Current Developments in Cancer Vaccines and Cellular Immunotherapy

By Antoni Ribas, Lisa H. Butterfield, John A. Glaspy, and James S. Economou

Abstract: **This article reviews the immunologic basis of clinical trials that test means of tumor antigen recognition and immune activation, with the goal to provide the clinician with a mechanistic understanding of ongoing cancer vaccine and cellular immunotherapy clinical trials. Multiple novel immunotherapy strategies have reached the stage of testing in clinical trials that were accelerated by recent advances in the characterization of tumor antigens and by a more precise knowledge of the regulation of cell-mediated immune responses. The key steps in the generation of an immune response to cancer cells include loading of tumor antigens onto antigen-presenting cells in vitro or in vivo, presenting antigen in the appropriate immune stimulatory environment, activating cytotoxic lymphocytes, and blocking autoregulatory control mechanisms. This knowledge has opened the door to antigen-specific immunization for cancer using tumor-derived proteins or RNA, or synthetically generated peptide epitopes, RNA, or DNA. The critical step of antigen presentation has been facilitated by the coadministration of powerful immunologic adjuvants, the provision of costimulatory molecules and immune stimulatory cytokines, and the ability to culture dendritic cells. Advances in the understanding of the nature of tumor antigens and their optimal presentation, and in the regulatory mechanisms that govern the immune system, have provided multiple novel immunotherapy intervention strategies that are being tested in clinical trials.**

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IMMUNOBIOLOGY OF T-CELL RESPONSES TO CANCER

Antigen Presentation to the Immune System

The immune system responds to intracellular events in target cells by the recognition of intracellularly derived protein fragments presented on the cell surface by major histocompatibility complex (MHC) molecules. Circulating T lymphocytes can potentially engage these peptide-MHC complexes through their T-cell receptors (TCR). This mechanism allows the immune system to differentiate abnormal intracellular processes from normally functioning cells expressing so-called self proteins. For example, if a cell is infected by a virus, the virus will use the host cell machinery to produce viral proteins. Some of these nonself proteins will be degraded by the proteasome and then presented on the cell surface restricted by MHC molecules as short virally-derived peptide (Fig 1), thereby alerting the immune system to the intracellular viral infection.

MHC

The immune system recognizes antigens presented by two types of MHC molecules: MHC class I and II. These are transmembrane glycoproteins with the role of acquiring intracellular peptide antigens and displaying them on the cell surface. They have four domains: a peptide-binding domain with a central cleft where a linear peptide sequence from the potential antigen resides, an immunoglobulin-like domain, a transmembrane region, and a cytoplasmic tail (see review in[1,2]). In humans, MHC class I molecules correspond to the HLA-A, -B, and -C molecules, and MHC class II molecules correspond to HLA-D molecules.

MHC class I molecules present eight- to 11-amino acid–long peptides derived from intracellular proteins digested by the proteasome complex. These complexes are displayed on the surface of the majority of cells and are recognized by $CD8^+$ T cells (Fig 1). MHC class II molecules have a more restricted distribution, and are mainly expressed on the surface of so-called professional antigen-presenting cells (APCs), such as dendritic cells, macrophages, and activated B cells (Fig 2). The peptides presented by MHC class II are longer, usually 10 to 34 amino acids, and derive from exogenous proteins endocytosed into endosome-lysosome compartments.[1] Under certain conditions there is deviation from these primary physiologic antigen presentation pathways because exogenous peptides can be presented by MHC class I molecules and endogenous peptides can be presented by MHC class II molecules.[3]

From the Divisions of Hematology-Oncology, Departments of Medicine, Surgical-Oncology, Surgery, and Microbiology, Immunology, and Molecular Genetics, University of California Los Angeles, Los Angeles, CA.

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Address reprint requests to Antoni Ribas, MD, Division of Hematology-Oncology, 11-934 Factor Bldg, UCLA Medical Center, 10833 Le Conte Avenue, Los Angeles, CA 90095-1782. email: aribas@mednet.ucla.edu.

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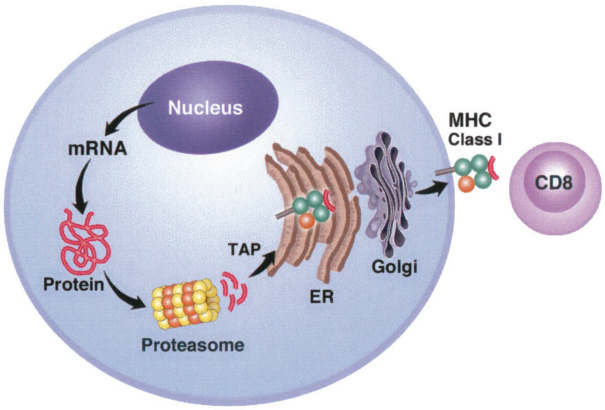

Fig 1. Major histocompatibility complex (MHC) Class I Presentation. Intracellular proteins, most commonly proteins with abnormal sequences, undergo degradation by the proteasome complex, are brought into the endoplasmic reticulum (ER) where they bind to major histocompatibility complex (MHC) class I molecules and traffic through the Golgi apparatus in their way to the cell surface. On the cell surface are recognized by CD8+ T lymphocytes.

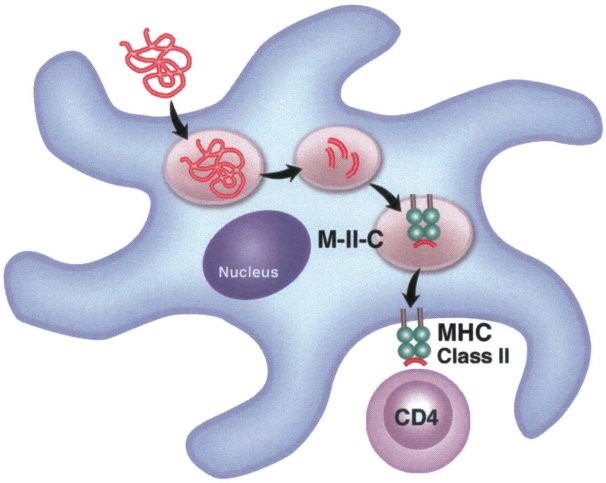

Fig 2. Major histocompatibility complex (MHC) Class II Presentation. Exogenous proteins enter the endosomes/lysosome vesicles in antigen presenting cells after internalization by phagocytosis or endocytosis. Here they are degraded into peptides by proteases. These peptides bind to MHC class II molecules in MHC class II compartments (MIIC), from where they are transferred to the cell surface by transport vesicles, where they are recognized by CD4+ T lymphocytes.

