The Therapeutic Implications of Intratumoral Regulatory T Cells

Commentary on Wolf et al. p. 8326

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In this issue of Clinical Cancer Research, Wolf and colleagues present compelling evidence that underscores a key role for regulatory T cell–induced immune suppression in tumor pathogenesis (1). Although most cancer patients mount innate and adaptive antitumor reactions, the evolution of clinically evident disease implies a failure of host defense. The analysis of endogenous antitumor responses may thus yield insights into the mechanisms underlying cancer escape from immune control. As recent work in murine models established that some host reactions mediate tumor protection (2), deciphering the pathways that restrain tumor immunity in cancer patients should further the development of immunotherapy.

The crafting of genetic and biochemical techniques to characterize tumor antigens led to the realization that most cancer patients harbor in their peripheral blood T cells and antibodies that manifest specificity for autologous tumor. In some cases, primary and metastatic tumor deposits also elicit significant lymphocyte infiltrates. Together, these findings indicate that many cancers are sufficiently immunogenic to provoke nascent host responses.

Longitudinal clinicopathologic investigations revealed that some endogenous reactions are of prognostic importance (3). Brisk intratumoral (but not peritumoral) T-cell infiltrates in early-stage melanomas are correlated with a reduced incidence of recurrent disease and decreased mortality after complete resection. Intratumoral T cells in follicular lymphomas and colon, renal cell, and ovarian carcinomas similarly are coupled to improved patient outcomes after surgery and chemotherapy. High-titer antibodies to the tumor-associated antigen MUC-1 are also associated with increased survival in patients with early-stage breast cancer. Collectively, these results imply that whereas spontaneous host reactions might be inadequate to prevent tumor formation, some are linked to more profound and durable clinical benefits with conventional cancer therapy. An intriguing issue for further exploration is whether endogenous tumor immunity contributes in some way to the efficacy of standard treatments.

What are the mechanisms that restrain the potency of nascent host responses? Detailed studies of CD8+ T-lymphocyte infiltrates in the metastases of patients with melanoma uncovered striking defects in cellular differentiation and cytotoxic pathways (4). Although some of these abnormalities might reflect, at least in part, impaired dendritic cell tumor antigen presentation and/or a lack of productive T-cell help, accumulating evidence portrays an active system of negative immunoregulation. Indeed, the pioneering experiments of Robert North and colleagues two decades ago illustrated that the implantation of transplantable tumors into normal mice engendered an early cytotoxic T-cell response, but a subsequent CD4+ T-cell expansion attenuated the lymphocyte-mediated killing, thereby unleashing tumor growth (5). This novel inhibitory population seemed to function in opposition to effector CD4+ T cells, which supported the development of antitumor CD8+ T lymphocytes.

Progress in unraveling this suppressor activity in more detail required a deeper understanding of CD4+ regulatory T-cell function, which emerged from basic investigations in autoimmunity. Sakaguchi and colleagues showed that depletion of CD4+CD25+ T cells (which account for 5-10% of all CD4+ T cells) from otherwise normal mice precipitated a variety of organ-specific inflammatory diseases, including diabetes, gastritis, thyroiditis, glomerulonephritis, orchitis, and oophoritis (6). These findings unveiled an extrinsic mode of peripheral immune tolerance, in which a specialized population of T cells acts to restrain autoreactive lymphocytes that escape deletion in the thymus or bone marrow. Interestingly, animals depleted of CD4+CD25+ T cells resembled the scurfy strain of mice in their spectrum of autoimmune lesions; positional cloning revealed that scurfy mice harbored a mutation in the forkhead winged-helix transcription factor Foxp3 (7). Further studies established Foxp3 as a master regulator of regulatory T-cell development and activity; Foxp3 knockouts proved devoid of regulatory T cells, and enforced Foxp3 expression in effector CD4+ T cells triggered their reprogramming into regulatory T cells (8). Moreover, humans with the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, a constellation of organ-specific inflammatory diseases, including diabetes, gastritis, thyroiditis, glomerulonephritis, orchitis, and oophoritis (6). These findings unveiled an extrinsic mode of peripheral immune tolerance, in which a specialized population of T cells acts to restrain autoreactive lymphocytes that escape deletion in the thymus or bone marrow. Interestingly, animals depleted of CD4+CD25+ T cells resembled the scurfy strain of mice in their spectrum of autoimmune lesions; positional cloning revealed that scurfy mice harbored a mutation in the forkhead winged-helix transcription factor Foxp3 (7). Further studies established Foxp3 as a master regulator of regulatory T-cell development and activity; Foxp3 knockouts proved devoid of regulatory T cells, and enforced Foxp3 expression in effector CD4+ T cells triggered their reprogramming into regulatory T cells (8). Moreover, humans with the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, a constellation of pathologies akin to scurfy mice, also manifested profound regulatory T-cell deficiencies due to mutations in the human Foxp3 gene (9), thus highlighting the extensive conservation of this tolerance mechanism.

