Abstract
Transforming growth factor-β (TGF-β) is a key player in malignant disease through its actions on host tissues and cells. Malignant cells often secrete large amounts of TGF-β that act on nontransformed cells present in the tumor mass as well as distal cells in the host to suppress antitumor immune responses creating an environment of immune tolerance, augmenting angiogenesis, invasion and metastasis, and increasing tumor extracellular matrix deposition. Cells of the innate immune system contribute to the high concentrations of TGF-β found in tumor masses. In addition, dendritic cell subpopulations secreting TGF-β contribute to the generation of regulatory T cells that actively inhibit the activity of other T cells. Elevated levels of plasma TGF-β are associated with advanced stage disease and may separate patients into prognostically high-risk populations. Anti-TGF-β therapy could reverse the immunosuppressive effects of this cytokine on the host as well as decrease extracellular matrix formation, decrease angiogenesis, decrease osteolytic activity, and increase the sensitivity of the malignant cells to cytotoxic therapies and immunotherapies. Phase I clinical trials of an inhibitor of TGF-β receptor type I kinase activity and a TGF-β neutralizing antibody are under way.

Background
Despite all that we have learned about genetic alterations that occur in malignant cells, the development of highly effective anticancer therapeutics remains a stunningly difficult task because malignant cells are so similar to normal cells. This statement is true and yet untrue. The untruth comes from our knowledge that the immune system can recognize and eliminate cells that are slightly abnormal through immune surveillance. The truth comes from our knowledge that frankly malignant cells suppress the immune system and survive and thrive by coopting varied nearby and distal host cells to support the malignant disease (1–4). An effective immune system recognizes and destroys a wide diversity of pathogens, recognizes and destroys altered or damaged self, including apoptotic and necrotic cells, exhausted cells and malignant cells, and is tolerant to self. The theory of immune surveillance is that the immune system continuously recognizes and eliminates tumor cells. When a tumor escapes immune surveillance, it is frankly malignant and can grow to become lethal to the host.

Transforming growth factor-β (TGF-β) is a key player in malignant disease through its actions on host tissues and cells. Aberrant expression of TGF-β results in profound changes in the genetic stability of cells leading to alteration of both the differentiation state of the cells, altered interaction of the cells with the host environment, and the generation of therapy-resistant disease. Malignant cell resistance to TGF-β is frequently due to loss, silencing, or mutational inactivation of genes in the TGF-β signaling pathway including the type I and type II receptors and receptor-associated and common-mediator Smads (5, 6).

In cells with intact canonical TGF-β signaling, the binding of a TGF-β (isoform-1, -2, or -3) to the TGF-β type II receptor enables the formation of a heteromeric complex between TGF-β type I and type II receptors. The type I receptor is phosphorylated by the type II receptor serine/threonine kinase. The activated type I receptor phosphorylates selected receptor-activated Smads that then complex with Smad4. The Smad complexes translocate into the nucleus. Activated Smad complexes regulate the transcription of target genes through direct or indirect interaction with DNA-binding transcription factors or coactivators. Activation of receptor-activated Smads can be inhibited by Smad6 or Smad7 (5, 6).

TGF-β knockout mice suffer from a lethal multifocal inflammatory disease that shows the importance of TGF-β in maintaining immune system homeostasis (7–9). The blockade of TGF-β signaling in T cells by transfection with a dominant-negative type II receptor or in bone marrow by conditional knockout of the TGF-β type II receptor, results in similar multifocal inflammatory responses (10, 11). On the other hand, transgenic expression of a dominant-negative type II receptor specifically in T cells of C57BL/6 mice prevented the growth of the syngeneic tumors EL-4 thymoma (i.p.) and B16F10 melanoma (i.v.) in the animals (12).

Malignant cells often secrete large amounts of TGF-β that act on nontransformed cells present in the tumor mass as well as distal cells in the host to suppress antitumor immune responses creating an environment of immune tolerance, augmenting angiogenesis, invasion and metastasis, and increasing tumor extracellular matrix deposition. In addition, within the tumor...
and tumor vicinity, TGF-β may be released from the extracellular matrix or secreted by mesenchymal cells, resident leukocytes, or by monocytes and macrophages recruited to the tumor (13–16).