Tumor Antigens

The majority of tumors are ignored by the immune system, and it was thought for a long time that tumor antigens did not exist. In the late 1980s, Boon et al in Belgium and Rosenberg et al in Bethesda, MD, independently recognized that tumor-infiltrating lymphocytes (TIL) obtained from different HLA-matched subjects with melanoma were capable of lysing HLA-matched melanoma cell lines (see review in[4]). This provided evidence that melanoma antigens might be shared and led to the characterization of their gene sequences and the immunogenic amino acid sequences presented by MHC molecules on the cell surface.

Since then, the number of tumor antigens has increased rapidly and can be categorized in the following groups: (a) MAGE, BAGE, RAGE, and NY-ESO are nonmutated antigens expressed in the immune-privileged areas of the testes and in a variety of tumor cells; (b) lineage-specific tumor antigens, such as the melanocyte-melanoma lineage antigens MART-1/Melan-A (MART-1), gp100, gp75, mda-7, tyrosinase and tyrosinase-related protein, or the prostate specific membrane antigen and prostate-specific antigen, which are antigens expressed in normal and neoplastic cells derived from the same tissue; (c) epitopes derived from genes mutated in tumor cells or genes transcribed at different levels in tumor compared to normal cells, such as mutated *ras*, *bcr/abl* rearrangement, Her2/*neu*, mutated or wild-type *p53*, cytochrome P450 1B1, and abnormally expressed intron sequences such as *N*-acetylglucosaminyltransferase-V; (d) clonal rearrangements of immunoglobulin genes generating unique idiotypes in myeloma and B-cell lymphomas; (e) epitopes derived from oncoviral processes, such as human papilloma virus proteins E6 and E7; and (f) nonmutated oncofetal proteins with a tumor-selective expression, such as carcinoembryonic antigen and alpha-fetoprotein. Although the immune system has been widely exposed to some of these antigens in fetal life or later, responses can still be generated to these proteins when adequately presented in an immunostimulatory context.

Only short peptide sequences of the entire tumor antigen protein are immunogenic. These peptide sequences (called epitopes) are presented by MHC molecules according to a set of rules derived from the proteasome cleavage sites, the affinity of transporters associated with antigen processing (TAP), and the anchoring pockets in the peptide-binding groove of the MHC molecule.[5,6] New technologies have the potential to allow a rapid characterization of new antigens. Microarray and gene chip analysis can provide lists of genes that are differentially displayed in tumor cells compared with their normal tissue counterparts.[7] Computer algorithms that take into account the proteasome cleavage sites and the preferred and incompatible amino acids at the anchor positions of MHC binding allow the screening of protein amino acid sequences for candidate epitopes.[8] This may result in the recognition of multiple new antigens for cancers in each HLA subtype, thereby making epitope-based immunotherapy strategies more broadly applicable in the next several years.

Antigen Recognition by T Cells, Central Tolerance, Antigen Crypticity, and Subdominant Epitopes

T lymphocytes circulate between lymphoid organs, peripheral blood, and nonlymphoid organs searching for their specific MHC-peptide complex. Once the complex is recognized, T cells lyse the target cell. The extreme polymorphism of the MHC-peptide interaction and the heterogeneity and fine specificity of the TCR allow for virtually unlimited T-lymphocyte specificities. However, the host needs to protect itself from potentially harmful T cells. Strongly autoreactive T cells that recognize dominant self antigens are deleted in the thymus in a process known as negative selection. Otherwise, vertebrates would be plagued by a high incidence of autoimmune diseases. Autoreactive T cells with weak MHC recognition die of neglect. Together, these two processes are known as central tolerance. This

leaves a window of autoreactive T cells that escape thymic selection because of their recognition of sequestered or cryptic antigens that are usually hidden from the immune system, or antigens that are presented at subthreshold levels (subdominant epitopes), which stimulate weak T-cell responses.[9] These cryptic and subdominant self epitopes can be recognized by peripheral T cells under conditions where they are optimally presented to the immune system. Therefore, the immune system has the ability to recognize self as well as nonself antigens.

Self Versus Nonself, and Peripheral Tolerance

Cancer cells have frequent derangement of gene expression,[7] but only a minority of tumors express truly foreign proteins.[4] The recognition of nonmutated self antigens on tumor cells indicates that the immune system has the adequate T-cell repertoire to generate antitumor responses,[10,11] which are in fact antiself responses.[12] The self-reactive T cells that escaped central tolerance in the thymus circulate in the periphery of normal individuals, but are maintained in a tolerant or ignorant state by the lack of recognizable antigen (cryptic or subdominant) or by homeostatic processes in what is called peripheral tolerance.[13] To initiate an immune response and overcome peripheral tolerance the antigen, whether self or nonself, the antigen has to be presented with the appropriate immune activating signals.[14]

Two-Signal Model

Immunologists have long recognized that two signals are necessary for the initial activation of naïve T cells. The two-signal model[15] predicts that when an antigen is presented by an MHC molecule (signal 1) together with the costimulatory molecules B7.1 and B7.2 (signal 2), an immune response will be generated. This event will usually happen only if the antigen is presented by a professional APC; the most efficient professional APC is a dendritic cell. Conversely, if only signal 1 is presented, which is the case for the majority of tumor cells, an immune response will not be initiated because signal 2 is lacking, which helps maintain peripheral tolerance or ignorance of tumor antigens.

