Tumour immunity: effector response to tumour and role of the microenvironment

Alberto Mantovani, Pedro Romero, A Karolina Palucka, Francesco M Marincola

Substantial evidence shows that inflammation promotes oncogenesis and, occasionally, participates in cancer rejection. This paradox can be accounted for by a dynamic switch from chronic smouldering inflammation promoting cancer-cell survival to florid, tissue-disruptive inflammatory reactions that trigger cancer-cell destruction. Clinical and experimental observations suggest that the mechanism of this switch recapitulates the events associated with pathogen infection, which stimulate immune cells to recognise danger signals and activate immune effector functions. Generally, cancers do not have danger signals and, therefore, they cannot elicit strong immune reactions. Synthetic molecules have been developed that mimic pathogen invasion at the tumour site. These compounds activate dendritic cells to produce proinflammatory cytokines, which in turn trigger cytotoxic mechanisms leading to cancer death. Simultaneously, dendritic cells capture antigen shed by dying cancer cells, undergo activation, and stimulate antigen-specific T and B cells. This process results in massive amplification of the antineoplastic inflammatory process. Thus, although anti-inflammatory drugs can prevent onset of some malignant diseases, induction of T cells specific for tumour antigen by active immunisation, combined with powerful activation signals within the cancer microenvironment, might yield the best strategy for treatment of established cancers.

Introduction

During the past two decades, tumour immunology matured as a discipline with the unequivocal identification of tumour-specific antigens recognised by antibodies and T cells. This result suggests that the immune system naturally acquires the ability to recognise cancer cells and yet cannot control malignant growth. This paradox can be accounted for by a generalised immunodeficiency associated with the tumour-bearing status or by modulatory properties of cancer or bystander cells that hamper immune function within the tumour microenvironment. Generalised immunodeficiencies in cancer patients have been discussed elsewhere. Here, we will focus on immune interactions happening within the tumour microenvironment that promote or inhibit cancer growth.

Under certain conditions, inflammation seems to promote carcinogenesis, whereas in other situations it seems to have antitumour effects. The intensity and nature of the inflammation could explain this apparent contradiction. Inflammation ranges from milder processes such as those seen in some autoimmune disorders and chronic infections to more vigorous acute inflammatory processes as seen with pathogen clearance or allograft rejection. In most cases, inflammation associated with cancer is similar to that seen with chronic inflammation, which includes the production of growth and angiogenic factors that stimulate tissue repair—factors that can also promote cancer-cell survival, implantation, and growth. Occasionally, however, and for unclear reasons, the pendulum shifts towards an inflammatory process, similar to acute inflammatory processes, that favours an immune effector mechanism capable of inducing spontaneous or treatment-induced cancer regression.

Cancer-related inflammation is driven mainly by cells of the innate immune system—a term describing our inborn defences that sense potential threats independent of previous exposure. The acquired (also called adaptive) immune response also seems to participate in this process: B lymphocytes contribute to carcinogenesis in animal models, and identification in cancer patients of tumour-antigen-specific antibodies and lymphocytes lends further support to this idea in human beings. Thus, both innate and adaptive immune responses can promote carcinogenesis and tumour growth or have anticancer effects.

At the same time, however, mice deficient in interferon γ and mice deficient in T cells develop tumours with high frequency. Congenital and acquired immunodeficiencies are also associated with increased prevalence of malignant diseases. These findings indicate that the immune system also has an active protective role in surveillance against cancer. The relationship between the immune system surveillance and cancer is, however, dynamic. Immune surveillance can control or eliminate some premalignant lesions and proto-cancers (early cancers). However, with time, tumour cells under selective pressure from immune surveillance can undergo a process referred to as immune editing, and become resistant to this first-line of defence and develop a phenotype capable of manipulating immune cells through secretion of chemokines and growth factors.

Search strategy and selection criteria

We searched PubMed with the keywords “tumor microenvironment” and “tumor immunity”. We also looked for papers describing the characterisation of immune responses specific to tumour antigens in individuals and for reports detailing the relation between adaptive and innate immune responses in the context of cancer, with preferential interest in human models but not excluding preclinical ones. We reviewed all abstracts and selected the most relevant papers. Searches were done from November, 2005, to July 1, 2006. We did not restrict the search by language or date.
cytokines (figure 1). At this point, tumour-associated macrophages and B cells can then interact to foster cancer growth by producing factors that promote tissue remodelling and neoangiogenesis.

In theory, immunotherapy could alter this balance by activation of antigen-presenting cells and by recruitment and activation of both T cells—including cytotoxic T lymphocytes—and natural killer cells and, thus, turn an indolent inflammatory process that favours tumour growth into an acute process that promotes tumour destruction. When a threshold necessary for tumour rejection is reached, lesions can regress and, if all cancer cells are destroyed, disappear. However, should some tumour cells escape immune recognition, the tumours could progress or recur (figure 1).

Clinical trials with tumour-antigen vaccines, systemic administration of cytokines, adoptive transfer of T cells specific for tumour antigen, or antibodies show that the immune system is capable of rejecting established tumours. The focus of the rest of this Review will be on how inflammatory processes that often promote tumour growth can be induced to have a therapeutic antitumour effect. In particular, we will discuss: how chronic inflammation and innate immunity favour tumour growth; how the cross-talk linking innate and adaptive immunity through tumour-antigen presentation could affect the antitumour component of the immune response; how functional characteristics of adaptive immune responses in the tumour microenvironment might account for the immune system's limited ability to destroy cancer cells; and how commonalities with the immune processes leading to autoimmune reactions, acute allograft rejection, and pathogen clearance might offer ideas about ways to improve the efficiency of antitumour immune response.

