Less Yin, More Yang: Confronting the Barriers to Cancer Immunotherapy

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Abstract

Clinical trials involving T-cell–based immunotherapy for the treatment of human cancer have shown limited degrees of success. In cancer vaccine trials conducted at multiple centers worldwide, immunization has often resulted in the robust elicitation of T cells that specifically recognize antigens expressed on the surface of tumor cells. However, to date, objective clinical responses resulting from these approaches have remained relatively rare. By contrast, adoptive transfer of laboratory-expanded T cells into patients has had more success, producing impressive clinical regressions in a subset of advanced metastatic melanoma patients. The failure of activated T cells to consistently induce clinical responses in many other patients has pushed us toward a deeper understanding of natural immunoregulatory mechanisms that are directly responsible for diminishing tumor-specific T-cell activation, migration, and effector function in vivo. Such immunosuppressive factors likely evolved to prevent autoimmunity, but are frequently co-opted by tumors to evade tumor-specific immune responses. With this knowledge, it now becomes imperative to develop specific clinical interventions capable of eliminating tumor-specific immunosuppression, with the goal of shifting the balance to favor effector T-cell function and tumor cell killing.

Although immunotherapeutic approaches for the treatment of cancer are now gaining wider acceptance as viable alternative therapies for treating certain tumor types, such was not always the case. Serious doubts existed for many years among oncologists and immunologists alike as to whether the immune system is capable of eliminating human cancer. One of the first indications that immune responses could effectively alter the clinical course of established, invasive human cancers came from studies of the administration of interleukin-2 (IL-2) to patients with metastatic melanoma (1). Although IL-2 does not directly affect the growth of cancer cells, it does activate and induce the proliferation of CD8+ T lymphocytes, which possess potent cytolytic capacities (2, 3). Approximately 15% to 20% of metastatic melanoma patients treated with IL-2 alone experience objective clinical regressions, many of which are long lasting and curative (4, 5). Thus, IL-2 has provided a strong impetus to define the means by which T cells can recognize tumor cells and how they can be best manipulated to reliably induce tumor regressions.

Subsequent studies over the past two decades in both humans and animal models have allowed for the elucidation of the mechanisms behind the immune recognition of tumor cells at the molecular level. T cells, through their T-cell receptors, specifically recognize antigens presented as small peptides in the groove of surface human leukocyte antigen molecules (6, 7). Peptide antigens expressed on the surface of cancer cells have been shown in multiple studies to induce T-cell recognition leading to tumor cell killing and/or the release of helper cytokines (8, 9). In the case of melanoma, several antigens have now been identified that can be recognized by both CD8+ cytotoxic T cells and CD4+ T-helper cells, including MART-1, gp100, MAGE-1, tyrosinase, TRP-1, TRP-2, and NY-ESO-1 (10, 11). Two of these antigens, MART-1 and gp100, seem to be expressed in the majority of fresh and cultured melanomas (12). Because these are nonmutated, "self" antigens that are also expressed in normal melanocytes, it is likely that immune tolerance mechanisms prevent or dampen the ability of the immune system to naturally recognize and react to these antigens (13, 14). Although such tolerance mechanisms have emerged as formidable barriers to immunotherapy, as discussed below, our knowledge of tumor-associated antigens for melanoma and other tumor types has provided potential molecular targets for T-cell–based immunotherapy, and has allowed for the design of strategies to break immune tolerance for patients with cancer.

Cancer Vaccines

The common goal of cancer vaccination strategies is to induce tumor regressions through the in vivo activation of tumor-specific T lymphocytes. A number of different immunization approaches have been used using murine models and many are also currently under way as part of clinical trials (15–17). Several studies have attempted to vaccinate with peptides comprising the recognized tumor antigens themselves. Although such approaches are relatively nontoxic and have resulted in increased numbers of circulating antigen-specific
T cells in peripheral blood (especially after long series of vaccinations over many months), rarely have they led to objective regressions of bulky, established solid tumors (18–23). It has been speculated that the failure of this strategy stems from the fact that optimal activation of naive T cells requires antigen presentation by activated professional antigen-presenting cells (pAPC) displaying appropriate costimulatory molecules, such as B7-1 and B7-2 (24, 25). Peptide presentation by other cell types, including tumor cells, often leads instead to T-cell tolerance (26).