Danger

The demonstration that lineage-specific epitopes can serve as tumor antigens indicates that a self antigen can be presented in a context in which it can be viewed as a nonself antigen, making the question of self versus nonself less relevant. This indicates that a host that seemed to be functionally tolerant to a self antigen, as in the case of a tumor antigen, is in fact simply ignorant of the antigen's presence. The danger hypothesis avoids the self versus nonself distinction by focusing on the environment of antigen presentation and not the nature of the antigen.[14] This hypothesis postulates that an epitope does or does not stimulate an immune response depending on how it is presented to the immune system. If presented in a context of danger, it stimulates the immune system; if presented in a context of nondanger, it induces tolerance or nonreaction to that epitope.[14] This hypothesis presents a coherent explanation of the recognition of self antigens in cancer cells and supports a rational approach to the development and testing of strategies to present these antigens in an immune-stimulatory context.

Cross-Presentation, Cross-Priming, and Cross-Tolerance

Because cancer cells only have signal 1 and usually lack nonself epitopes that would create a danger environment, tumor antigens need to be presented by other types of cells if they are going to stimulate the immune system. This can be achieved by a process known as cross-presentation, which is a general mechanism for the induction of T cells.[16] Cross-presentation is involved in responses to viral infections, transplanted organs, and cancer cells (see review in[17]). Tumor antigens released by tumor cells are taken up by APCs, which process and present them in the APC cell surface restricted by their own MHC class I or II molecules. APCs such as dendritic cells can efficiently prime T cells when they display MHC-antigen complexes (signal 1) together with costimulatory molecules (signal 2), which activates naïve T cells in a process known as cross-priming. However, this same process of cross-presentation has been shown to induce T-cell tolerance, a process known as cross-tolerance, which may have an important role in maintaining tolerance to self antigens.[17]

Tumor and Antigen Location

Even in the setting of danger and with the appropriate delivery of the two signals by tumor antigens cross-presented by host APCs, cancers may not stimulate an immune response. Zinkernagel et al have postulated and tested the hypothesis that the location of the primary tumor and how its tumor antigens reach the lymph nodes are the critical steps that guide immune responses to cancer (see review in[18]). Tumor cells, or their antigens presented by APCs, may reach T-cell areas of the lymph nodes optimally located for T-cell activation, or may reach the lymph nodes but be walled off from T cells. This may help explain observations of different response rates to immune therapies according to different metastatic sites.[19]

Determinant Spreading

A major immunopathogenic event in autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, or insulin-dependent diabetes is the spread of reactivity from a single epitope to other epitopes derived from the same antigen (intramolecular spreading) or other antigens derived from the same tissue (intermolecular spreading).[9,20] An initial driver T-cell clone for a self antigen leads to tissue destruction. The debris from the immunologic attack is taken up and cross-presented by host APCs. This leads to the immunostimulatory presentation of additional and possibly cryptic or subdominant self epitopes, which provide novel determinants for T-cell recognition.[20] This mechanism of immune diversification, termed determinant spreading, provides a pathway for the generation of highly complex autoimmune responses that potentially originate from a single autoreactive determinant. This phenomenon may also be implicated in immune responses to cancer because it has been noted in preclinical models of cancer vaccines[21] and in clinical responders in immunotherapy trials.[22-25]

APCs and Dendritic Cells

Dendritic cells have emerged as the most powerful APCs to stimulate naïve T cells.[26] These infrequent bone marrow–derived leukocytes are ideally prepared for antigen presentation and stimulation of immune cells because they have the greatest surface density of MHC and costimulatory molecules, together with a high motility (which allows them to traffic from the site of antigen to the T-cell areas of lymph nodes), and have the ability to produce immunostimulatory cytokines and chemokines (see review in[26,27]). Dendritic cells are the most efficient cell type that is able to process exogenous antigen into the MHC class I pathway to stimulate naïve $CD8^+$ T lymphocytes by cross-priming. In the majority of other cell types, the MHC class I pathway only presents endogenous antigens and is therefore unable to cross-present exogenous antigens.

The ability to differentiate dendritic cells in ex vivo cell culture from bone marrow precursors or circulating monocytes (both in mice and in human subjects) using cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) has allowed their testing as natural immunologic adjuvants to initiate antitumor immune responses in preclinical models and pilot clinical trials (see reviews in[28,29]). Dendritic cells are specialized in antigen presentation and stimulation of both the innate and adaptive immune system because of their ability to interact with $CD4^+$ and $CD8^+$ T cells (adaptive), natural killer (NK) cells, and natural killer T (NKT; innate, see effector cells of the immune system, below) cells. The interaction between dendritic cells and cells of the adaptive immune response occurs by antigen presentation in a multimolecular complex called the immunologic synapse, which forms between a professional APC and a T lymphocyte. The immunologic synapse contains an antigen epitope bound to MHC and flanked by receptor-ligand interactions from costimulatory and adhesion molecules (see review in[30]).

EFFECTOR CELLS OF THE IMMUNE SYSTEM

$CD4^+$ T Cells

The principal role of $CD4^+$ T cells is helping APCs activate and maintain $CD8^+$ T cell-mediated responses. $CD4^+$ T cells recognize specific peptide sequences presented by MHC class II. MHC-antigen recognition requires the presence of costimulatory molecules and adhesion molecules (mainly intercellular adhesion molecule-1) in the immunologic synapse, leading to signal amplification in the APCs.[30] Activated $CD4^+$ T cells mediate help by activating APCs through the CD40-L (on the surface of the $CD4^+$ T cell), which cross-links the CD40 receptor on the APC.[31-33] Other similar receptor-ligand mechanisms of T-cell help have been described under certain conditions.