**Role of tumour-associated macrophages in cancer-promoting inflammation**

Over the past several years, there has been a renaissance of research into the connection between inflammation and cancer. Findings of epidemiological studies have shown that chronic inflammation predisposes to different cancers—colon cancer being the prototype. The triggers of chronic inflammation that increase cancer risk include microbial infections (eg, *Helicobacter pylori* for gastric cancer and mucosal lymphoma), autoimmune diseases (eg, thyroiditis for papillary thyroid carcinoma, inflammatory bowel disease for colon cancer), and cryptogenic inflammatory disorders (eg, prostatitis for prostate cancer). Furthermore, work has shown that use of non-steroidal anti-inflammatory drugs decreases the incidence of several tumours.

Inflammatory processes associated with cancers include: leucocyte infiltration, predominantly tumour-associated macrophages; expression of cytokines, such as tumour necrosis factor α (TNFα) or interleukin 1, and chemokines, such as chemokine (C-C motif) ligand 2 (CCL2); and tissue remodelling and angiogenesis. As early as the 1970s, researchers noted that tumour-associated macrophages promoted malignant growth in vitro and in vivo and that in most human cancers a high frequency of macrophages was associated with poor prognosis. With the advent of genomics, the mechanisms accounting for these pathological findings have been clarified. For example, genes associated with leucocyte or macrophage infiltration—eg, *CD68*—are part of molecular signatures that herald poor prognosis in lymphomas and breast carcinomas. Similarly, functional polymorphisms of master genes of inflammation (TNFα and interleukin 1) are associated with increased cancer risk or progression.

Transgenic mice, including models with cell-specific gene inactivation, have allowed the dissection of inflammatory pathways that lead to tumour promotion and the molecular characterisation of the elements involved in specific steps of tumour progression. The essential elements in carcinogenesis or acquisition of a metastatic phenotype in skin, liver, mammary gland, and intestine cancers include TNFα, interleukin 1, the macrophage growth and attractant factor CSF1, CCL2 (a chemokine originally described as a tumour-derived macrophage attractant), cyclo-oxygenase 2, the master inflammatory transcription factor NFκB, and enzymes involved in tissue remodelling.

Heterogeneity and plasticity are hallmarks of cells of the monocyte-macrophage lineage. In response to cytokines and microbial products, mononuclear phagocytes express specialised and polarised functional properties. Mirroring the T helper 1 and 2 nomenclature (Th1 and Th2), many researchers refer to polarised macrophages as M1 and M2 cells. Classically activated M1 macrophages are induced by interferon γ alone or in concert with microbial stimuli (ie, lipopolysaccharide) or cytokines (ie, TNFα and granulocyte-macrophage colony-stimulating factor). Interleukin 4 and interleukin 13, long thought to be simple inhibitors of macrophage activation, are now known to induce an alternate form, called M2. The designation M2 covers

---

**Figure 1:** Postulated interactions between immune and cancer cells at various stages of carcinogenesis and progression.
several forms of macrophage activation distinct from M1, including cells exposed to interleukin 4, interleukin 13, or glucocorticoid or seco steroid hormones.53,54

M1 cells have a phenotype of interleukin 12 high, interleukin 23 high, interleukin 10 low. They are efficient producers of reactive oxygen and nitrogen intermediates and inflammatory cytokines (including interleukin 1, TNFα, interleukin 6). M1 cells participate as inducer and effector cells in polarised Th1 responses. Finally, they mediate resistance against intracellular parasites and tumours. M2 macrophages, on the other hand, share a phenotype of interleukin 12 low, interleukin 23 low, interleukin 10 high, with variable capacity to produce inflammatory cytokines depending on the signal used. M2 cells have high amounts of scavenger, mannose, and galactose-type receptors. Further, arginine metabolism is shifted towards ornithine and polyamines. Differential regulation of components of the interleukin 1 system55 takes place in polarised macrophages, with low interleukin 1αβ and low caspase 1, high interleukin 1 receptor antagonist, and high decoy type II receptor in M2 cells. M1 and the various forms of M2 cells have distinct chemokine and chemokine receptor repertoires.56 In general, M2 cells participate in polarised Th2 reactions, promote killing and encapsulation of parasites,57 are present in established tumours, promote their tumour progression, tissue repair, and remodelling,58 and have immunoregulatory functions.59 Inflammatory chemokines (ie, CCL2), CSF1, and vascular endothelial growth factor A (VEGFA) recruit tumour-associated macrophages and sustain their survival in cancers. Hypoxia-dependent upregulation of chemokine (C-X-C motif) receptor 4 (CXCR4) probably induces accumulation of these macrophages in hypoxic areas of tumours50 and promotes angiogenesis.59