Because inflammation is known to comprise an important component of cell-mediated immune responses, alternate strategies have been developed that introduce tumor antigens in vivo using viral vector delivery systems (27, 28). Antigens delivered in this fashion will presumably be presented by pAPCs to optimally activate naive T cells, with the added benefit that viral components, including Toll-like receptor agonists, activate innate immunity, thereby inducing inflammatory cytokines and leading to an enhancement of the tumor-specific immune response. Although this strategy holds promise, it is somewhat limited by the natural development of humoral immunity to viral components, frequently rendering sequential vaccinations ineffective (29). There are also potential dangers inherent in using viruses in human subjects, especially for patients receiving lymphodepleting chemotherapy. Although several clinical trials involving viral vector–mediated delivery of tumor antigens have been successful at generating antigen-specific T lymphocytes in vivo, clinical response rates have generally been disappointing (16, 30, 31).

Perhaps one of the most promising strategies currently in use for vaccination against tumors involves the infusion of autologous dendritic cells. Dendritic cells are the most potent pAPCs known, and have an unparalleled capacity for efficient activation of naive T cells (32, 33). As such, they may be useful as clinical reagents for inducing antitumor immunity by directly activating tumor-specific T lymphocytes in vivo that are capable of mediating tumor destruction (34, 35). Several approaches have been taken to load dendritic cells with tumor antigens for presentation to T cells. These include pulsing dendritic cells with antigenic peptides, whole proteins, tumor cell lysates, transduction of dendritic cells with viral vectors expressing tumor antigens, expression of tumor-derived mRNA in dendritic cells, and fusion of dendritic cells with tumor cells (36–41). All of these approaches have been shown to enhance activation and proliferation of tumor antigen–specific T cells and several of them have been shown in mouse models to induce regression of tumors. Several clinical trials are currently in progress using antigen-loaded dendritic cells as clinical reagents to immunize cancer patients. Although many of the trials have resulted in encouraging tumor antigen–specific T-cell reactivity, reports of complete regressions of established tumors in humans have remained largely anecdotal (30, 42, 43).

Although cancer vaccines have not yet fulfilled their promise, recent results strongly suggest that combination therapies, such as those involving immunization combined with adoptive T-cell transfer, may produce synergistic antitumor responses that are considerably more potent than either approach used in isolation (44, 45).

### Adoptive T-Cell Transfer

The technological capability to expand tumor-reactive T cells to large numbers in the laboratory has led to the development of adoptive cell transfer (ACT) protocols for patients with metastatic melanoma. Tumor-infiltrating lymphocytes derived from resected tumors and expanded in vitro were shown to be capable of specifically recognizing tumor antigens, particularly MART-1 and gp100, in over two thirds of melanoma patients (46, 47). Such tumor-infiltrating lymphocytes, when expanded to large numbers and adoptively transferred to patients along with IL-2, resulted in an objective response rate of ~30% (48). However, most of these clinical responses were transient, and a limited persistence of the transferred cells was observed in vivo.

Mounting evidence suggests that the host immune environment significantly affects the efficacy of ACT therapy (49). Preclinical models have shown that sublethal doses of irradiation before adoptive transfer of tumor antigen–specific T cells substantially increases the persistence and antitumor activity of the transferred cells (50). With these results in mind, a clinical trial investigated the addition of a lymphodepleting conditioning regimen before ACT therapy in patients with metastatic melanoma. Patients received a nonmyeloablative, lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine before infusion of expanded, tumor-reactive, tumor-infiltrating lymphocyte cells and IL-2 therapy (51, 52). This approach resulted in durable objective responses in ~50% of patients treated, with regressions observed in metastatic deposits in the liver, lungs, cutaneous and subcutaneous tissues, and lymph nodes. Interestingly, a number of responding patients showed marked evidence of lymphoproliferation of the transferred cells, some of which persisted at high levels in the peripheral blood for several weeks (53, 54). Subsequent studies showed that clinical responses were highly correlated with in vivo persistence of the transferred cells (55). These results have highlighted the significant potential of lymphodepleting regimens to enhance the efficacy of ACT.

### How Does Lymphodepletion Improve Adoptive T-Cell Therapy?

While the exact mechanisms by which lymphodepletion regimens lead to improved responses to adoptive T-cell transfer remain to be defined, three nonmutually exclusive models have been proposed. Under one scenario, prior depletion of lymphocytes may create “space” for the adoptively transferred cells within the lymphocyte compartment (56). In this model, homeostatic mechanisms result in increased proliferation and enhanced survival of the transferred T cells, largely through increased access to endogenous common γ-chain cytokines, such as IL-2, IL-7 and IL-15 (57, 58).