$CD8^+$ T Cells

$CD8^+$ T cells are the principal effector cells of the adaptive immune response, which mediates antigen-specific, MHC-restricted cytotoxic effects. $CD8^+$ T cells recognize peptides presented by MHC class I molecules through their TCR complex (Figs 1 and 2). Antigen-specific $CD8^+$ T cells become activated by the TCR-MHC class I peptide interaction on an APC, together with help from activated $CD4^+$ T cells. This leads to a clonal expansion of antigen-specific cytotoxic T lymphocytes (CTL) that will specifically lyse target cells that express this same peptide-MHC class I complex. $CD8^+$ T-cell–mediated cytotoxicity induces programmed cell death mainly through two mechanisms: the predominant Ca^{2+}-dependent perforin–granzyme B mechanism and the Fas-Fas ligand pathway, which requires the expression of Fas receptor on the target cell surface and Fas ligand on the effector CTL surface.

NK Cells

NK cells are innate effectors serving as a first line of immunologic defense. They eliminate their targets without prior sensitization, but lack peptide antigen specificity because they do not express a TCR. Their cytotoxic activity is tightly regulated by a diverse repertoire of activating and inhibitory receptors that recognize specific ligands on the surface of the target cells.[34,35] NK cells characteristically lyse MHC mismatched cells (tissue transplants, pregnancy) or cells having low levels of or lacking MHC expression (a frequent finding in cells after viral infections or in cancer cells). Allogeneic MHC molecules are recognized as nonself by NK cells regardless of their peptide epitope. Type I membrane glycoproteins or immunoglobulin-like receptors on NK cells recognize the allogeneic MHC, leading to a cytotoxic effect. However, in most physiologic situations, the dominant signal received by NK cells is inhibitory because of the recognition of syngeneic MHC molecule expression by inhibitory receptors on NK cells.[36] This protects normal host cells from NK attack and eliminates cells with aberrant MHC class I expression, according to the hypothesis of the missing self of Karre et al.[37] For tumors with low to undetectable MHC molecule expression, NK cells may be the predominant immune effector mechanism of defense.[38] In addition, NK cells can kill tumor cells that express normal levels of MHC class I through the interaction between activation of natural cytotoxicity receptors and specific MHC-like ligands on target cells.

NKT Cells

NKT cells coexpress a TCR (characteristic of T cells) and the NK1.1. receptor (characteristic of NK cells). These cells have been considered a remnant of an evolutionary primitive form of immune system (see review in[39]). They recognize a limited array of peptide and nonpeptide (glycolipid) antigens presented by the nonpolymorphic MHC-like molecule CD1, which is widely expressed in most APCs and several tissues. After CD1-antigen recognition, NKT cells immediately produce large amounts of type 1 (interferon gamma [IFNγ]) or type 2 (IL-4) cytokines (see cytokine profile below).[39] Because this cytokine milieu is thought to be a key component of the immune system response to a specific antigen, NKT cells have been proposed as the triggers of a polarized type 1 or 2 response. However, their exact role in shaping the acquired immune response is not fully understood. The activation of NKT cells by glycosylceramides presented by dendritic cells in CD1 molecules may be an initiation step for an adequate adaptive immune response.

AUTOREGULATORY CONTROL OF THE IMMUNE SYSTEM

A balance exists between immune response and tolerance or ignorance of tumor antigens. Dendritic cells are at the center of this balance, guiding the immune system toward acceptance or rejection of tumor cells by making a judgment of what should be presented and recognized as nondangerous self, dangerous self, or nonself.[26,40] Once T cells are activated, the immune system makes a great effort to keep them under control. Uncontrolled exponential expansion of lymphocytes after antigen stimulation would quickly overwhelm the lymphoid organs, and unchecked cytokine production and cytotoxic activity may lead to autoimmunity. An understanding of these control mechanisms of immune activation may help to optimize the design of immunotherapy interventions and cancer vaccine development.

Cytokine Profile

On the basis of the patterns of cytokine production in $CD4^+$ T cell clones, Mosmann and Coffman[41] originally described two distinct populations. T helper 1 (Th1) clones produce IL-2, IFNγ, and tumor necrosis factor alpha (TNF-α; type 1 cytokines), and Th2 clones produce IL-4, IL-5, IL-6, and IL-10 (type 2 cytokines). Th1 clones mediate cytotoxic and delayed-type hypersensitivity reactions, whereas Th2 cells are more potent helpers for antibody production and humoral responses.[42] Deviation from a type 1 to a type 2 cytokine profile has been associated with decreased protection to tumors.[43] The production of helper cytokines is not a function restricted to $CD4^+$ T helper cells. Other immune cells are able to produce polarized type 1 and type 2 cytokines, including $CD8^+$, NK, NKT, and dendritic cells.

Dendritic Cell Subsets

Dendritic cells have been reported to have both stimulatory and inhibitory effects on the immune system.[26,40] The nature or the activation state of the dendritic cell presenting the antigen may determine the type of immune response generated. There is considerable controversy about the origin of activating and tolerance-inducing dendritic cell subsets. The so-called myeloid dendritic cells and plasmacytoid dendritic cells (also known plasmacytoid monocytes, or lymphoid dendritic cells in mice) are the two principal subpopulations of human dendritic cells (see review in[44]). These two lineages of dendritic cells have been shown to derive from a common committed hematopoietic precursor, without either one having a clearly distinct role in immune activation or tolerance. However, the maturation status of the dendritic cells, although not clearly defined, does have an impact on their ability to generate immunity or tolerance.[45]

Cytokine Deprivation

IL-2 is a critical cytokine for the survival of activated T cells. When a T cell is activated after the recognition of its specific antigenic epitope presented by the appropriate MHC molecule, it starts dividing to generate a large pool of clonal T cells specific for that antigen. This leads to a several-fold increase in the number of T cells in that clone, which occupy a large space in lymphoid organs and peripheral blood. To maintain this clonal expansion of T cells under control, activated T cells become exquisitely sensitive to the lack of IL-2, leading to cell death under conditions of IL-2 deprivation (see review in[46]).

Activation-Induced Cell Death

MHC-antigen–TCR recognition has the dual ability to stimulate and inhibit T-cell responses, which helps prevent the accumulation of activated lymphocytes that are no longer needed. This process is known as activation-induced cell death and functions as a homeostatic mechanism for the establishment and maintenance of tolerance to endogenous antigens (see review in[47]). When resting T cells first recognize an MHC-peptide complex, they become activated and are resistant to apoptotic death to allow adequate development of an immune response. However, after repetitive or continuous antigen exposure, the activated T cells upregulate the proapoptotic Fas receptor and Fas ligand molecules, leading to T-cell death.