In the tumour microenvironment (figure 2), macrophages are skewed to an M2 phenotype.60 The immature myeloid cells with immunosuppressive functions present in lymphoid organs and neoplastic tissues share properties and their gene-expression profile with M2 polarised tumour-associated macrophages.61,62 The relation between M1 and M2 cells and tumour-infiltrating dendritic cells remains to be established. Immature and activated myeloid dendritic cells could correspond to M2 and M1 cells. In this context, it is noteworthy that immature monocyte-derived dendritic cells and macrophages show great plasticity, with each cell type having the capability to convert into the other until late in the differentiation and maturation process.63 Accordingly, the cytokine environment and presence of differentiation or other stimulatory signals could be the so-called final decision-making factors determining whether these cells will acquire dendritic-cell or macrophage characteristics and functions, as exemplified by interleukin-6-mediated skewing towards macrophage64 and TNFα-mediated skewing towards dendritic cells.65

We do not yet know which signals lead to M2 polarisation of tumour-associated macrophages, but interleukin 10, transforming growth factor β, and CSF1 might play a part. In a human papillomavirus-driven carcinogenesis model, genetic elimination of T and B lymphocytes blocks recruitment of innate immune cells, tissue remodelling, and angiogenesis, with arrest of carcinogenesis at the stage of epithelial hyperplasia.66 B cells, which do not infiltrate lesions, act as remote orchestrators of innate immune cells in situ. Circumstantial evidence suggests that this remote control mechanism of cancer-promoting inflammation operates via deposition of immunoglobulins in the extracellular matrix. Immune complexes participate in induction of M2 macrophage activation.67

The smouldering inflammation68 driven by M2-skewed tumour-associated macrophages promotes tumour proliferation and progression,69 stromal deposition and remodelling, stimulates angiogenesis60,70 and lymphangiogenesis, and disorients or inhibits adaptive immunity.71 Accordingly, mice deficient in Src homology 2-containing-inositol 5-phosphate, which show spontaneous drift towards M2 polarisation, have increased growth of transplanted tumours.72 Findings of studies addressing genetic links between inflammation and cancer support a central role for NFκB in inflammation-induced liver and colon cancer.73,74 Selective inactivation of IKKβ (inhibitor of kappa light polypeptide gene enhancer in B cells, kinase beta) in inflammatory cells was protective against colitis-driven tumour development.75 Ras-induced interleukin 8 was reported to drive tumour growth and
Rearrangements of the RET receptor tyrosine kinase specific to papillary thyroid carcinoma is a frequent, causative, and sufficient genetic event in the pathogenesis of human papillary carcinoma of the thyroid. This rearrangement activates a proinflammatory transcriptional programme, including production of CCL2 and colony-stimulating factor in primary human thyrocytes, which correlates with in-vivo metastatic potential in human beings. These results identify a direct link between a genetic event causing human cancer and the resulting tumour-promoting inflammatory microenvironment.

Interventions that target tumour-promoting inflammation are in their infancy. Therapeutic strategies for inhibiting angiogenesis can also interfere with phagocyte recruitment. For instance, angiogenesis inhibitors such as angiotatin-related kringle 5 act, at least in part, by affecting tumour-associated macrophage-based tumour promotion. Strategies aimed at interfering with macrophage recruitment, survival, and polarisation yielded encouraging results, suggesting that identification of the cellular and molecular mechanism linking inflammation and cancer, with tumour-associated macrophages as key orchestrators, offers promise for the development of novel therapeutic strategies.

Tumour-disruptive inflammatory responses

Whereas tumour-associated macrophages promote tumour growth through innate immune mechanisms, dendritic cells—the so-called professional antigen-presenting cells that modulate adaptive immunity by controlling activation of T cells, B cells, natural killer cells, and natural killer T cells—can induce M1 activation of macrophages leading to tumour-disruptive inflammation. Dendritic cells reside in all tissues, where they are poised to capture, process, and—after migration into the secondary lymphoid tissues—present antigens to lymphocytes. They are composed of subsets with distinct functions. Blood contains CD14+CD11c+ monocytes, which are precursors of myeloid dendritic cells, and LINnegCD11c− interleukin 3 receptor α+ plasmacytoid dendritic cells, which are natural, type 1, interferon-producing cells. Immature (non-activated) dendritic cells induce tolerance by presenting self-antigens to T cells, whereas mature antigen-loaded cells launch antigen-specific immunity by promoting T-cell proliferation and differentiation into helper and effector cells. Maturation of dendritic cells includes coordinated events such as translocation of antigen-presenting and costimulatory molecules to the cell surface. Several members of the tumour necrosis receptor family (TNFSF9 and TNFRSF4), Notch ligands such as Delta and Jagged, and secreted cytokines including interleukin 12 and interleukin 23 have a role in activation of dendritic cells. Tolerance versus priming might be determined by the threshold of activation of signalling events through either CD80/CD86 and cytotoxic T lymphocyte antigen 4, CD74 and programmed cell death (PDCD1), or immunoglobulin-like transcript 3 and 4. Although mature dendritic cells generally induce Th1 immune responses and are regarded as anticancer effectors, they can also expand regulatory and suppressor T cells, therefore participating in the induction of central and peripheral tolerance.

Observations from many clinical studies have noted infiltration of tumours with dendritic cells. Immature dendritic cells are present in more than 90% of breast cancers, whereas mature cells—recorded in 60% of samples—are confined to peritumoural areas. In some cases, T cells cluster around mature dendritic cells, resembling the dendritic cell and T-cell clusters of secondary lymphoid organs characteristic of ongoing immune reactions. Presence of mature dendritic cells outside lymphoid organs is linked with inflammation and can be recorded in aseptic synovial inflammation in rheumatoid arthritis or in the blood of patients with systemic autoimmunity. Whether the presence of mature dendritic cells in tumours is beneficial remains to be established, bearing in mind the postulated role of chronic inflammation in cancer promotion.

