Cancer Immunotherapy and Breaking Immune Tolerance: New Approaches to an Old Challenge

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Abstract

Cancer immunotherapy has proven to be challenging as it depends on overcoming multiple mechanisms that mediate immune tolerance to self-antigens. A growing understanding of immune tolerance has been the foundation for new approaches to cancer immunotherapy. Adaptive transfer of immune effectors such as antitumor mAb and chimeric antigen receptor T cells bypasses many of the mechanisms involved in immune tolerance by allowing for expansion of tumor-specific effectors ex vivo. Vaccination with whole tumor cells, protein, peptide, or dendritic cells has proven challenging, yet may be more useful when combined with other cancer immunotherapeutic strategies. Immunomodulatory approaches to cancer immunotherapy include treatment with agents that enhance and maintain T-cell activation. Recent advances in the use of checkpoint blockade to block negative signals and to maintain the antitumor response are particularly exciting. With our growing knowledge of immune tolerance and ways to overcome it, combination treatments are being developed, tested, and have particular promise. One example is in situ immunization that is designed to break tolerance within the tumor microenvironment. Progress in all these areas is continuing based on clear evidence that cancer immunotherapy designed to overcome immune tolerance can be useful for a growing number of patients with cancer. Cancer Res; 75(1); 5–10. ©2014 AACR.

Introduction

Rapid growth in our understanding of immunology over the past several decades has resulted in more effective vaccines against a variety of infectious diseases. This led to hope that similar successes would follow in cancer immunotherapy. Responses to cancer immunotherapy were largely disappointing, in large part because inducing an antitumor immune response required breaking immune tolerance to self-antigens, and tools for doing so at the time were limited. Immune tolerance can be broadly classified into central tolerance based on immune editing that takes place in the thymus, and peripheral tolerance that involves suppression of autoreactive lymphocytes that have entered the periphery (1). In this review, we will discuss immune tolerance and how it is addressed by current approaches to cancer immunotherapy.

A discussion of immunotherapy strategies designed to break tolerance would benefit from a brief review of the immune response and how immune tolerance impacts on each point in the process. An effective antitumor immune response requires processing of tumor-associated antigens by dendritic cells (DC), presentation of antigens to antigen-specific T cells, activation and proliferation of those T cells, and maintenance of the T-cell response long enough for the T cells to effectively eliminate the cancer (Fig. 1; ref. 2). Immune tolerance can result from suppression at any point in this process. A suboptimal immune response can result from limited antigen uptake and presentation (3). T cells capable of responding to specific tumor antigens may be significantly reduced because of immune editing (4). The ability of tumor-specific lymphocytes to be fully activated and to proliferate may be limited by a lack of effective costimulatory signals (5). Even if a robust immune response is generated, it may not last long enough to induce tumor regression. Activated T cells need to efficiently traffic and accumulate at the tumor site (6). They then need to resist exhaustion and immunosuppression in the tumor microenvironment. Multiple mechanisms used by tumor cells, including alteration of the antigen presentation machinery or secretion of immunosuppressive factors that can induce apoptosis of lymphocytes or activate negative regulatory pathways, can induce tolerance and limit the effectiveness of the immune response (7). Tumor cells that enhance immune tolerance either directly or indirectly have a selective survival advantage thereby resulting in their outgrowth (4).

Cancer immunotherapy strategies designed to break tolerance can be broadly classified on the basis of the point in this process where the intervention takes place. Such strategies include: (i) adoptive transfer of immune effectors, (ii) vaccination, and (iii) immunomodulatory therapy.

Adaptive Transfer of Immune Effectors

The underlying advantage of adoptive transfer as an immunotherapeutic strategy is that immune effectors (such as antibodies and T cells) can be generated in large numbers ex vivo, bypassing the need for in vivo antigen presentation and immune effector proliferation.

Tumor-specific monoclonal antibodies

mAbs represent an extremely valuable and successful class of cancer therapeutics. mAbs targeting the HER-2 (trastuzumab), CD20 (rituximab), and the VEGF (bevacizumab) are top-selling drugs, and a number of other mAbs have received FDA approval
for the treatment of various solid tumors and hematologic malignancies (8–10). In addition to modifying tumor cell signaling cascades or tumor-stroma interactions, mAbs can also mediate antitumor activity via antibody-dependent cell-mediated cytotoxicity, phagocytosis, and complement-dependent cytotoxicity. There is growing evidence that, in some cases, mAb-induced tumor cell lysis can enhance uptake and cross-presentation of tumor antigens by DCs, leading to the generation of adaptive immune responses (9, 10). This illustrates how manipulation of the immune system in one manner can impact on other aspects of the immune response.