Costimulation and CTL Antigen 4 (CTLA-4)

The natural ligand for signal 2 provided by the costimulatory molecules B7.1 (CD80) and B7.2 (CD86) is the CD28 molecule, constitutively present on both $CD4^+$ and $CD8^+$ T cells. When these T cells are activated, they upregulate the CTLA-4, which is a homologue of CD28. CTLA-4 competes and successfully displaces CD28, since CTLA-4 has a much higher affinity for B7 molecules, compared to CD28. CTLA-4 functions as a counter-regulatory receptor that attenuates T-cell responses. Cross-linking of CTLA-4 by B7 inhibits T cell activation, IL-2 production, and T-cell proliferation by directly inhibiting TCR signal transduction (see review in[48]). In animal models, blockade of CTLA-4 enhances antitumor responses and potentiates the activity of cancer vaccines.[48]

Regulatory T Cells

The existence of peripheral lymphocytes with a professional suppressor function has been suspected for many years, but better characterization of these cells is recent. A distinct population of 5% to 10% of the total $CD4^+$ T cells has a constitutive expression of CD25 (the alpha chain of the IL-2 receptor), which is only expressed by other $CD4^+$ T cells after TCR activation. These constitutive $CD4^+$ and $CD25^+$ T cells actively suppress autoreactivity because in their presence other lymphocytes specific for self antigens fail to react to them. The depletion of these cells leads to the development of autoimmune diseases such as colitis or encephalitis and the potentiation of antitumor responses (see review in[49]). These cells have been remarkably conserved in evolution, with similar properties in different species. These properties include a limited ability to proliferate, constitutive expression of intracellular and surface CTLA-4, and an ability to produce the immune-suppressive cytokines IL-10 and transforming growth factor beta (TGF-β). Among $CD8^+$ T cells, a population with the phenotype of $CD8^+$ and $CD28^-$ may also have a suppressor role on immune responses.[50]

Altered Peptide Ligands

Altered peptide ligands are closely related epitopes with minimal differences in the amino acid sequence that have the

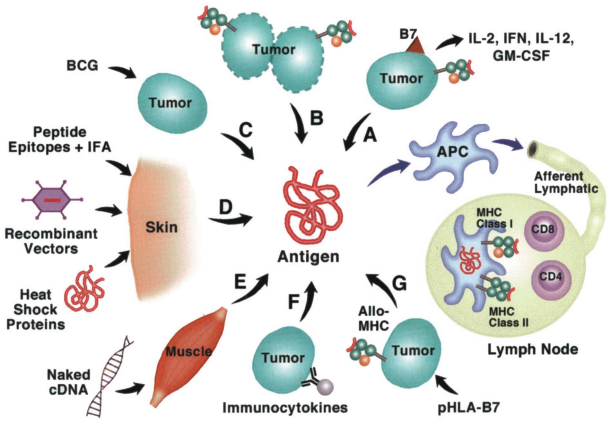

Fig 3. Cross-presentation of tumor antigens derived from cancer vaccines. Several immunologic manipulations lead to a common pathway of cross presentation of proteins derived from tumor antigens. (A) Gene-modified tumor vaccines, (B) whole-cell tumor vaccines; (C) bacillus Calmette-Guérin (BCG); (D) peptide epitope immunization, recombinant viral vector immunization, and heat shock proteins; (E) naked DNA immunization; (F) immunocytokines; and (G) HLA-B7 intratumoral plasmid injection, all lead to release of tumor antigens, which are picked up by host antigen-presenting cells (APCs). These APCs, the most powerful of which are the dendritic cells, circulate through the afferent lymphatic vessels to the T-cell areas of lymph nodes. There they cross-present the tumor antigen to T lymphocytes. IFA, incomplete Freund's adjuvant; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; MHC, major histocompatibility complex.

potential to generate opposing signals (see review in[51]). A heteroclitic APL is an analog of an immunogenic peptide with a modification of one or more amino acids at the TCR contact site, usually the amino acids in the middle of the peptide sequence. Heteroclitic peptides may function as agonists, partial agonists, or even antagonists for a certain TCR specific for the native peptide, which may lead to a change in the cytokine profile, cell proliferation, or cytotoxic activity (see review in[51]). Heteroclitic APLs with antagonistic activity may prove useful in inducing tolerance to antigens that generate autoimmune diseases, and heteroclitic APLs with higher ability to activate T cells have been tested in tumor immunotherapy.[52]

The Tumor Counterattack

Tumor cells have developed mechanisms aimed at avoiding the effects of the immune system, which include downregulation of tumor antigen expression or processing, interference with dendritic cell antigen presentation, direct inhibition of activated lymphocyte function and resistance to immune effector cell–mediated apoptosis. Tumor cells frequently have altered TAP transporter expression resulting in suboptimal peptide delivery to MHC class I molecules and decreased surface presentation of MHC molecules. This leads to impaired antigen presentation, which is known as immune escape because it avoids antigen recognition by tumor antigen-specific activated lymphocytes (see review in[53]).

Tumor cells also protect themselves from dendritic cell-mediated cross-presentation. Tumor cells attract immature APCs by the production of certain cytokines, which skew them to present tumor antigens in a tolerance-inducing setting. The tumor- or tumor stroma–derived soluble factors include IL-6, IL-10, monocyte colony-stimulating factor (M-CSF), prostaglandins, or vascular endothelial growth factor,[54] and prevent the normal differentiation and function of dendritic cells. In addition, tumor cells, or neighboring cells receiving signals from tumor cells, also produce soluble factors that directly interfere with activated lymphocyte function, such as TGF-β, IL-10, or prostaglandin E_2. In addition, tumor cells may become insensitive to apoptotic signals derived from effector immune cells, thereby escaping cell death. Therefore, the tumor microenvironment has developed means to protect itself from stimulatory antigen presentation and T-cell function.