TGF-β is a potent suppressor of the immune system. With broad activity over natural killer (NK) cells, T cells, monocytes/macrophages, and dendritic cells, TGF-β can affect the initiation and stimulation of both primary and secondary immune responses as well as suppress antitumor effector cells (17–25). TGF-β in lung and colorectal cancer patient plasma samples directly suppressed NK cell activity, a defect that could be reversed with anti-TGF-β antibodies (24).

**Effects on the Immune System**

Cells of the innate immune system including neutrophils, NK cells, monocytes, dendritic cells, and monocyte-derived macrophages contribute to the high concentrations of TGF-β found in tumor masses (Table 1). Tumor-infiltrating macrophages may be particularly instrumental to malignancy because they adopt a trophic role that results in extracellular matrix breakdown, angiogenesis, and tumor cell motility, thus facilitating tumor growth and metastasis (26). Active TGF-β binds to the TGF-β receptors in tumor-infiltrating lymphocytes including CD4+, CD8+, or NK cells and alters their phenotype, proliferation, and cytokine secretion (ref. 27; Fig. 1). Tumor-infiltrating dendritic cells secrete TGF-β and respond to TGF-β and interleukin 10 with markedly down-regulated expression of the costimulatory molecules CD80, CD86, and CD40, and markedly decreased secretion of tumor necrosis factor-α, interleukin 12, and CCL5/RANTES (28). Dendritic cell subpopulations (CD4+CD8− dendritic cells) secreting TGF-β have been implicated in the generation of CD4+ regulatory T cells (29). Regulatory T cell differentiation can be driven by immature or “tolerogenic” dendritic cells (30). Regulatory T cells actively inhibit the activity of other T cells. There are two classes of regulatory T cells: natural Tregs differentiate in the thymus and induced or “adaptive” Tregs that arise in the periphery. The most common phenotypes for Tregs are CD4+CD25+Foxp3+ and CD8+CD25+Foxp3+. However, this is a very active area of investigation and other potential phenotypes involve CTLA4 and TGF-β. The action of natural (innate) Tregs may be different from the mechanism of action of induced (adaptive) Tregs with one involving cell contact and the other involving secretion of TGF-β or other cytokines. TGF-β is important in both the induction of the regulatory phenotype in peripheral Tregs and in the effector function of Tregs. The presence of Tregs in a tumor produces an environment of immune privilege (15). By direct cell-to-cell contact, Tregs deliver inhibitory signals to CD4+, CD8+, and NK cells to produce host tolerance to tumors (31). Tregs migrate to and are retained in tumor tissue and may account for >20% of tumor-infiltrating T cells; thus, providing immune privilege for the malignancy (32, 33). Treg and TGF-β alter the innate immune response through direct interaction with NK cells and Th17 cells (15, 34).

**Clinical-Translational Advances**

The majority of tumors from patients with advanced breast cancer as well as several other malignancies have been reported to be refractory to TGF-β–induced growth inhibition and many produce large amounts of this cytokine (refs. 38, 39; Table 2). In addition, elevated levels of plasma TGF-β have been associated with advanced stage disease and might separate patients into prognostically high-risk populations (40–42). It is believed that active TGF-β produced by the tumor and local stroma contributes to the progression and metastatic potential of this cancer through autocrine and paracrine effects (43). As with breast cancer, TGF-β plasma levels are elevated in patients with prostate cancer, and these levels correlate with advanced stage, metastases, and poorer clinical outcome (44–47). Increased TGF-β expression has been observed in both tumor cells and in tumor stroma (48–50). Similarly, TGF-β plasma levels are elevated in patients with pancreatic cancer and these levels correlate with advanced stage, metastases, and poorer clinical outcome (51).

TGF-β plasma levels are elevated in patients with renal cell cancer (52, 53). TGF-β neutralization can be an effective therapy in animal models of renal cell cancer. In some animal models, the benefits from TGF-β neutralization may be additive or synergistic when combined with chemotherapy (54, 55). In addition, renal cell carcinomas are sensitive to antitumor immunotherapy including immunomodulators such as interleukin 2, lymphocyte-activated killer cells, tumor-infiltrating lymphocyte therapy, and vaccine approaches. It remains to be determined if neutralization of TGF-β could enhance immunity and be effective in combination with immunotherapeutic approaches (56, 57).