Early advances in mAb therapy included assessing mAbs with different specificities, and modifying their structure to reduce their immunogenicity (8, 10). Ongoing studies are exploring ways to modify their structure to enhance interactions with immune effector cells, including natural killer cells and DCs (11).

Immunoclonjugates can combine the specificity of mAbs with the potency of cytotoxic moieties. Immunoclonjugates...
include the anti-CD20 radioconjugates 131I-rituximab tiuxetan and 131I-tositumomab for lymphoma, and a number of antibody drug conjugates that are showing promise in both hematologic malignancies and solid tumors (12). Although some may not consider immunoconjugates to be "immunotherapy" because the mechanism of tumor cell lysis is not limited to immune-mediated lysis, immunoconjugates do rely on immune recognition of the target antigen and provide an effective approach to overcoming immune tolerance.

Bispecific mAbs harness the cytolytic potential of T cells by binding to tumor antigens with one arm and an antigen on immune effector cells with the other, thereby retargeting the T cell, irrespective of its natural specificity, toward malignant cells expressing the target antigen. Examples of bispecific antibodies include anti-CD19/anti-CD20 (blinatumomab) and anti-EPCAM/anti-CD3 (catumaxomab; refs. 8–11). The rapid clearance of bispecific antibodies necessitates continuous infusion, which represents an ongoing challenge in their clinical utility (2). However, bispecific mAbs do demonstrate clinical efficacy in some malignancies and development continues.

Adoptive T-cell transfer

Adoptive T-cell transfer involves removing lymphocytes from the tumor-bearing patient, expanding them ex vivo in the presence of various growth factors, and reinfusing them into the patient (13, 14). To enhance T-cell activation, IL2 coinfusion was used. To enhance tumor specificity and efficacy of transferred cells, tumor-infiltrating lymphocytes were used on the basis of the assumption that they are rich in antitumor T-cell populations. Examples of bispecific antibodies include anti-CD19/anti-CD20 (blinatumomab) and anti-EPCAM/anti-CD3 (catumaxomab; refs. 8–11). The rapid clearance of bispecific antibodies necessitates continuous infusion, which represents an ongoing challenge in their clinical utility (2). However, bispecific mAbs do demonstrate clinical efficacy in some malignancies and development continues.

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Whole-cell vaccines

Cell-based vaccines allow the host to generate immune responses against a variety of tumor-associated antigens and so lead to a more diverse immune response, which is helpful if immune tolerance is stronger for one antigen than another (19). An example of such vaccines is GVAX, which is composed of two irradiated prostate cancer cell lines that secrete granulocyte-macrophage colony stimulating factor (GM-CSF). Clinical evaluation of GVAX failed to show a survival benefit in randomized clinical trials although some of the correlative science suggested development of an immune response (19). GVAX involved immunization with allogeneic cell lines, which might not be the best way to generate an immune response directed toward antigens expressed by the patient's tumor. Use of modified autologous cells is more challenging logistically. Nevertheless, ongoing studies are exploring this possibility. There is also active investigation into further genetic modification of the tumor cell, fusion of the tumor cell with antigen-presenting cells, and use of various immune adjuvants to enhance its ability to break tolerance (19, 20).

Vaccination with protein antigen or peptides

Immunization using intact peptides derived from tumor-associated antigens has the potential to overcome immune tolerance by bypassing the need for antigen processing. Such peptides can bind directly to MHC molecules (most commonly class I) on DCs, thereby providing a robust activating signal to T cells (21). Although peptides have been found to be safe and relatively easy to synthesize, initial clinical trials of immunization with free...
peptides demonstrated limited efficacy (2). Addition of immune stimulants to further enhance activation of peptide-specific T cells may help some in this regard. Positive results were seen in a study exploring the combination of short peptides derived from glycoprotein 100 (gp100), a melanocyte differentiation antigen, with high-dose IL2 for patients with advanced melanoma. In this phase III clinical trial, the response rate was higher and progression-free survival longer with vaccine and IL2 than with IL2 alone (22). Another successful approach is a phase III clinical trial that evaluated subcutaneous GM-CSF with a keyhole limpet hemocyanin (KLH) anti-idiotypic fusion antibody (Id-KLH + GM-CSF) in patients with follicular lymphoma. The patient-specific Id protein vaccine significantly prolonged disease-free survival compared with the control vaccine (KLH + GM-CSF; ref. 23).