CELLULAR IMMUNOTHERAPY AND CANCER VACCINES

Table 1 summarizes cellular immunotherapy and cancer vaccine approaches. Recognition of the central role of the APC in presenting defined or undefined tumor antigens allows the classification of strategies that focus on tumor antigen presentation to effector cells. Other strategies attempt to bypass the antigen presentation step by directly activating effector cells, either nonspecifically or in an antigen-specific fashion. The recognition of the important autoregulatory mechanisms in limiting the activation of the immune system allows the development of strategies that block these so-called off switches with a net result of immune activation. In addition, tumor-derived immune-suppressor molecules can be blocked to avoid the tumor escape from activated lymphocytes. Lastly, vaccine strategies not directly designed for T-cell activation occasionally have been found to generate T-cell responses. Tables 2 and 3 describe ongoing cancer vaccine clinical trials listed in the National Cancer Institute's Physician Data Query (PDQ) Web site,[55] the NIH Office of Biotechnology Activities' Human Gene Transfer Clinical Trials Database Web site,[56] or presented in major scientific meetings (American Society of Clinical Oncology, American Association for Cancer Research, and American Society of Hematology).

In Vivo APC-Based Vaccines

Intratumoral bacillus Calmette-Guérin (BCG). Intratumoral injection of the BCG may be one of the earliest forms of cellular immunotherapy tested in cancer.[57] The immunologic basis of this phenomenon is that BCG generates an inflammatory process ideal for the attraction of APCs, which pick up tumor antigens released by the tumor cells damaged by the bacterial infection and cross-present them in a so-called danger environment (Fig 3). This form of treatment generates occasional antitumor immune responses. However, its utility in clinical practice has been limited; it is currently restricted to the treatment of superficial bladder cancer.

Intratumoral HLA-B7. The intratumoral injection of the alloantigen HLA-B7 in HLA-B7–negative subjects (this haplotype is not common in the general population) leads to an innate response to the foreign HLA molecule.[58] As in the case of the intratumoral injection of BCG, the recognition of a powerful alloantigen by cells with NK activity allows the recruitment of APCs, among other inflammatory cells, which will pick up tumor antigens released by the HLA-B7–transfected cells and

Table 1. Cellular Immunotherapy for Cancer

In vivo APC
Bacillus Calmette-Guérin: BCG live, Pacis, Biochem Pharma, Inc, Sainte-Foy, Quebec, Canada
Whole-cell cancer vaccines: Melacine, Corixa Corp, Seattle, WA; Cancvaxin, CancerVax Corporation, Carlsbad, CA; OncoVax, Intracel LLC, Frederick, MD; MVAX, AVAX, Overland Park, KS; ONYCR1-3, ONYVax, London, UK; CMVAC
Gene modified cancer vaccines: GVAX, Genesys Inc, Foster City, CA
Recombinant cytokines that increase the availability of APC: GM-CSF (Leukine and Flt-3L, Amgen/Immunex, Seattle, WA; IL-4, Schering-Plough, Kenilworth, NJ)
HLA-B7: Allovectin-7, Vical Inc, San Diego, CA
Heat shock proteins: Oncophage, Antigenics Inc, Woburn, MA Javelin, Mojave Therapeutics, Hawthorne, NY; Oncocine HspE7, Stressgen Biotechnologies Corp, Victoria, BC, Canada
Synthetic peptide epitopes in immunological adjuvants: Multiple Peptide Systems, San DIego, Ca; Epimmune, San Diego, CA; Cytel Inc, San Diego, CA; Peptide Technologies Inc, Gaithersburg, MD, U.C.B. Bioproducts, Braine-l'Allleud, Belgium; United Biochemical Inc, Seattle, WA
Naked plasmid DNA immunization: Leuvectin, Vical Inc, San Diego, CA (Synchrovax SEM, CTL Immunotherapies Corp, Chatsworth, CA; ZYC300, Zycos Inc, Lexington, MA)
Immunization with recombinant viral vectors (ALVAC-B7.1, Aventis, Bridgewater, NJ; CEA-TRICOM and rF-gp100p209, Therion Biologics Corp, Cambridge, MA; MVA-BNtyr, TA-HPV and DISC-GMCSF, Xenova, Berkshire, UK; OncoVax-P, TroVax, Oxford BioMedica, Oxford, UK; TG1041)
Prime-boost strategy (PROSTVAC-VF, Therion Biologics Corp, Cambridge, MA)
Ex vivo APC
Peptide-pulsed dendritic cells (Proverge and Myloverge, Dendreon, Seattle, WA; CaPVax and DCVax-Prostate, Northwest Biotherapeutics, Bothell, WA)
Dendritic cells pulsed with tumor lysates (DCVax-Lung and DCVax-Brain, Northwest Biotherapeutics, Bothell, WA)
Dendritic cells transfected with tumor-derived messanger RNA
Gene-modified dendritic cells
Dendritic cell/cancer cell hybrids
Exosomes (Dexosomes, Anosys, Menlo Park, CA; Dendritophages, Immuno-Designed Molecules, Paris, France)
Nonspecific immunologic stimulants
Recombinant cytokines: IL-2, Proleukin, Chiron Therapeutis, Suresnes, France; IFN-α2, Intron-A, Schering Corp, Kenilworth, NJ; Roferon A, Hoffman LaRoche, Nutley, NJ; Infergen, Amgen, Thousand Oaks, CA; Alferon N, Interferon Sciences Inc., New Brunswick, NJ; Pegasys, Hoffmann-La Roche, Nutley, NJ; Peg-Intron, Schering-Plough, Kenilworth, NJ; IFN-γ, Immukin, Boehringer Ingelhiem, Ingelheim, Germany; IL-12 (Genetics Institute, Cambridge, MA), IL-18, Schering-Plough, Kenilworth, NJ; CD40-L (Avrend, Amgen/Immunex, Seattle, WA)
Intratumoral plasmid injection: IL-2, Leuvectin, Vical Inc, San Diego, CA; IFNα, IL-12, Valentis, Burlingamee, CA
Immunocytokines (KS-IL2 and h14.18-IL2, EMD Pharmaceuticals, Durham, NC)
Adoptive transfer
Allogeneic stem cell transplant
Minidose allogeneic transplant
Donor leukocyte infusion
Lymphokine-activated killers
Tumor-infiltrating lymphocyte infusion
Cloned CTL
Gene-modified CTL
Blockade of immunosuppressors
Anti-CTLA4 (MDX010, Medarex, Princeton, NJ)
TGF-β antisense (TGF-β2, NovaRX, San Diego, CA)
COX-2 inhibition: Celebrex, Pfizer, Groton, CN; Vioxx, Merck, Whitehouse Station, NJ
Non–T-cell–directed vaccines
Anticarbohydrates GMK, Progenics Pharmaceuticals Inc, Tarrytown, NJ; Theratope, Biomira, Edmonton, Alberta, Canada
Anti-idiotype antibodies: OvaRex, AltaRex, Waltham, MA; CeaVax, TriAb, TriGem, Titan Pharmaceuticals Inc, South San Francisco, CA
Antihormones: Gastrin 17, Aphton Corp, Woodland, CA; Avicine, AVI Biopharma, Portland, OR
Antisurface proteins: Rituxan, Herceptin, Genentech Inc, South San Francisco, CA