As Paulos et al. (58) report in this issue, myeloablative lymphodepletion regimens involving total body irradiation also induce superior antitumor responses when combined with adoptive T-cell transfer, as shown in both human cancer patients and in animal models. Radiation induces damage to the gut, leading to the translocation of microbes from the gut and the systemic liberation of bacterial-derived lipopolysaccharide. This
Toll-like receptor 4 agonist then acts to activate the innate immune system, leading to increased levels of proinflammatory cytokines, activated pAPCs, and enhanced antitumor function of adoptively transferred T cells.

The third mechanism by which lymphodepletion is thought to augment ACT is through the depletion of immune cells that act to suppress immune responses \textit{in vivo} (14). Regulatory T cells are a subset of CD4+ T cells that are known to inhibit both the activation and proliferation of cytotoxic and helper T cells, and are likely to play a major role in the induction of functional immune tolerance to tumors often observed in cancer patients (59). Other regulatory CD4+ T-cell subsets likely targeted by lymphodepletion include Tr1 and Th3 cells, which produce the immunosuppressive cytokines IL-10 and transforming growth factor-\(\beta\) (TGF-\(\beta\)), respectively (60, 61). Increased numbers of regulatory T cells in cancer patients has been correlated with an unfavorable prognosis, arguing that elimination of these cells may result in an improved efficacy of adoptive immunotherapy (62). Although lymphodepletion is effective at eliminating regulatory T cells \textit{in vivo}, recent studies have shown that these cells repopulate the periphery very rapidly, highlighting the transient and possibly limiting nature of this approach (63).

Observing the benefits of lymphodepletion in the context of ACT, combined with the recent discovery and characterization of diverse regulatory immune cell subsets, has forced tumor immunologists to confront the dualistic nature of the immune system. Although, historically, we have been almost exclusively focused on generating more “Yang” (i.e., effector T cells capable of killing tumor cells), it has become clear that the inseparable “Yin” (i.e., immunoregulatory mechanisms that suppress T-cell function) also needs to be acknowledged and addressed in a more comprehensive manner. In other words, it probably does not matter how many effector T cells we can generate or how well they can kill tumor cells \textit{in vitro} if they are being effectively shut down \textit{in vivo}. Moreover, the immune system seems to react to any activation stimulus with a corresponding response that tends to be immunoregulatory in nature. It is becoming apparent that only through understanding the totality of the immune system can we begin to design truly rational treatment strategies designed to tip the balance in favor of immune activation.

### Barriers Faced by the Tumor-Specific T Cell

Tumor antigen–specific T lymphocytes face several barriers \textit{in vivo} that can act to abrogate their antitumor function, regardless of whether they were elicited naturally, through vaccination, or acquired through adoptive transfer (Fig. 1). All of these barriers probably originally evolved naturally to prevent autoimmunity by providing critical “brakes” for potentially uncontrolled immune responses. We will discuss these barriers in the context of three phases of the T-cell response: (a) priming of naïve T cells, including activation, proliferation, and survival; (b) migration of the activated T cells to tumor sites; and (c) T-cell effector function within the tumor microenvironment.

During the priming phase, T cells require antigen presentation by pAPCs expressing the appropriate costimulatory molecules to achieve full activation status. This positive costimulation is normally provided by the B7 family members...
B7-1 and B7-2, which bind to CD28 expressed on the naive T-cell surface, leading to T-cell activation (24, 64). Upon activation, however, T cells up-regulate expression of CTLA-4, which effectively competes with CD28 for binding to B7 molecules, and results in a suppression of T-cell activation (65). As discussed by Zang and Allison (in this issue; ref. 25), several clinical trials are currently under way to test CTLA-4 blocking antibodies for antitumor efficacy.

Lack of innate immune activation due to an absence of Toll-like receptor signaling is also a barrier to optimal T-cell priming and activation. Pathogen-derived Toll-like receptor agonists lead to pAPC activation that, in turn, generates effective adaptive immune responses and pathogen clearance (66). Tumors, by contrast, lack such innate activation signals and, consequently, spontaneous antitumor immune responses are comparatively weak. Expression of the immunosuppressive cytokines IL-10, TGF-β, and vascular endothelial growth factor also significantly inhibit pAPC activation, thereby constituting another set of barriers to effective T-cell priming (62).