Many challenges remain if peptide-based vaccines are to be effective. These include selecting the right peptide, heterogeneous expression of the peptide by tumor cells, enhancing activation of DCs at the site of immunization to optimize activation of T cells, avoiding development of peripheral tolerance once an immune response has been generated, and blocking the immunosuppressive mechanisms used by tumors. It is likely peptide vaccines will need to be combined with other approaches to overcome immune tolerance that go beyond the use of traditional immune adjuvants. Such approaches might include using agents that could impact on immune tolerance induced by the tumor microenvironment, using substances produced by tumors, including IL10 and VEGF, can block the differentiation of myeloid DCs and lead to accumulation of immature DCs with reduced expression of costimulatory molecules (CD80/CD86), leading to T-cell anergy (Fig. 1; refs. 2, 7, 30, 31).

Efforts geared toward selectively targeting the immunosuppressive cells in the tumor microenvironment are ongoing (2), but to date have proven challenging. Alternatively, a broad variety of agents that could impact on immune tolerance induced by the tumor microenvironment have been used to tip the balance from immune tolerance to immune reactivity.

Vaccination with antigen-pulsed DCs

DC-based vaccines were developed on the basis of the realization that DCs are required to process and present tumor antigens but may be functionally defective, or present in inadequate numbers, in patients with cancer (24). To overcome this problem, DCs are generated from various patient-derived precursors by culturing them in vitro in the presence of cytokine and growth factor cocktails that induce DC expansion and maturation. DCs can then be loaded with tumor antigens, peptides, or genetically modified to produce a tumor-associated antigen, and administered back to the patient (24). Despite the complexities of ex vivo manipulation, some evidence for clinical efficacy has been observed with DC-based vaccines, and a vaccine for metastatic hormone-refractory prostate cancer (sipuleucel-T) was granted FDA approval in 2010. This vaccine uses a fusion protein of prostate acid phosphatase and GM-CSF for loading DCs. Sipuleucel-T prolonged median survival by 4.1 months in a phase III trial (25).

DC biology is extremely complex, and DC subsets vary considerably in their ability to process and present antigen and activate T cells (24). Some subsets of DCs can actually enhance immune tolerance. Various approaches to improving DC-based vaccines by enhancing antigen uptake, inducing maturation, and increasing antigen presentation in an immunogenic instead of a tolerogenic context have been used. These include modifying the manner of DC exposure to antigen and provision of other activation signals, including Toll-like receptor (TLR) agonists (TLR4, TLR7, and TLR9), several interleukins, and immunostimulatory mAbs (26–29). Although promising, their clinical utility as components of therapeutic DC-based vaccines for the treatment of cancer remains unclear.

Immunomodulatory Therapy

A growing understanding of mechanisms of immune tolerance has led to identification of cell populations in the tumor microenvironment that contribute to local immunosuppression. Myeloid-derived suppressor cells (MDSC) can be abundant in the tumor microenvironment and have profound suppressive effects on T cells. MDSCs can also activate regulatory T cells (Treg), which inhibit the function of effector T cells via multiple mechanisms. The presence of MDSCs and Tregs at the tumor site or in peripheral blood has been shown to correlate with poor prognosis in several types of cancer. Other substances produced by tumors, including IL10 and VEGF, can block the differentiation of myeloid DCs and lead to accumulation of immature DCs with reduced expression of costimulatory molecules (CD80/CD86), leading to T-cell anergy (Fig. 1; refs. 2, 7, 30, 31).

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Enhancing T-cell activation

Interleukins, including IL2, IL7, IL12, and IL15 have been investigated either as single agents or in combinatorial vaccine approaches. They each have complex effects, but in general stimulate T-cell activation. Immunogenic T-cell activation also depends on receiving a costimulatory signal from DCs that would lead to T-cell activation and proliferation. mAbs capable of delivering costimulatory signals are being clinically evaluated. Examples include CD40,OX40 (CD134), 4-1BB (CD137), and GITR (CD357; refs. 28, 29).

Checkpoint blockade

Immune checkpoints tightly regulate the magnitude of the T-cell response and are critical for avoiding autoimmunity. However, they also limit the robustness and duration of desirable antitumor immune responses. Molecules that play a key role in checkpoint regulation include the T-cell surface molecules cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death-1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), and lymphocyte activation gene-3 (LAG-3). In the tumor microenvironment, the expression of these markers by intratumoral lymphocytes results in hyporesponsiveness sometimes described as immune exhaustion. A number of these molecules are also highly expressed on Tregs and are used to suppress effector T cells (32). As such, these molecules are highly attractive as targets for reversing immune tolerance.