Cancers could subvert dendritic cell function and escape immune effectors by polarising cell maturation towards a phenotype that prevents specific immunity, induces tolerance, and triggers suppressive pathways (figure 3). Activation of signal transducer and activator of transcription 3 (STAT3) in myeloid cells results in increased production of vascular endothelial growth factor (VEGF), which interferes with dendritic cell maturation. Several cytokines besides VEGF can suppress dendritic cells in the tumour microenvironment.

Tumour-derived interleukin 10 in melanoma yields tolerogenic dendritic cells that induce tumour-specific anergy. Interleukin 6 secreted by breast cancer cells skews monoocyte differentiation into tumour-associated macrophages at the expense of dendritic cells. Arachidonic acid metabolites, including prosta-104 glandins and thromboxanes synthesised by cyclo-oxygenases 1 and 2, might contribute to malignant progression not only through enhanced angiogenesis but also through direct inhibitory effects on dendritic cells. Antigen capture and presenting pathways can be targeted by cancers to prevent or skew development of T-cell immunity. Tumour glycoproteins, such as carcino-embryonic antigen and mucin 1 (MUC1), interact with C-type lectins on the surface of the dendritic cell. MUC1 glycoproteins, secreted by breast cancer cells, are endocytosed by dendritic cells, in which they are mostly retained in early endosomes leading to inefficient processing and presentation to T cells. In this dynamic interaction, dendritic cells can fight back. Under certain circumstances, different subsets express cytoktoxic molecules, including granzyme by plasmacytoid dendritic cells and surface expression of TNFSF10 by type 1 interferon-stimulated myeloid or plasmacytoid dendritic cells, both enabling tumour-cell
in patients with cancer, providing proof of principle of T cells have been used as vaccines to enhance immunity and expressed them on their surface for presentation to that have taken up tumour antigens provided in culture to fight against malignant diseases. These cells could extending the armamentarium of dendritic cells in their related to the tissue origin of dendritic cells.

mice show that induction of tissue-specific immunity is lymphoid organs. Furthermore, findings of studies in are expressed in lymphatic vessels and secondary appropriate ligands such as CCL19 and CCL21, which the capacity of the dendritic cell to respond to expression of CCR7 by dendritic cells, hence augmenting signals—eg, PGE2 could induce preferential killing. Murine interferon-producing killer dendritic cells with cytotoxic activity have been identified, thus extending the armamentarium of dendritic cells in their fight against malignant diseases. These cells could prevent tumour outgrowth on adoptive transfer, using TNFSF10 for cytotoxic activity. Another subset of mouse interferon-producing killer dendritic cells can destroy cancer cells with natural killer-activating receptors. The existence and function of these dendritic cell types in human tumours remains to be established.

Cancer vaccines aim to induce both effector and memory T cells that are specific for tumour antigen; these T-cell types can reduce the malignant mass and control cancer relapse, respectively. Ex-vivo-generated dendritic cells that have taken up tumour antigens provided in culture and expressed them on their surface for presentation to T cells have been used as vaccines to enhance immunity in patients with cancer, providing proof of principle of their effectiveness. Several groups have now used dendritic cells that are either conditioned with interleukin 4, obtained from blood, or derived from CD34+ haemopoietic progenitor cells. Loading human leucocyte antigen (HLA) class I and class II molecules with peptides derived from defined tumour antigen is the most frequently used strategy for dendritic-cell-based vaccination, although some techniques also include recombinant proteins, exosomes, viral vectors, plasmid DNA, RNA transfection, immune complexes, and antibodies specific for surface molecules on dendritic cells, sometimes exploiting the capacity of these cells to cross-prime—ie, to present exogenous antigens on HLA class I.

Findings of migration studies showed that only a small proportion (<1%) of labelled dendritic cells localise in regional lymphatic vessels after intradermal injection, and this process can be enhanced experimentally by conditioning the injection site with TNF-α. Furthermore, distinct maturation and activation signals—eg, PGE2—could induce preferential expression of CCR7 by dendritic cells, hence augmenting the capacity of the dendritic cell to respond to appropriate ligands such as CCL19 and CCL21, which are expressed in lymphatic vessels and secondary lymphoid organs. Furthermore, findings of studies in mice show that induction of tissue-specific immunity is related to the tissue origin of dendritic cells.

Functional characterisation of adaptive immune responses in the tumour microenvironment and draining lymph nodes

Identification of tumour antigens recognised by CD8+ cytotoxic T lymphocytes and CD4+ helper T cells has enabled development of specific assays such as fluorescent tetrameric HLA–epitope complexes to monitor T cells specific for tumour antigen in complex populations. Such reagents are prepared by assembly of recombinant soluble HLA class I molecules with peptides mimicking tumour antigen. In combination with fluorescent antibodies directed against cell-surface molecules, tetrameric HLA–epitope complexes allow enumeration of cytotoxic T lymphocytes specific for tumour antigen, phenotyping of these cells, and their isolation by fluorescence activated cell sorting.