As discussed by Gajewski (in this issue; ref. 67), lack of T-cell migration to tumor sites also plays a major role in the failure of activated T cells to cause tumor regression. This can be due to several factors, including lack of inflammation at the tumor site (Fig. 1). During pathogen infections, expression of inflammatory cytokines at the infection site normally facilitates trafficking of T cells to infected or inflamed tissues. Because the tumor microenvironment often does not show such inflammation, T cells tend to not migrate there in substantial numbers.

Because T cells are normally known to migrate in response to chemokine gradients, much work has focused on defining the chemokines naturally produced within the tumor microenvironment (68). However, studies have shown that T cells usually do not naturally express the corresponding chemokine receptors that can facilitate their migration to tumors (69). As might be expected, T cells naturally tend to express alternate chemokine receptors that do not recognize tumor-derived chemokines. Two potential clinical interventions for remedying this situation are to induce inflammation at the tumor site(s) by exogenous

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**Fig. 2.** Immunosuppressive factors within the melanoma tumor microenvironment. Immunohistochemistry of paraffin-embedded melanoma tumor sections reveals (A-C) the presence of immunosuppressive cytokines TGF-β and IL-10 (>10 magnification) and (D and E) infiltration of regulatory cells, as identified by nuclear staining of the transcription factor Foxp3 (>40 magnification). IgG1 indicates tumor staining with a nonspecific, isotype control antibody.
means, or to genetically modify transferred T cells to express chemokine receptors that will respond to chemokines naturally produced by tumors.

Even if a tumor-specific T-cell successfully migrates into the tumor microenvironment, there are several factors present within tumors that may act on the T-cell to diminish or abrogate its effector function (Fig. 1). Regulatory immune cells, including regulatory T cells, Tr1 cells, Th3 cells, and myeloid lineage suppressor cells, have all been described to play roles in immune suppression within the tumor microenvironment (62). The cytokines IL-10 and TGF-β, secreted by subsets of these regulatory cells, not only inhibit the activation of pAPCs, but also directly suppress the function of effector T cells (70, 71). As shown in Fig. 2, IL-10 and TGF-β can also be produced by tumor cells themselves, providing an inhospitable environment for effector T-cell function. Wrzesinski et al. (in this issue; ref. 72) discuss in detail the important role TGF-β plays in general immune suppression and in the context of regulatory T-cell generation.

Tumor cells also express several other soluble and membrane-bound factors that can inhibit effector T-cell function. These include the so-called coinhibitory members of the B7 family such as PD-L1 (also known as B7-H1), a ligand for the programmed death-1 receptor expressed on T cells (73). As discussed by Zang and Allison (in this issue; ref. 25), newly discovered B7 family members B7-H3 and B7-x may also provide relevant, tumor-associated coinhibitory signals for T cells, although their receptors have yet to be identified.

Three metabolic enzymes have also been identified as contributing to immunosuppression within the tumor microenvironment. Indoleamine 2,3-dioxygenase, produced by tumor cells or dendritic cells within the tumor microenvironment, depletes local tryptophan and inhibits T-cell proliferation (74, 75). Arginase degrades another essential amino acid, arginine, leading to inhibition of T-cell activation (76, 77). Inducible nitric oxide synthase leads to the production of nitric oxide, which has detrimental effects on T-cell priming, proliferation, and cytotoxicity (78). Furthermore, inducible nitric oxide synthase expression by tumors has also been directly linked to poor prognosis of melanoma patients (79).

### Conclusion

With a clearer view of how the immune system naturally regulates its own activation and duration and intensity of responses, we have now started to gain a higher level of understanding that will drive the next generation of cancer immunotherapeutics. The next challenge clinically will be to develop agents that will effectively “break down the barriers” and pave the way for optimal antitumor immune responses to occur. In the context of treatment, it will also be critical to determine which immunosuppressive mechanisms exist in and are relevant for each individual patient. For example, we have observed that there exists a significant degree of patient-to-patient variability in the tumor microenvironment of melanoma patients with respect to which immunoregulatory mechanisms are present. Another challenge will be how to reduce immune suppression regionally within tumor sites, because systemic interventions can lead to autoimmune manifestations, as seen with anti–CTLA-4 treatment (25, 80). In summary, it is only by acknowledging and managing the Yin side of the immune response that we can ensure that the Yang side is optimally effective in inducing tangible tumor regressions, and even complete cures for our future patients.

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