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Creating Therapeutic Cancer Vaccines: Notes from the Battlefield

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Abstract

With the identification of tumor antigens and a knowledge of how to vaccinate against them, the field of tumor immunology faces new challenges. In this article, the authors argue that successful immunotherapies of the future will activate anti-tumor T cells without inducing their anergy or apoptotic death.

It is now abundantly clear that the immune system can mediate the regression of even large tumor burdens in patients who have cancer. Target antigens recognized by T and B cells have been identified, and the cellular and molecular basis for immune recognition of tumors is understood at a complex level. Despite this progress, the design of a cancer vaccine that is reproducibly effective is still an elusive goal.

Melanoma antigens

A decade ago, it seemed clear that our burgeoning knowledge of the molecular identities of tumor-associated antigens would point the way to an effective therapeutic cancer vaccine. Since the cloning of the murine P1A antigen and the human melanoma antigen MAGE-1, progressive technical improvements have resulted in a long and growing list of antigens from a large variety of tumors[1,2].

Since melanomas clearly respond to immunotherapy[2], one approach has been to target melanocyte differentiation antigens (MDAs) with therapeutic anticancer vaccines in mice and humans[1-3]. MDAs appear to be the predominant antigens that are recognized by T cells isolated directly from malignant human melanomas[4]. The correlation of autoimmune destruction of normal melanocytes, termed vitiligo, with antimelanoma activity in mice and humans also validates MDAs as vaccine targets[5-7].

Immunization strategies

Many cancer vaccines currently under investigation are based on recombinant immunogens such as viruses and bacteria. In animal models, these vaccines can prime T-cell responses and elicit powerful immune responses that lead to destruction of tumor cells[8]; however, several obstacles remain in the translation of these strategies to the clinic. For example, many cancer patients have high pre-existing, neutralizing titers to vaccines based on adenoviruses and vaccinia viruses, the result of the ubiquitous environmental presence of adenoviruses and the worldwide immunization program to eradicate smallpox[9]. One way of circumventing pre-existing immunity is the use of viruses whose natural hosts are non-mammalian, such as the avian poxviruses[10]. Yet a remaining problem is that immunity to antigenically complex vaccine vectors may interfere with the induction of reactivity to the encoded tumor antigen through the poorly understood mechanisms of immunodominance[11].

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The use of vaccines based on 'naked' plasmid DNA vaccines (i.e. DNA without associated protein) may circumvent both pre-existing immunity and immunodominance. Although effective in many animal models, our own clinical work has shown no evidence of immunization or anti-tumor effect of 'naked' DNA immunization against the MDA gp100 (our unpublished data).

The administration of professional antigen-presenting cells (APCs) such as dendritic cells (DCs) loaded with tumor antigens has been heralded as a direct way of stimulating T cells *in vivo*. Although DC-based therapies are successful in mice, the outcomes of clinical trials using DCs have ranged from 'poor' to 'promising'[12-14].

To date, the most effective immunization strategy in our patients with advanced melanoma has been vaccination with peptide emulsified in incomplete Freund's adjuvant (IFA). Immunization with a gp100-derived peptide modified to enhance its binding to major histocompatibility complex (MHC) HLA-A2 dramatically increased levels of peptide-specific $CD8^+$ T cells in the peripheral blood. Importantly, these T cells recognized and killed a variety of melanoma cells that expressed the gp100 melanoma antigen and the restriction element HLA-A *0201 after culture *in vitro*[15]. Administration of interleukin 2 (IL-2) following peptide immunization resulted in significantly more-objective tumor regressions than observed after IL-2 treatment alone[15]. However, many of these responses are partial and transient.

Tumor escape

Although the loss of HLA or antigen, or the ability to process antigen, can certainly occur after a response to treatment[16,17], 'tumor escape' by these mechanisms does not occur in the majority of patients that fail to respond to T-cell-based therapies. Indeed, the overwhelming majority of tumors from a large cohort of productively immunized yet non-responding patients retain the ability to be efficiently lysed by T cells *in vitro* (F.M. Marincola, pers. commun.).

