vaccine therapies in non-small-cell lung cancer. *Clin Lung Cancer* 2008;9(Suppl 1):S20–S27.

147. Vansteenkiste J, Zielinski M, Linder A, et al. Final results of a multi-center, double-blind, randomized, placebo-controlled phase II study to assess the efficacy of MAGE-A3 immunotherapeutic adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC). *J Clin Oncol* 2007;25(Suppl 18):398.

148. Sangha R, Butts C. L-BLP25: a peptide vaccine strategy in non small cell lung cancer. *Clin Cancer Res* 2007;13:s4652–s4654.

149. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J Clin Oncol* 2005;23:6674–6681.