Overcoming obstacles to the effective immunotherapy of human cancer

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Cancers can grow and spread in humans despite their expression of cancer-associated antigens and the presence, within the tumor, of immune lymphocytes that can recognize those antigens. One of the possible explanations for this paradox is discussed in the article by Bai *et al*. (1) in this issue of PNAS. They demonstrate that anti-tumor T cells can be tolerized in the tumor microenvironment and thus down-regulate their effector functions. This is but one element in a complex story.

It has been known since the mid-1960s that the cellular arm of the immune response is responsible for the rejection of experimental tumors and organ allografts. The predominant effector element in the cellular immune system is the T lymphocyte, which contains surface molecules called T cell receptors that can recognize antigenic peptides presented on the surface of tumor cells. Thus, attempts to develop effective immunotherapies for cancer have emphasized the stimulation, *in vivo*, of T cells capable of recognizing and destroying cancer cells that express these antigens (reviewed in ref. 2) These attempts at immunotherapy fall into three main classes. They are (*i*) nonspecific immune stimulation with the goal that T cells reactive against the cancer will also be increased; (*ii*) active immunization of the tumor-bearing host designed to increase and activate the numbers of preexisting anti-tumor T cell precursors; and (*iii*) adoptive cell transfer (or adoptive immunotherapy), which involves the transfer to the tumor-bearing host of activated immune T cells capable of recognizing and destroying cancer cells.

At present, the effectiveness of cancer immunotherapy in humans is quite limited. IL-2, a cytokine that stimulates T cells and is approved by the Food and Drug Administration for patients with metastatic melanoma or renal cancer, can mediate cancer regression in ≈15–20% of these patients (3). Cancer vaccines are currently ineffective for the therapy of patients with established cancer except in rare and very sporadic cases (4). In contrast, cell transfer approaches can be very effective and can mediate cancer regression in 50–70% of patients with metastatic melanoma. This latter approach has provided important clues to the requirements for the successful immunotherapy of cancers in general (5, 6).

To mediate anti-tumor effects *in vivo*, T cells of sufficient avidity for recognition of tumor antigens must be present in sufficient quantities, traffic to the tumor site, extravasate from the circulation, and then mediate effector functions to cause destruction of cancer cells. All of these criteria must be met if a treatment is to be effective.

The presence of even large numbers of T cells capable of recognizing tumors is not sufficient to mediate tumor regression. Tumors can grow normally in T cell receptor transgenic mice, all of whose T cells are capable of recognizing the tumor antigen (7). In humans, studies of active immunization have shown that reactive T cells can be generated against antigens present on the tumor, and yet have no impact at all on tumor growth. In some cases, up to 30% of all circulating $CD8^+$ T cells can be shown to have anti-tumor reactivity in tumors that grow normally (8). The paper by Bai *et al*. (1) clearly demonstrates that T cells in the tumor microenvironment can be suppressed, whereas similar cells at other sites can exhibit profound effector function. The mechanisms of this local down-regulation are not clear, although many hypotheses have been tested, including the local presence of inhibitory cytokines such as IL-10 (9) or TGF-β (10), the presence of other cell types capable of actively suppressing immune reactions such as T regulatory cells (11) or myeloid-derived suppressor cells (12), or the stimulation of inhibitory cell surface components on infiltrating lymphocytes such as PD-1 (13) and CTLA-4 (14) that result in lymphocyte suppression. Lymphocytes can undergo apoptosis when encountering antigen under unique conditions, including lack of costimulation.

Profound lymphodepletion of the host substantially increases the effectiveness of cell transfer therapy.

Overcoming Obstacles

Adoptive immunotherapy experiments in both mice and humans have identified factors involved in overcoming the local immunosuppression at the growing tumor site. Profound lymphodepletion of the host substantially increases the effectiveness of cell transfer therapy in part by eliminating many of the cellular elements responsible for local suppression such as T regulatory cells, as well as myeloid-derived suppressor cells (15). The absence of these regulatory cells at a time when activated T cells are present in the tumor results in substantial enhancement of anti-tumor activity. Lymphodepletion has the added advantage of eliminating lymphocytes that compete with the transferred cells for homeostatic cytokines such as IL-7 and IL-15 (15). Thus, the homeostatic influence of these cytokines is apparent only on the transferred cells with anti-tumor activity.

An important factor that explains much of the failure of active immunization against cancer is the absence of precursor cells with very high affinity for recognition of tumor antigens. The majority of tumor antigens that have been described are self antigens that are selectively expressed or overexpressed on the tumor (16, 17). Because these determinants have been present during thymic development, negative selection mechanisms in the thymus eliminate those clonotypes with high reactivity against these antigens. Negative selection is essential to ensure that cells with high levels of autoimmune activity are deleted and cannot cause destruction of normal tissues in the adult. Thus, with rare exceptions only low-affinity T cell receptors capable of recognizing these antigens persist in the host. It is these cells that are largely stimulated by cancer vaccines, and the expansion of these low-affinity T cells cannot be stimulated by tumor antigens in the suppressive

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microenvironment of the tumor. This is a major obstacle to the development of effective cancer vaccines.

Adoptive immunotherapy allows the identification of rare cells with high affinity for tumor antigen that can be selected *in vitro* and expanded before transfer to the host. The anti-tumor cells can be activated *ex vivo* and directly administered, thus avoiding the tolerizing factors present at the tumor site. This approach using tumor-infiltrating lymphocytes has resulted in objective regression rates of 50–70% in patients with metastatic melanoma, including bulky, invasive tumors at multiple sites, including liver, lung, soft tissues, and brain (2). These studies have taught us that the administration to a lymphodepleted host of large numbers of activated high-affinity T cells capable of recognizing tumor antigens can overcome host inhibitory factors and mediate effective cancer immunotherapy in humans.

Prospects

The recent ability to genetically modify lymphocytes has opened possibilities for the *in vitro* creation of lymphocytes with appropriate therapeutic properties (18, 19). High-affinity T cell receptors can be introduced into a patient's normal lymphocytes and the administration of these cells to the lymphodepleted patient has now been shown to be capable of mediating cancer regression (20). The ability to further modify these lymphocytes, to make them less subject to the suppressive influences present in the tumor micro-environment such as the introduction of genes encoding dominant-negative TGF-β, or inhibitory RNAs to prevent the expression of inhibitory molecules such as CTLA-4 and PD-1, can potentially enhance the activity of the transferred cells.

Studies of cell transfer therapy are leading to an understanding of the factors that limit effective cancer immunotherapy and are suggesting experimental manipulations that can lead to the development of effective immunotherapies for the treatment of patients with cancer.

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