Malignant melanoma has increased TGF-β expression in tumor cells, which is not observed in benign or in situ lesions (58). Higher expression of TGF-β is associated with metastatic lesions and deeper invasion (worse prognosis, Clark’s level 3 and higher) in this disease (58). In transgenic models in which T cells are rendered insensitive to TGF-β, animals are able to...
completely eradicate tumors such as B16 melanoma. Similarly, anti–TGF-β antibodies enhance tumor-specific immune responses, but have not been successful in completely eradicating tumors (12, 59, 60). Gorelik and Flavell suggested that this might be due to the incomplete neutralization of TGF-β (12).

Elevated serum levels of TGF-β have been observed in patients with myeloma and have correlated with higher levels of serum β2-microglobulin, which is an adverse prognostic marker of this disease (61). Sorted CD38+CD45RA- myeloma cells secrete significantly more TGF-β than peripheral blood mononuclear cells, splenic B cells, or CD40 ligand-activated B cells. In addition, TGF-β secretion by myeloma bone marrow stromal/mononuclear cells was significantly greater than by normal bone marrow mononuclear cells. Therefore, in patients with myeloma, the source of TGF-β is clearly from the malignant cells as well as bone marrow stromal cells (62).

TGF-β levels are elevated in non–Hodgkin’s lymphoma and are markedly elevated in high-grade lymphomas, cutaneous T cell lymphomas with a T-regulatory phenotype, and in splenic marginal zone lymphomas presenting as myelofibrosis (63–65). Non–Hodgkin’s lymphomas are sensitive to anti-tumor immunotherapy including antibody therapies and cell therapies, as well as vaccine approaches. The idiotype proteins on B cell non–Hodgkin’s lymphomas are tumor-specific antigens, which are effective targets for monoclonal antibodies as well as for vaccine approaches (66–70). Unmaintained remissions of >10 years have been seen in patients treated with specific anti-idiotype antibodies or idiotype vaccines (67, 68). The reversal of TGF-β–induced immunosuppression might improve both the induction of primary immune responses and the maintenance of effective cytotoxic T cell activity against these malignancies.

Malignant disease grips the host influencing the behavior of cells in the vicinity of the tumor and distal cells and tissues as well. TGF-β, a secreted protein, is a key player in the malignant process. Anti–TGF-β therapy could reverse the

Table 2. Human malignant diseases in which elevated levels of TGF-β have been implicated as contributing to poor outcome

<table>
<thead>
<tr>
<th>Patients/tumor type</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Breast cancer</td>
<td>(38–43)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>(44–50, 76)</td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>(52, 53)</td>
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<tr>
<td>Melanoma</td>
<td>(58)</td>
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<tr>
<td>Pancreatic cancer</td>
<td>(51)</td>
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<tr>
<td>Multiple myeloma</td>
<td>(61, 62)</td>
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<tr>
<td>Non–Hodgkin’s Lymphoma</td>
<td>(63–65)</td>
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<tr>
<td>Non–small cell lung cancer</td>
<td>(77)</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
<td>(78, 79)</td>
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<tr>
<td>Colorectal cancer</td>
<td>(80)</td>
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<tr>
<td>Ovarian cancer</td>
<td>(81)</td>
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<tr>
<td>Cervical cancer</td>
<td>(82)</td>
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<tr>
<td>Bladder cancer</td>
<td>(83, 84)</td>
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<tr>
<td>Kaposi sarcoma</td>
<td>(85)</td>
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<tr>
<td>Glioma</td>
<td>(86)</td>
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<tr>
<td>Head and neck cancer</td>
<td>(87)</td>
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<tr>
<td>Thyroid cancer</td>
<td>(88)</td>
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<tr>
<td>Esophageal cancer</td>
<td>(89)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>(90)</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>(91, 92)</td>
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immunosuppressive effects of this cytokine on the host as well as decrease extracellular matrix formation, decrease angiogenesis, decrease osteolytic activity, and increase the sensitivity of the malignant cells to cytotoxic therapies and immunotherapies (71–75). Phase I clinical trials are under way, a small molecule inhibitor of TGF-β receptor type I kinase activity and with a fully human monoclonal antibody that neutralizes the three isoforms of TGF-β.


Clinical Cancer Research

Transforming Growth Factor-β and the Immune Response to Malignant Disease

Beverly A. Teicher


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