Tetrameric HLA–epitope complexes have been used to characterise acquired T-cell responses against tumours, in particular in malignant melanoma. In general, T cells specific for tumour antigen are not detectable ex vivo in peripheral-blood lymphocytes because of their low frequency. As an exception, the proportion of HLA-A2-restricted CD8 T cells recognising the melanoma antigen MLANA is large enough to allow their detection ex vivo via direct labelling of peripheral-blood lymphocytes by tetrameric HLA–epitope complexes. The antigenic determinant is a decapetide called MLANA presented to cytotoxic T lymphocytes in association with HLA-A*0201. Labelling peripheral-blood lymphocytes from healthy individuals identified MLANA-specific cytotoxic T lymphocytes in 80% of people, occurring at frequencies of one in 1400. Although such frequencies are characteristic of memory T cells, MLANA-specific CD8+ T cells have a naive phenotype with long telomeres indicative of limited cell division throughout their lifetime. These cells proliferate vigorously in vitro by stimulation with MLANA in the presence of interleukin 2, and undergo differentiation to cytolytic effectors able to kill MLANA-expressing tumour cells. Data from clonal analysis have indicated that these cytotoxic T lymphocytes

Figure 3: Role of dendritic cells in the tumour microenvironment. Cancer cells attract immature dendritic cells possibly through chemokines such as CCL20 or CXC12. Dendritic cells can then be either blocked or skewed in their maturation—eg, by vascular endothelial growth factor—leading to induction of polarised CD4+ T cells that promote expansion of cancer cells (procancer) at the expense of CD8+ T cells that can cause tumour regression (anticancer).
The fate of T cells specific for tumour antigen is unknown. A high rate of spontaneous apoptotic cell death has been reported. Work in melanoma metastases suggests that T cells specific for tumour antigen can survive and proliferate vigorously when cultured in vitro with low quantities of interleukin 2. Moreover, after a short period in culture, perforin and granzyme content increases, leading to full recovery of effector functions. Interleukin 2 can also reverse T-cell anergy in human melanoma cells, leading to full recovery of effector functions. In vitro, perforin and granzyme content increases, leading to full recovery of effector functions. Interleukin 2 can also reverse T-cell anergy in human melanoma cells, leading to full recovery of effector functions. In vitro, perforin and granzyme content increases, leading to full recovery of effector functions.

Assessment of tetrameric HLA–epitope complexes of T cells specific for tumour antigen in fixed malignant samples is impossible, because formalin fixation alters the structure of the T-cell receptor to which these complexes bind. Since no informative surveys of T-cell responses in situ are possible, in-situ monitoring has been restricted to analysis of tumour-infiltrating lymphocytes in freshly prepared single-cell suspensions from cancer fragments. As in peripheral-blood lymphocytes, most specificities thus far analysed are lower than the detection limit of tetrameric HLA–epitope complexes, with the exception of MLANA-specific tumour-infiltrating lymphocytes. Two-thirds of 20 melanoma metastases expressing HLA-A*0201 contained detectable amounts of MLANA-specific tumour-infiltrating lymphocytes, with values ranging from 0.1% to 16% of all cytotoxic T lymphocytes. Other work has confirmed the high prevalence of MLANA-specific tumour-infiltrating lymphocytes or other T cells specific for tumour antigen in draining lymph nodes. Although these frequencies seem high, their numerical value is dwarfed compared with the global cellular composition of a tumour mass. Cancer cells represent 50–90% of cell suspensions derived from melanoma metastases, whereas T cells comprise about 10%, of which a tenth are cytotoxic T lymphocytes. Thus, HLA-A*0201/MLANA-specific cytotoxic T lymphocytes represent 0.001–0.16% of a tumour's cell population and are greatly outnumbered by malignant and other normal cells.

There are striking differences in the functional competence of MLANA-specific cytotoxic T lymphocytes in peripheral-blood and tumour-infiltrating lymphocytes. Circulating MLANA-specific T cells express granzyme A and B and high amounts of perforin and they release interferon γ on exposure to antigen. MLANA-specific tumour-infiltrating lymphocytes express low concentrations of perforin and low or undetectable concentrations of granzyme A and B and fail to produce interferon γ in response to stimulation. Interferon γ hyporesponsiveness was antigen specific, because lymphocytes could still produce normal amounts of interferon γ if T-cell receptor signalling was bypassed (figure 4). Findings of other studies confirmed selective tumour-antigen unresponsiveness in tumour-infiltrating lymphocytes from various human lesions. Thus, T cells specific for tumour antigen are rendered functionally tolerant once they are present in the tumour microenvironment.

Several studies over the years, however, have yielded contradictory results about the functional status of cytotoxic T lymphocytes specific for tumour antigen in cancer patients. In one report, for example, lymphocytes recognising tyrosinase or cytokeratin 18 were noted to be deeply anergic, whereas in two other studies, fully functional cytotoxic T lymphocytes showing several characteristics of effector T cells were seen. Expansion of T cells specific for tumour antigen from peripheral-blood lymphocytes of cancer patients by in-vitro sensitisation is sufficient to raise their frequency to detectable amounts, but the process alters the functional characteristics of the cells, limiting its usefulness as a technique to study cell properties in vivo.

