

Regulatory T Lymphocytes: Pivotal Components of the Host Antitumor Response

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The role of host immunity in the development and progression of cancer has been a subject of speculation and considerable controversy for more than the last 50 years.¹ Tumor-infiltrating lymphocytes (TILs), the primary immune component infiltrating solid tumors, are considered to be a manifestation of the host antitumor reaction. The report by Gao et al² in this issue provides additional evidence for the prognostic significance of TILs in solid tumors in general and hepatocellular carcinoma (HCC) specifically.

Historically, tumor-associated lymphoid infiltrates were categorized descriptively as brisk, nonbrisk, or absent, based on morphologic observation alone.³ The survival advantage of a brisk lymphocytic infiltrate in malignant melanoma has been demonstrated by Clark et al,³ and confirmed by others.⁴ However, there is accumulating evidence that the specific type of immune cells, rather than their sheer quantity, governs the host-versus-tumor immune response. With development of immunohistochemical and flow cytometry techniques, it has been demonstrated that the majority of TILs in solid tumors are of the CD3⁺ T-cell phenotype. CD3⁺ T cells can be stratified further into CD4⁺ helper cells (including the Th1 and Th2 subtypes based on their cytokine profile), CD4⁺ regulatory T cells (Tregs), previously designated as suppressor cells, and CD8⁺ cytotoxic effector cells.

Initial studies that set out to characterize the antitumor immune response were directed toward CD8⁺ cytotoxic T lymphocytes (CTLs). CTLs destroy tumor cells via the triggering of apoptosis by means of cytotoxic granule exocytosis and/or the Fas/FasL receptor-mediated pathway.⁵ In the mid-1990s, antibodies that recognize cytotoxic molecules, including TIA-1, granzyme B, and perforin, became available for immunohistochemical studies. The number of CD8⁺ CTLs expressing TIA-1 and/or granzyme B cytotoxic granules infiltrating tumors has been shown to be associated with improved prognosis in certain human neoplasms, such as Epstein-Barr virus-associated gastric cancer and carcinomas of the colon, breast, uterus, and esophagus.⁶⁻¹⁰

The role of CD4⁺ T cells in the host antitumor response is an area of considerable debate. This subset may be considered as a double-edged immunologic sword, able to promote or inhibit tumor growth. CD4⁺ T-helper cells are subdivided into Th1 cells, which induce cellular immunity, and Th2 cells, which elicit a humoral immune response. The Th1 response is associated with CTL activation and is considered to be beneficial for antitumor

immunity.¹¹ In contrast, a predominant Th2-like cytokine profile is associated with a metastasis-inclined microenvironment in HCC and other tumors.^{12,13}

A subpopulation of CD4⁺ T cells, Tregs, which accounts for 5% to 10% of all CD4⁺ cells, is generating intense interest in tumor immunology, autoimmunity, and infectious disease.^{14,15} Historically, Tregs were first hypothesized as suppressive T cells in early 1971 by Gershon and Kondo,¹⁶ however, subsequent studies were hindered because of a lack of specific molecular markers and difficulties in their isolation and culture. The renaissance of these regulatory cells came a few years ago, when FOXP3, a member of the forkhead family of DNA transcription factors, was cloned.¹⁷ There is now considerable evidence that FOXP3 is a key control molecule for Treg development and function, and is an excellent marker for the study of Tregs. Recent studies have demonstrated that Tregs are beneficial for the prevention of autoimmune disease, such as type I diabetes, graft-versus-host disease, transplant rejection, and tissue destruction during infection.^{14,15} In contrast, Tregs seem to be a detrimental factor in the generation of host-versus-tumor immunity via suppression of tumor-specific effector T-cell responses and development of immune tolerance to neoplastic cells.

The classic Treg is a thymus-derived CD4⁺CD25⁺FOXP3⁺ T lymphocyte. In addition to these markers, Tregs express cell surface CTL-associated antigen 4 (CTLA-4), and the glucocorticoid-induced tumor necrosis factor alpha receptor and secrete immunosuppressive cytokines such as transforming growth factor beta and interleukin 10 (IL-10).¹⁸ Initially it was demonstrated that the number of CD4⁺CD25⁺ T-cells were increased in several solid tumors and in certain hematologic malignancies.¹⁴ However, subsequent studies provided evidence that CD25, which is an IL-2 α receptor subunit, is induced on activation in all T-cells, not just Tregs. Recently, there has been an sudden increase of immunohistochemical studies using FOXP3 as a more specific marker of Tregs. Increased numbers of FOXP3⁺ Tregs infiltrating tumor cell nests have been demonstrated in ovarian, lung, breast, pancreatic, hepatocellular, head and neck, and anal carcinomas, and lymphomas.¹⁹⁻²⁸ In most of the solid tumors studied, accumulation of FOXP3⁺ Tregs predicts a striking reduction of patient survival¹⁹⁻²⁴; however, paradoxically, increased FOXP3⁺ Tregs were found to be associated with improved prognosis in lymphoma patients.^{27,28}