NOTE. Examples of candidate products under development are noted in parentheses.

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; APC, antigen-presenting cells; IL-2, interleukin-2; IFNα2, interferon alfa-2; TGF-β, transforming growth factor beta; COX-2, cyclo-oxygenase-2; CTL, cytotoxic lymphocytes.

cross-present them to cytotoxic effector cells (Fig 3). These tumor antigen-specific $CD8^+$ CTLs would then be permitted to attack other tumor cells without the requirement of the presence of the alloantigen HLA-B7 on tumor cells. Clinical development of intratumoral plasmid injection has the limitation of low transduction efficiency of the target cells, and is usually restricted to cells located adjacent to the needle track. This leads to poor allo-MHC molecule expression in the tumor, resulting in weak immunologic responses.

Whole-cell tumor vaccines. Whole-cell tumor vaccines have undergone decades of clinical investigation. Allogeneic or autologous tumor cells are processed (lysates or irradiated cells) to optimize the release of their antigens, and are injected together with powerful immunologic adjuvants, or haptens (BCG, diphtheria toxin, dinitrophenyl, keyhole limpet hemocyanin, virus), or both, with the rationale of presenting the tumor antigens in an inflammatory context to attract host APCs (Fig 3). Whole-cell autologous tumor vaccines are personalized vaccines, and it can be assumed that they contain the relevant tumor antigens; however, the logistic drawback is that it is difficult to obtain and individually prepare vaccines for each patient. To avoid this problem, other tumor cell vaccines have been formulated as lysates of allogeneic laboratory cell lines containing shared tumor antigens.[59,60] The main drawback of both autologous and allogeneic whole-cell tumor vaccines is their limited ability to stimulate immune responses. In fact, the majority of completed randomized clinical trials using this class of tumor vaccines have not been able to reflect a clear antitumor activity.[59-62] Other large randomized trials are ongoing (Table 3).

Gene-modified tumor vaccines. Gene-modified tumor vaccines are usually composed of autologous tumor cells stably transfected with an immunostimulatory gene (see review in[63]). The original hypothesis was that the paracrine expression of cytokines such as IL-2 or IFNγ, or the costimulatory molecule B7.1, would allow the tumor cell to provide all of the signals for direct cytotoxic T cell activation, bypassing the need for host APCs and $CD4^+$ T lymphocyte help.[3,64] However, comparison of the antitumor capacity of gene-modified tumor vaccines in preclinical models was surprising in that the introduction of GM-CSF into tumor cells produced the most active vaccine.[65] Bone marrow chimeras were used to show that the GM-CSF gene-modified tumor vaccines attracted host APCs, which picked up tumor antigens and cross-presented them to the host immune system.[3] This same mechanism of action was implicated in gene-modified tumor vaccines expressing other immunostimulatory molecules, thereby providing a rational explanation for the superiority of tumor vaccines expressing GM-CSF (Fig 3).

These cytokine-modified autologous tumor cell vaccines have been tested in clinical trials for several years.[66-68] Their manufacture requires tumor cell cultures from each patient, followed by selection of cells that adequately express the transgene (which may take months). To avoid this long manufacture process, other avenues have been explored, including the use of allogeneic gene-modified tumor cell vaccines, transfection of autologous noncancerous cells that are easier to obtain and gene-modify (usually fibroblasts), the use of other bystander cells coinjected