What allows tumors to grow in the face of circulating, tumor-specific T cells? Tumor cells may ectopically employ normal immunosuppressive mechanisms, such as the production of transforming growth factor β (TGF-β). This cytokine is normally produced by certain immune and other somatic cells, but is also potentially antiproliferative for T cells and natural killer (NK) cells. IL-10 is another candidate immunosuppressor. It is normally produced by activated T cells, B cells, monocytes and keratinocytes, but may be produced by certain tumors and interfere with macrophage-mediated antigen presentation and other immune functions. There have been reports that tumor cells can kill T cells through expression of Fas ligand (FasL), which engages Fas on T cells, but these reports have been disputed[18,19]. Recent evidence suggests that another molecule called TRAIL (TNF related apoptosis inducing ligand) mediates tumor escape[20], but much work remains to be done to verify the role of this death receptor ligand in the failure of immunotherapies.

Substantial evidence points towards the specific requirements for T-cell activation as the central reason for the failed anti-tumor immune response. It is now clear that there are many ways in which triggering a T-cell receptor (TCR) can result in the ultimate inactivation or even demise of the T cell bearing it. TCR engagement without concurrent ligation of receptors such as CD28,4-1BB, CD154 (CD40L) and OX40 receptor can result in T-cell anergy and consequent unresponsiveness to TCR stimulation[21]. Like most normal cells in the body, tumor cells generally do not express these costimulatory molecules and thus can continually promote specific T-cell anergy.

On the other hand, overstimulation can terminate an otherwise effective T-cell response through activation-induced cell death (AICD)[22], fratricide[23] or killing of one T cell by another and clonal exhaustion[24]. TCR ligation can result in either enhanced, partial or abrogated TCR

signaling and T-cell function upon stimulation with antigenic peptides differing by as little as one amino acid[25,26]. New molecular mechanisms are being elucidated through which notoriously ill-defined regulatory T cells can powerfully suppress T-cell-mediated autoimmune disease and tumor rejection[27,28].

Overcoming tumor-specific T-cell tolerance

It is unclear which, if any, of the above mechanisms allows for the continued growth of tumors in the face of potentially tumor-reactive T cells. However, it has been shown from studies in animal models that the tumor environment somehow inhibits an efficient T-cell response to any antigen, 'self' or non-'self', that is expressed by a tumor cell. Even strong antigens such as viral, bacterial or xenogeneic proteins typically do not evoke and sustain a productive immune response when expressed by tumor cells[10,29,30]. Yet immunity to these proteins is possible, as shown by T-cell activation and rejection of tumors (and even normal tissues) when the antigen is presented in an immunogenic form such as during viral infection[6,29]. The difference between antigen presentation in the tumor environment and in a virally infected tissue is probably the activation of resident APCs, which are the scavengers and 'danger' sensors of the immune system. The lack of pro-inflammatory mediators that induce maturation of DCs, in conjunction with the abundant antigen presentation by non-costimulatory, tolerizing tumor cells, might tip the balance between T-cell activation and inactivation in favor of tumor-specific T-cell tolerance.

$CD4^+$ T cells may be a means to reverse or overcome the tolerizing effect of the tumor environment, both directly, through the production of T-cell-trophic and -chemotactic factors such as cytokines and chemokines, and indirectly, through activation and maturation of APCs (Refs 31-33). Animal models have demonstrated the power of $CD4^+$ T-cell help, but the application of these concepts to human cancer vaccines remains undeveloped.

Concluding remarks

In conclusion, it is clear that tumor immunologists have made great strides in understanding components of the successful immunotherapy of cancer. Antigens have now been cloned that are expressed by tumors, are processed and presented in the context of MHC class I and class II molecules, and are recognized by cells from the patient's own T-cell repertoire. We have also learned how to immunize and are now capable of significantly expanding precursor T cells with vaccination. However, in the absence of a truly effective therapeutic vaccine, the appropriate and continued activation of anti-tumor T cells may be the missing piece of the immunotherapy puzzle. Thus, the focus of tumor immunotherapy is shifting. The challenge now is to learn how to promote T-cell activation and proliferation while abrogating T-cell anergy and death in the context of a profoundly tolerogenic tumor environment.

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