Assessment of tetrameric HLA–epitope complexes of T cells specific for tumour antigen in fixed malignant samples is impossible, because formalin fixation alters the structure of the T-cell receptor to which these complexes bind. Since no informative surveys of T-cell responses in situ are possible, in-situ monitoring has been restricted to analysis of tumour-infiltrating lymphocytes in freshly prepared single-cell suspensions from cancer fragments. As in peripheral-blood lymphocytes, most specificities thus far analysed are lower than the detection limit of tetrameric HLA–epitope complexes, with the exception of MLANA-specific tumour-infiltrating lymphocytes. Two-thirds of 20 melanoma metastases expressing HLA-A*0201 contained detectable amounts of MLANA-specific tumour-infiltrating lymphocytes, with values ranging from 0.1% to 16% of all cytotoxic T lymphocytes. Other work has confirmed the high prevalence of MLANA-specific tumour-infiltrating lymphocytes or other T cells specific for tumour antigen in draining lymph nodes. Although these frequencies seem high, their numerical value is dwarfed compared with the global cellular composition of a tumour mass. Cancer cells represent 50–90% of cell suspensions derived from melanoma metastases, whereas T cells comprise about 10%, of which a tenth are cytotoxic T lymphocytes. Thus, HLA-A*0201/MLANA-specific cytotoxic T lymphocytes represent 0.001–0.16% of a tumour's cell population and are greatly outnumbered by malignant and other normal cells.

There are striking differences in the functional competence of MLANA-specific cytotoxic T lymphocytes in peripheral-blood and tumour-infiltrating lymphocytes. Circulating MLANA-specific T cells express granzyme A and B and high amounts of perforin and they release interferon γ on exposure to antigen. MLANA-specific tumour-infiltrating lymphocytes express low concentrations of perforin and low or undetectable concentrations of granzyme A and B and fail to produce interferon γ in response to stimulation. Interferon γ hyporesponsiveness was antigen specific, because lymphocytes could still produce normal amounts of interferon γ if T-cell receptor signalling was bypassed (figure 4). Findings of other studies confirmed selective tumour-antigen unresponsiveness in tumour-infiltrating lymphocytes from various human lesions. Thus, T cells specific for tumour antigen are rendered functionally tolerant once they are present in the tumour microenvironment.

Several studies over the years, however, have yielded contradictory results about the functional status of cytotoxic T lymphocytes specific for tumour antigen in cancer patients. In one report, for example, lymphocytes recognising tyrosinase or cytokeratin 18 were noted to be deeply anergic, whereas in two other studies, fully functional cytotoxic T lymphocytes showing several characteristics of effector T cells were seen. Expansion of T cells specific for tumour antigen from peripheral-blood lymphocytes of cancer patients by in-vitro sensitisation is sufficient to raise their frequency to detectable amounts, but the process alters the functional characteristics of the cells, limiting its usefulness as a technique to study cell properties in vivo.

Assessment of tetrameric HLA–epitope complexes of T cells specific for tumour antigen in fixed malignant samples is impossible, because formalin fixation alters the structure of the T-cell receptor to which these complexes bind. Since no informative surveys of T-cell responses in situ are possible, in-situ monitoring has been restricted to analysis of tumour-infiltrating lymphocytes in freshly prepared single-cell suspensions from cancer fragments. As in peripheral-blood lymphocytes, most specificities thus far analysed are lower than the detection limit of tetrameric HLA–epitope complexes, with the exception of MLANA-specific tumour-infiltrating lymphocytes. Two-thirds of 20 melanoma metastases expressing HLA-A*0201 contained detectable amounts of MLANA-specific tumour-infiltrating lymphocytes, with values ranging from 0.1% to 16% of all cytotoxic T lymphocytes. Other work has confirmed the high prevalence of MLANA-specific tumour-infiltrating lymphocytes or other T cells specific for tumour antigen in draining lymph nodes. Although these frequencies seem high, their numerical value is dwarfed compared with the global cellular composition of a tumour mass. Cancer cells represent 50–90% of cell suspensions derived from melanoma metastases, whereas T cells comprise about 10%, of which a tenth are cytotoxic T lymphocytes. Thus, HLA-A*0201/MLANA-specific cytotoxic T lymphocytes represent 0.001–0.16% of a tumour's cell population and are greatly outnumbered by malignant and other normal cells.

There are striking differences in the functional competence of MLANA-specific cytotoxic T lymphocytes in peripheral-blood and tumour-infiltrating lymphocytes. Circulating MLANA-specific T cells express granzyme A and B and high amounts of perforin and they release interferon γ on exposure to antigen. MLANA-specific tumour-infiltrating lymphocytes express low concentrations of perforin and low or undetectable concentrations of granzyme A and B and fail to produce interferon γ in response to stimulation. Interferon γ hyporesponsiveness was antigen specific, because lymphocytes could still produce normal amounts of interferon γ if T-cell receptor signalling was bypassed (figure 4). Findings of other studies confirmed selective tumour-antigen unresponsiveness in tumour-infiltrating lymphocytes from various human lesions. Thus, T cells specific for tumour antigen are rendered functionally tolerant once they are present in the tumour microenvironment.
Adoptive transfer of rapidly expanded tumour-infiltrating lymphocytes from metastatic melanoma can mediate regression of large tumours if several conditions are met: first, that tumour-infiltrating lymphocytes that are highly reactive for tumour antigen are selected; second, that lymphopenia is induced by cyclophosphamide and fludarabine; and, third, that high doses of interleukin 2 are given. Whether all three conditions are indispensable for tumour rejection remains unclear; however, the rate of objective clinical responses with this strategy was 51% in 35 treated patients.