Our knowledge of these negative regulators and our ability to use checkpoint blockade to turn off suppressive signals, are one of the most rapidly moving and exciting areas in both cancer immunology and clinical oncology. Checkpoint blockade mAbs that recognize receptor or ligand, and interfere with their interaction, can enhance the antitumor immune response and have been found to be effective clinically in some recent studies (33). The anti–CTLA-4 mAb ipilimumab (Bristol-Myers Squibb) received FDA approval in 2011 for the treatment of metastatic melanoma based on a phase III trial demonstrating the first overall survival benefit in this population of patients with metastatic melanoma (34). Autoimmune side effects to anti–CTLA-4 therapy include enteropathies and endocrinopathies (11, 32). Preliminary preclinical and clinical studies exploring PD-1 blockade that target
complex, preliminary studies suggest that it can be effective
enhancement of antigen uptake and presentation, facilitates T-cell
response. Although this is
various aspects in the tumor microenvironment for
imunization to be effective. Ideally, such an approach would
generate immune responses capable of eradicating distant
disease at sites outside the
eld of radiation (abscopal effect; ref. 36).
An ongoing challenge is learning how to use these agents to
optimize tumor regression while avoiding unacceptable toxicity
due to enhanced autoreactivity of T cells to benign cells.
Despite this challenge, there is no doubt that checkpoint
blockade is a successful immunotherapeutic strategy, and will
be increasingly valuable in the clinic. Medical oncologists are
accustomed to dealing with the toxicities of cytotoxic drugs,
but at present are less familiar with identifying and managing
the autoimmune toxicities of checkpoint blockade. This
will need to change over the next decade as we learn more
about how to use checkpoint blockade, and it becomes a
component of cancer immunotherapy for an increasing num-
ber of patients.

In situ immunization
Many of the processes that contribute to immune tolerance
take place, at least in part, in the tumor microenvironment. As
we learn more about such processes, there is increased interest
in altering the microenvironment directly in a manner that
overcomes immune tolerance and allows for development of a
systemic antitumor response. Such modification of the tumor
microenvironment with the goal of enhancing the antitumor
response is not a new idea. Over a century ago, Dr. William
Coley injected microorganisms that we now know include a
number of TLR agonists into tumors and observed systemic
antitumor responses (2). Similarly, radiation therapy can
generate immune responses capable of eradicating distant
disease at sites outside the field of radiation (abscopal effect; ref. 36).
Combination therapy is likely to be needed to modify various aspects in the tumor microenvironment for in situ immunization to be effective. Ideally, such an approach would include therapy that induces immunogenic cell death, enhances antigen uptake and presentation, facilitates T-cell activation, and maintains the T cell response. Although this is complex, preliminary studies suggest that it can be effective (37).

Table 1. Summary of how various immunotherapeutic strategies impact on immune tolerance

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<thead>
<tr>
<th>Immunotherapeutic strategy</th>
<th>Antigen release and uptake by DCs</th>
<th>Antigen processing</th>
<th>Antigen presentation</th>
<th>Immune effector expansion</th>
<th>Immune Effector maintenance</th>
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<tr>
<td>Tumor-specific mAbs</td>
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<td>Vaccination with antigen-pulsed DCs</td>
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<td>Enhancing T-cell activation</td>
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<td>Checkpoint blockade</td>
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<td>In situ immunization and combination therapies</td>
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NOTE: Therapeutic approaches to cancer therapy are designed to either bypass immune editing (central tolerance) or address mechanisms that contribute to peripheral tolerance (outlined in Fig. 1).

Conclusion
We are currently in an incredibly exciting time in cancer immunotherapy, as we learn more about immune tolerance and how to overcome it. Decades of disappointing clinical trials of cancer vaccines that were based on poor understanding of the immune system (2), led some oncologists and cancer researchers at the turn of the century to view cancer immunotherapy as a "failed hypothesis." No one would contend that today. The value of mAbs that target cancer is undeniable. Checkpoint blockade mAbs that do not target the cancer directly but instead modify the immune response have clear clinical efficacy. CAR T-cell therapy has shown to be incredibly effective in select cancer types, and will surely have a role in the cancer immunotherapy of the future. There are still challenges with using vaccination to treat established cancers, but a growing number of studies suggest that we are making progress there as well.

These various approaches to breaking immune tolerance toward cancer have their impact at different points in the complex interaction between the malignancy and the immune system. Therapeutic approaches to cancer therapy are designed to either bypass immune editing or address mechanisms that contribute to peripheral tolerance (Table 1). Over the next 10 years, we will learn more about the value of each of these approaches individually, and more importantly, how to combine them into comprehensive strategies that enhance the immune response to cancer.

Disclosure of Potential Conflicts of Interest
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