Two populations of Foxp3-expressing regulatory T cells have been characterized. One lineage, designated as natural suppressor cells, arises in the thymus in a pathway that requires T cell receptor stimulation by peptide-loaded major histocompatibility class II proteins, interleukin 2, and CD28, and might be mediated through dendritic cells associated with Hassall’s corpuscles (10). The maintenance of these cells after thymic export involves additional interleukin 2, CD40, and probably transforming growth factor-β. A second group of regulatory T cells might be induced in the periphery from Foxp3-negative CD4+ T cells; prolonged, noninflammatory antigen exposure and high local concentrations of transforming growth factor-β promote this conversion (11). Together, these developmental

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pathways endow regulatory T cells with a T-cell receptor repertoire that may be comparable in diversity to effector CD4+ T cells.

Whereas regulatory T cells require T-cell receptor triggering for activation, their suppression of other CD4+ and CD8+ T lymphocytes and B cells proceeds in an antigen nonspecific, but contact-dependent manner, perhaps involving transforming growth factor-β and CTL-associated antigen-4 (CTLA-4; ref. 12). Antibody production together with T cell proliferation, cytokine secretion, and cytotoxicity are all subject to regulation. Regulatory T cells may be tonically stimulated in lymph nodes, where encounters with dendritic cells charged with self-antigens acquired in the tissues likely contribute to tolerance preservation (13). Activated regulatory T cells also modulate innate and adaptive responses to foreign antigens, thereby playing an additional role in microbial defense, where these cells help establish an appropriate balance between pathogen control and untoward inflammatory pathology (14).

What are the functions of regulatory T cells during tumor development? Experiments employing a T-cell receptor transgenic system and a model cancer antigen showed that regulatory T cells attenuate tumor-specific CD8+ T-cell killing in a transforming growth factor-β–dependent fashion (15), thus confirming North’s earlier observations. Cancers may engender regulatory T cells through promoting the constitutive presentation of self-antigens by immature dendritic cells; high levels of transforming growth factor-β and prostaglandin E2 (derived in part from elevated COX-2 expression) in the tumor microenvironment may also enhance the conversion of FoxP3-negative CD4+ T-cell effectors to FoxP3-positive regulatory T cells (16).

Expression cloning strategies have disclosed that the targets of tumor-reactive regulatory T cells include mutated proteins, cancer-testis gene products, and wild-type, overexpressed self-antigens (17). In addition to inhibiting CD8+ T-cell cytotoxicity, regulatory T cells also restrain the activities of antitumor CD1d-restricted NKT cells (18), a key source of IFN-γ and other proinflammatory cytokines. In transplantable tumor models, the administration of antibodies to CD4 or CD25, which effectively antagonize regulatory T-cell function, established a critical role for regulatory T cell–mediated immune suppression at both early and late stages of disease, as these manipulations evoked impressive tumor regressions and protection against subsequent tumor challenges (19, 20).

Parallel studies in cancer patients revealed frequent increases in regulatory T-cell populations in peripheral blood, lymph nodes, and tumor deposits (21). Diverse malignancies stimulate these expansions, including lung and gastrointestinal cancers, head and neck tumors, ovarian carcinomas, and melanomas; furthermore, these cells manifest efficient suppression in situ. Regulatory T cells are also the targets of transformation in some T-cell lymphoproliferative disorders.

Curiel and associates were the first to delineate the prognostic significance of regulatory T-cell reactions in women with advanced ovarian carcinoma (22). These investigators documented the accumulation of FoxP3-expressing CD4+CD25+ T cells in tumors and ascites at the time of initial debulking. This regulatory T-cell recruitment involved the production of CCL22 by tumor cells and infiltrating macrophages, which triggered a CCR4-mediated chemotactic response. FoxP3-positive cells were juxtaposed with CD8+ T cells in immunohistochemistry, raising the possibility that contact-dependent suppression might be operative in situ. Indeed, regulatory T cells purified from these lesions inhibited the in vitro proliferation of autologous Her-2/neu-specific T cells (generated ex vivo with dendritic cell stimulation) and prevented these effectors from controlling tumor growth following adoptive transfer in a xenogeneic tumor transplant model. The physiologic relevance of this suppressive function was vividly illustrated in the inferior survival of patients manifesting the largest numbers of intratumoral regulatory T cells.

In this issue of Clinical Cancer Research, Wolf and colleagues extend these provocative findings in several important ways (1). The new experiments analyze a cohort of 99 ovarian carcinoma patients who underwent surgery and cytotoxic therapy and were then followed for a median of 5.8 years. FoxP3 expression in tumor resection samples was determined by real-time PCR and compared with values in normal ovaries removed at the time of surgery for nonmalignant disease. Although FoxP3 transcripts were detected in normal ovaries, consistent with the previously delineated role for regulatory T cells in preventing oophoritis in mice, the ovarian carcinoma patients showed a 1.8 ± 0.7-fold overall increase in levels. Because FoxP3 transcripts constituted a continuous function, the investigators compared cancer patients in the top and bottom quintiles to assess a possible prognostic relationship. Strikingly, subjects in the highest FoxP3 quintile relative to those in the lowest showed a marked diminution in both overall (28 versus 77 months) and progression-free survival (18 versus 57 months). Decreased FoxP3 expression was associated with a 60% reduction in the likelihood of disease recurrence or death, and this advantage was retained in multivariate analysis.