Why are tumour-infiltrating lymphocytes hypo-functional? Chronic antigen exposure in a poorly stimulatory context can lead to clonal exhaustion. The tumour/cytotoxic T lymphocyte ratio is equal to or higher than 10:1. Data from experimental mouse models of chronic viral infection also support this hypothesis and suggest that reduction in tumour load might restore T-cell functional competence. Researchers have noted that uncoupling of the PDCD1 pathway inducer of T-cell apoptosis reverses T-cell unresponsiveness in a model of chronic murine viral infection, suggesting a novel strategy of immune intervention because many lesions constitutively express CD274, the ligand for PDCD1.

Other tolerance-inducing mechanisms include production of interleukin 10, VEGF, or transforming growth factor β by cancer cells. Tumour cells or tumour-associated macrophages can express the enzyme indoleamine oxidase, which depletes the aminoacid tryptophan—essential for T-cell fitness. As previously discussed, tumour-associated macrophages dampen T-cell function through production of free radicals due to T regulatory cells. Various therapeutic interventions—including depletion of T-regulatory cells before vaccination, combination of vaccination and blockade of the CD274–PDCD1 interaction, and sustained administration of cyclooxygenase 2 inhibitors—are presently being tested in preclinical models or phase I clinical trials.

The immunological constant of rejection

What turns an indolent immune response into a potent inflammatory reaction capable of decisively clearing the pathogenic process during immunotherapy? The answer could lie in immune processes characterised by a similar biphasic pattern. Symptomatic acute hepatitis B and C virus infections result in viral clearance, whereas asymptomatic infections lead to chronic unresolving outcomes. Allograft rejection can follow an indolent course for years until sudden events trigger acute rejection. Autoimmune diseases follow a waxing and waning course whose basis remains unknown. Could these patterns be accounted for by a common immunological constant, which is a shared combination of factors necessary to convert chronic inflammatory processes into acute inflammatory processes, whether they be involved in infection, graft rejection, autoimmune disease, or tumour-related immune responses?

The so-called danger model of immunity predicts that antigen exposure in the absence of costimulation is not sufficient to activate and sustain effective immune responses. In this model, danger signals trigger innate immune responses leading to recruitment, activation, and sustained survival of immune cells in the affected organ. Clearly, most cancers do not have the requisite danger signals. Thus, the need for T-cell activation in the target malignant tissue remains the limiting step needed for cancer immune rejection. The need for T-cell activation is well exemplified by efforts to induce immune responses specific to tumour antigen by anticancer vaccines; although this work has been quite successful in inducing cytotoxic T lymphocytes specific for tumour antigen, the expected anticancer effects have been disappointing. This outcome is partly attributable to the fact that circulating vaccination-induced cytotoxic T lymphocytes do not have effector functions in direct ex-vivo assays. These quiescent lymphocytes can readily recover full effector function when exposed in vitro to tumour antigen and interleukin 2 in combination, whereas neither substance is sufficient alone. This process is a physiological behaviour of cytotoxic T-lymphocyte responses, as shown by data from experimental animal models; antigen exposure is followed by a short period during which effector T-cell responses are active, ensued by a contraction phase in which T cells progressively lose effector function. Teleologically, to prevent collateral damage, immune cells exposed to antigen need to dampen their effector function when they leave the immunogenic site so that only immunological memory is preserved in case of future encounters. Similarly, after vaccination, effector T-cell responses can last for a limited time at the site of vaccination or in the draining lymph nodes. However, most vaccination-induced cytotoxic T lymphocytes that reach the systemic circulation and subsequently the tumour might already have lost their effector function. This way, reactivation of these cells is needed at the tumour site by combined exposure to antigen and costimulatory signals. However, contrary to acute viral infections, in which cytotoxic T lymphocytes are reactivated selectively in the affected organ, because
antigenic stimulation and acute inflammation caused by the pathogenic process arise simultaneously, tumours might not provide sufficient costimulation.161,164 These clinical or experimental observations provide a physiological explanation of why T cells specific for tumour antigen—whether naturally present or induced by immunisation—coexist with cancer cells in the tumour-bearing host, which most frequently does not provide acute inflammatory signals.

What most likely comprises the immunological constant of rejection? Evidence is mounting that immune-mediated tissue destruction encompasses at least two separate factors: activation of interferon-stimulated genes; and immune-effector mechanisms of the innate and adaptive immune response. Global transcript analysis has provided a broad view of biological processes associated with immune-mediated tissue destruction and identified convergent characteristics. Neoplastic inflammation shares similarities with the inflammation seen in chronic hepatitis C virus infection,10 and it is characterised by expression of interferon-stimulated genes. Similar signatures can be identified in liver biopsy samples from patients with chronic hepatitis C virus infection16 and in chronic allograft rejection controlled with standard immune suppression.16 Moreover, interferon-stimulated genes are expressed consistently in melanoma metastases after systemic administration of interleukin 2, independent of clinical outcome.14 Thus, these genes are seemingly part of immunological processes associated with lingering unresolving inflammation, which could be necessary but not sufficient to induced tissue rejection. Genes associated with cytotoxic effector function are expressed rarely in chronically inflamed tissues but arise consistently when the inflammatory process causes destruction of tumour,17 allograft,18 liver in hepatitis-C-virus-induced cirrhosis,19 or gut during flares of Crohn’s disease.19