In this issue, Gao et al² take a more comprehensive approach in determining the prognostic significance of FOXP3⁺ Tregs.² Gao et al demonstrate that the type (ratio of activated cytotoxic granzyme B-positive CD8⁺ T lymphocytes to FOXP3⁺ Tregs), density (high v low expression of cytotoxic or regulatory molecules), and location (lymphocytes within HCC tumor cell nests as opposed to the surrounding stroma) are all critical factors in the assessment of TILs and in the determination of their prognostic impact. In this study, the overall 5-year survival and disease-free survival rates were only 24% and 20% in the groups with high levels of intratumoral Tregs and low numbers of activated CTLs, as compared with 64% and 59% for the group with low numbers of intratumoral Tregs and high numbers of activated CTLs, respectively. This study is in agreement with a recent report by Sato et al,²⁰ which demonstrated that the ratio of CD8⁺/Treg cells is an independent prognostic factor for longer survival in ovarian cancer. In both of these studies, the impact of this balance on patient survival was larger than that of the number of intraepithelial CD8⁺ or Treg cells alone.

In lymphoid neoplasms, Tregs appear to play a different role. There is accumulating evidence that a high density of Tregs predicts improved survival of classical Hodgkin's lymphoma and follicular lymphoma patients.^{27,28} A plausible explanation for this paradox is that Tregs have numerous lymphoid targets, including CD8⁺ T cells, CD4⁺CD25⁺ T cells, B cells, natural-killer cells, and dendritic cells. This mechanism allows Tregs to control the immune response, and when misguided, may induce autoimmune disease. What if the target cells in certain hematologic malignancies are actually malignant cells recognized and destroyed by Tregs? It has been demonstrated that Tregs may suppress B-cell proliferation by inducing cytotoxic-dependent cell death.²⁹ Carreras et al²⁸ hypothesized that the favorable clinical impact of a high number of Tregs in follicular lymphoma may be due to a direct inhibitory effect on neoplastic B-cells.

The mechanisms governing the recruitment of tumor-associated Tregs, as well as those that trigger their activation, are under intense study. Increased frequencies of tumor-associated Treg cells may be related to the induction of chemokines produced by tumor-derived macrophages, such as CCL22.¹⁹ Activation of Tregs can be triggered specifically by recognition of tumor-specific antigens in association with the T-cell receptor. Nishikawa et al³⁰ found that the cancer testis antigen, NY-ESO-1, is one such tumor-specific antigen for which tumor-specific immunity is controlled by Tregs. Interestingly and relevant to the study by Gao et al, cancer testis-specific antigens are frequently expressed in HCC; MAGE-1 is expressed in up to 80% of patients, and NY-ESO-1 is expressed in 50% of patients.³¹

The possible mechanisms of Treg-induced immunosuppression have been addressed in numerous in vitro and in vivo studies; however, a definitive mode of action has not been discovered.³² Accumulating evidence suggests that the key mechanisms of Tregs suppression include *IL2* gene expression inhibition and thereby decreased proliferative activity of target cells; inhibition of *IL2* gene through induction of tryptophan catabolism in antigen presenting cells mediated by cell surface receptor CTLA-4; immunosuppressive cytokine secretion (transforming growth factor beta, IL-10); and that Tregs may kill their targets by means of cytotoxic granules, but independent of Fas/Fas.³²

There currently is great interest in determining the therapeutic implications of manipulating the Treg pathway as a means of cancer immunotherapy. Murine models have revealed that selective elimination of Tregs using antibodies against CD25 enhances antitumor T-cell responses and induces regression of experimental tumors.³³ However, nonselective depletion of Tregs may also result in significantly increased susceptibility to autoimmune diseases. Moreover, anti-CD25-depleting antibodies may reduce not only CD25⁺CD4⁺ Treg cells, but also CD25⁺-activated CD4⁺ helper and CD8⁺ effector cells. Therefore, current strategies directed toward selective depletion or inhibition of Treg cells include targeting local tumor versus peripheral blood; developing monoclonal antibodies to cell surface Treg molecules, including CD25, CTLA-4, and glucocorticoid-induced tumor necrosis factor alpha receptor; and combinations of Treg elimination with CTL expansion.¹⁴

The quantification of FOXP3⁺ Tregs and CTLs in solid tumors are straightforward immunohistochemical techniques that can be performed on formalin-fixed paraffin embedded tissues. Determining the ratio of these two cell populations may not only help to predict which patients are at highest risk of recurrence, thus facilitating patient selection for more aggressive treatment, but also serve to identify patients with tumor who may benefit by future immunotherapies targeting this pathway.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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