Wolf and colleagues also showed that FoxP3 transcripts were correlated with the expression of CD3, IFN-γ, and IRF-1 (an IFN-inducible gene). Although previous work revealed that high IFN-γ was a positive predictor of survival (23), perhaps reflective of a nascent Th1 cellular response, FoxP3 levels remained an important negative predictor even in this group. These findings may help reconcile the apparent discrepancy that whereas CD3 transcripts were inversely associated with survival in the current study, previous work of Zhang and associates in ovarian carcinoma delineated an improvement in clinical outcome with T cell infiltration (24). As Zhang and colleagues did not characterize the prognostic importance of CD4+ and CD8+ T-cell subsets separately, perhaps a comprehensive analysis of lymphocyte reactions will be more informative, with a high ratio of cytotoxic to regulatory T cells defining a group with the best outcome. Additional studies that fully integrate CD8+ T lymphocyte and regulatory T-cell responses should be undertaken in a broad range of malignancies.

What are the therapeutic implications of intratumoral regulatory T-cell infiltrates? The recent insights into immunoregulation discussed above raise the possibility that antitumor regulatory T-cell responses might be considered physiologic, part of an intricate mechanism devoted to maintaining tolerance to self-antigens and minimizing tissue damage in the face of persistent inflammation. Within this perspective, overcoming regulatory T cell–mediated immune suppression might accomplish substantial therapeutic benefit, albeit with a risk of precipitating serious autoimmunity. The therapeutic index for targeting regulatory T cells will likely depend on the relative thresholds for antagonizing negative regulation of antitumor versus autoreactive lymphocytes. The frequency,
affinity, and functional status of tumor-specific effectors may prove decisive in framing this window of intervention. Thus, patients with a strong antitumor CD8\(^+\) T lymphocyte reaction, generated either endogenously or as a consequence of tumor vaccination, may benefit the most from overriding regulatory T cells. In this context, immunization strategies that incorporate antigens with selective cancer cell expression should be advantageous. Key issues for further study are the extent to which regulatory T cell–mediated suppression in vivo is non–antigen-specific, whether cell contact is required for inhibition, and, if so, for what duration.

How might regulatory T cells be targeted therapeutically? First, it is tempting to speculate that part of the antitumor efficacy of current cytotoxic treatments might involve antagonizing regulatory T-cell function; previous work indicated that cyclophosphamide disrupts immune regulatory circuits (25), and thus patients with weak regulatory T-cell, but strong CD8\(^+\) T-cell reactions might respond to this manipulation. Nonetheless, robust regulatory T-cell infiltrates will likely require more potent and specific strategies, and a number of critical immunoregulatory molecules expressed on the surface of regulatory T cells may well be suited to this purpose. Indeed, initial clinical testing of a fully human monoclonal antibody that blocks CTLA-4 has already established the ability of this scheme to effectuate substantial tumor destruction, although at the expense of some autoimmunity (26). The spectrum of inflammatory toxicities observed thus far resembles the pathologies characteristic of the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, implying that CTLA-4 blockade targets regulatory T cells, at least in part, but additional effects on activated T cells are also possible (27). Preliminary findings suggest that administering anti-CTLA-4 antibodies to previously vaccinated patients might increase the therapeutic index by preferentially augmenting antitumor responses. Other antibodies specific for regulatory T-cell surface moieties including CD25, programmed death-1 (PD-1), and glucocorticoid-induced tumor necrosis factor receptor family–related gene (GITR) are likely to enter phase I clinical trials in the near future. Small molecules that perturb CCL22/CCR4 signaling might diminish regulatory T-cell recruitment, whereas nucleotide derivatives that trigger toll-like receptor 8 (TLR8) might also override regulatory T-cell inhibitory activity (ref. 28; Fig. 1).

Lastly, although substantial evidence supports regulatory T cell–mediated immune suppression as a significant obstacle to tumor control, hints of a more complex role for regulatory T cells in tumorigenesis are emerging. As chronic inflammation is a potent tumor promoter (29), deficiencies of regulatory T cells that result in unresolved inflammation might enhance tumor formation in some cases. Consistent with this idea, the adoptive transfer of regulatory T cells into mice with inflammation-associated colon carcinoma attenuates tumor development (30). Moreover, intratumoral regulatory T cells seem to be a favorable prognostic factor in patients with Hodgkin's lymphoma (31), perhaps indicating that the malignant Reed-Sternberg cells, a small minority among the prominent host infiltrate, receive trophic signals from the surrounding lymphocytes. Whereas these challenging findings await further confirmation, the biology of regulatory T cells undoubtedly retains many intricacies yet to be discovered, replete with therapeutic implications.

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**Fig. 1.** FoxP3-expressing regulatory T cells suppress tumor-specific CD8\(^+\) CTLs. Tumor-derived CCL22 recruits regulatory T cells through CCR4 signaling. Regulatory T-cell function might be attenuated with monoclonal antibodies that target CTLA-4, CD25, PD-1, or GITR. TLR8 engagement with nucleotide derivatives might also inhibit regulatory T-cell suppressor activity.
References


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