We have previously described overexpression of genes associated with cytotoxic function of T cells and natural killer cells in a melanoma metastasis that regressed in response to interleukin 2 treatment.19 Similar signatures were noted in renal tissues from which biopsy samples were taken during acute allograft rejection.19 Furthermore, of these genes, IL32 was reported as a central mediator of Crohn’s disease20 and liver damage during hepatitis C virus infection.21 This gene encodes an interleukin 2 and interleukin 18 inducible inflammatory factor that amplifies cytokine production.22 IL32 is constitutively expressed by natural killer cells but only by activated cytotoxic T lymphocytes.23 Moreover, we have noted that IL32 and other genes associated with cytotoxic function were expressed preferentially in metastatic melanoma compared with other less immune-responsive cancers.24 These surveys lead to the conclusion that immune-mediated tissue destruction encompasses at least two components: a baseline cluster of interferon-stimulated genes that seem necessary but insufficient to induce tissue rejection; and a less common signature including cytotoxic effector functions more tightly associated with rejection.

Key to successful cancer treatment is specificity. Antibodies specific for tumour antigen and T cells elicited by immunisation provide unprecedented anticancer specificity.17,180 However, effectiveness of monoclonal antibodies is limited by their pharmacodynamics, and T-cell function is dependent on environmental stimuli. Thus, vaccines specific to tumour antigen have been very successful in induction of T cells181 but tumour antigen exposure is not sufficient in itself to activate cytotoxic T lymphocytes in the target organ, and secondary stimulations are necessary to activate properly their effector functions when and where it counts.182 Because cancers do not provide this costimulation, T cells specific for tumour antigen remain quiescent on their encounter with tumour cells.183 Indirect evidence suggests that vaccine-induced T cells reach the lesion site184 and recognise tumour cells producing interferon γ. However, this mechanism is not sufficient for tumour rejection because other effector processes are not activated at the same time.172 Thus, immunisation successfully affects the afferent loop of the immune response by eliciting T cells specific for tumour antigen but cannot have an effect on T-cell activation at the target site.169,170 The challenge then is to deliver the danger signals necessary for T-cell activation to the tumour site. Dermatologists use Toll-like receptor 7 (TLR7) agonists to treat skin carcinomas, which destroy cancer cells selectively through an immune-mediated mechanism.175–177 TLR agonists mimic single-stranded RNA of viruses178 and activate plasmacytoid dendritic cells to secrete type I interferons and induce T-cell and natural-killer-cell activation, which in turn secretes interferon γ. Simultaneously, effector mechanisms in activated T cells and natural killer cells induce destruction of target cells, feeding tumour antigen to dendritic cells for presentation to upcoming T cells and B cells attracted by the inflammatory process (figure 5).175,177 Interferon-producing killer dendritic cells include all these tasks within a cellular unit.86 Transcriptional signatures characteristic of these cells are enriched by expression of genes associated with effector function recorded during acute tissue rejection. This finding suggests that interferon-producing killer dendritic cells could have a central role in immune rejection complementing that of plasmacytoid dendritic cells.

The cancer specificity of TLR agonists is based on preferential attraction of plasmacytoid dendritic cells to chronically inflamed tissues and their enhanced recruitment.176 Thus, the ability of TLR agonists to provoke acute inflammation capable of overcoming anti-inflammatory regulatory mechanisms shows how it could be possible to make T cells induced by vaccination active at the tumour site. We have noted transcriptional activation induced by a TLR7 agonist in a placebo-controlled, double-
blind, randomised trial. These findings confirmed that regression of basal-cell carcinoma mediated by TLR agonists demonstrated a pattern consistent with the immunological-constant-of-rejection model. That pattern includes not only expression of interferon-stimulated genes but also a consistent second signature of immune-effector functions, which recapitulated those seen in allograft rejection, autoimmunity, and acute antiviral responses.

Concluding remarks

Inflammation is an integral part of cancer biology, whether it fosters or hampers tumour growth. Immunisation, administration of cytokines, or treatment with antibodies or T cells specific for tumour antigen can clear large malignant burdens. The findings discussed in this Review indicate that, through activation of the host immune response, elimination of a cancer is possible. Observations lend support to the view that an immunological constant is needed for immune rejection. The eff erent arm of the immune system requires activation by appropriate signals at the tumour site to undertake its functions. Such triggers are exemplified by danger signs produced by pathogens during acute infectious processes. Cancers do not have such signals and, therefore, do not produce the combination of factors needed to initiate, sustain, and complete rejection. This deficiency results in a chronic lingering immune response that perpetuates and can even promote the neoplastic process through tissue remodelling mechanisms associated with chronic inflammation. However, eradication of cancer might be possible by shifting the balance towards an acute inflammatory response by cytokine administration, reduction of regulatory mechanisms through lymphodepletion, or the selective activation of plasmacytoid dendritic cells through TLR signalling.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

AM is supported by the Italian Association for Cancer Research, CARIPLO NOBEL, European Commission, Italian Ministry of Health, and Italian Ministry of University and Research. PR is supported in part by the National Center for Competence in Research (NCCR), Molecular Oncology, Epalinges, Switzerland. AKP is supported by grants from Baylor Health Care Systems Foundation and the National Institutes of Health (PO1 CA84512, U19 AI097234, CA78846, CA085540, and CA89440). The funding sources had no role in preparation or writing of this Review.
References


Review