Table 2. Ongoing Phase I or II Nonrandomized Trials of Cancer Vaccines

Type of Vaccine	Type of Cancer	Vaccine Composition	Adjuvant
Whole-cell or lysate cancer vaccines	Advanced	Autologous or allogeneic cancer cells	IFNα
	Advanced	Autologous cancer cells + IFN	GM-CSF
	ALL	Autologous cancer cells	KLH
	ALL	Autologous cancer cells	KLH + CD40-L
	NHL	Autologous cancer cells	IL-2
	NHL	Autologous cancer cells	KLH + GM-CSF
	NHL	Autologous cancer cells	IL-2
	Melanoma	Autologous cancer	DNP + BCG
	Melanoma	Allogeneic cancer cells	BCG
	Myeloma	Autologous cancer cells	GM-CSF
	Brain	Autologous cancer cells	GM-CSF
	Cervical	Autologous HPV-positive cancer cells	
	GI	Autologous cancer cells	BCG
	GI	Allogeneic cancer cells	BCG
	GI	Allogeneic melanoma cells	
Gene modified cancer cells	Neuroblastoma	Autologous or partially HLA-matched allogeneic cancer cells + IL-2	
	NSCLC	Autologous cancer cells + GM-CSF	
	NSCLC	K562-GM-CSF + autologous cancer cells	
	NSCLC	Allogeneic cancer cells + B7.1-HLA	
	AML	Autologous cancer cells + GM-CSF	
	CLL	Autologous cancer cells + AdCD40-L	
	Myeloma	Autologous cancer cells + GM-CSF	
	NSCLC	Allogeneic cancer cells + IL-2 + TGF-β antisense	
	Pancreatic	Autologous cancer cells + GM-CSF	
	Pancreatic	Allogeneic cancer cells + IFN	GM-CSF
	Prostate	Autologous cancer cells + GM-CSF	
	Breast	Allogeneic cancer cells + B7.1	GM-CSF
	Ovarian	Autologous cancer cells + B7.1	IFNα
	Ovarian	Autologous cancer cells + GM-CSF	
	RCC	Autologous cancer cells + B7.1	IL-2
Ex vivo–activated lymphocytes	Advanced	Ras peptide-specific lymphocytes	IL-2
	CNS	Vaccine-primed lymphocytes	SEA + IL-2
	CNS	PBMC postautologous tumor vaccine	IL-2
	EBV-positive cancer p53- or Ras-positive	EBV-specific T lymphocytes p53- or Ras-PAL	IL-2
	NHL	Cloned CD20-specific $CD8^+$ lymphocytes	
	Melanoma	gp-100–activated T lymphocytes	IL-2
	RCC	Vaccine-primed lymphocytes	Anti-CD3 + IL-2
	RCC	IL-12–activated T lymphocytes	
	GI	IL-12–activated T lymphocytes	
Gene-modified lymphocytes	Neuroblastoma	CD8 lymphocytes + CE7R scFvFc:zeta immunoreceptor	
	NHL	Lymphocytes + CD20 immunoreceptor	
	Melanoma	Lymphocytes + MART-1 TCR	IL-2
	Melanoma	Lymphocytes + IL-2	
	Ovarian	Lymphocytes + folate-binding protein TCR	
	GI	Lymphocytes + CEA TCR	
Heat shock proteins	Sarcoma	Autologous Hsp-96	
	CLL/NHL	Autologous Hsp-70	
	NHL	Autologous Hsp-96	
	CML	Autologous Hsp-70	
	Breast	Autologous Hsp-70	
	Melanoma	Autologous Hsp-70	
	Melanoma	Autologous Hsp-96	IFNα + GM-CSF
	Gastric	Autologous Hsp-96	
	GI	Autologous Hsp-96	
	Pancreatic	Autologous Hsp-96	
Immunocytokines	Epithelial	KSA-IL2	
	Melanoma	GD2-IL2	

Table 2. Ongoing Phase I or II Nonrandomized Trials of Cancer Vaccines (Cont'd)

Type of Vaccine	Type of Cancer	Vaccine Composition	Adjuvant
Naked DNA	Advanced	CYP1B1 plasmid	
	Head and neck	HLA-B7 plasmid	
	Melanoma	gp100 plasmid	
	Melanoma	gp100 plasmid	IL-2
	Melanoma	HLA-B7 plasmid	
	Melanoma	MUC-1 plasmid	
	Melanoma	Tyrosinase plasmid	
	Melanoma	MART-1 plasmid	
	RCC	IL-2 plasmid	
	Prostate	IL-2 plasmid	
	Skin metastasis	IL-12 plasmid	
Viral vectors	Advanced	HSV + GM-CSF	
	CNS	Adenovirus + IFN-β	
	Head and neck	Fowlpox-CEA-B7-ICAM-LFA	
	NSCLC	Adenovirus - CD40-L	
	Melanoma	Adenovirus - IFNγ	
	Melanoma	Adenovirus - MART-1 + adenovirus-gp100	IL-2
	Melanoma	Fowlpox-gp100	
	Melanoma	Vaccinia-tyrosinase	
	Melanoma	Vaccinia-B7.1	
	Melanoma	Vaccinia-CEA-B7-ICAM-LFA	
	Melanoma	Vaccinia-GM-CSF	
	GI	Fowlpox-CEA-B7-ICAM-LFA	
	GI	Fowlpox-CEA-B7-ICAM-LFA and Vaccinia-CEA-B7-ICAM-LFA	IL-2
	GI	Vaccinia-CEA-B7-ICAM-LFA → avipox-CEA-B7-ICAM-LFA	GM-CSF
	Colon	Vaccinia-5T4 antigen	
	Breast	Vaccinia-DF3/MUC-1	
	Cervical	Vaccinia-HPV-IL-2	
	Cervical	Vaccinia-HPV E6-E7	
	Prostate	Adenovirus-IL-12	
	Prostate	Adenovirus-PSA	Gelfoam
	Prostate	Vaccinia-PSA	
	Prostate	Vaccinia-MUC-1-IL-2	
	Prostate	Vaccinia-PSA + vaccinia-B7.1 → fowlpox-PSA	GM-CSF + IL-2
	Prostate	Baculovirus-PSA	GM-CSF + BCG + CY
Peptides	Advanced	Ras peptide	Detox PC + IL-2 + GM-CSF
	MAGE-12–positive	MAGE-12 peptide	Montanide ISA-51
	NY-ESO–positive	NY-ESO peptide	GM-CSF
	Sarcoma	NY-ESO peptide	GM-CSF
	Leukemia	PR1 peptide	Montanide ISA-51
	CML	bcr/abl peptide	QS-21
	Myeloma	Idiotype peptide	KLH + GM-CSF
	Melanoma	gp100 peptide	IL-2
	Melanoma	GnT-V peptide	
	Melanoma	MART-1 + gp100 peptides	Montanide ISA-51
	Melanoma	gp100 + tyrosinase peptides	Montanide ISA-51 + GM-CSF
	Melanoma	Tyrosinase + gp100 + MART-1 peptides	Montanide ISA-51 + GM-CSF
	Melanoma	MART-1 + gp100 + tyrosinase peptides	Flt-3L
	Melanoma	MART-1 + tyrosinase + NY-ESO peptides	Flt-3L
	Melanoma	MART-1 + gp100 + MHC class II epitopes peptides	Montanide ISA-51
	Melanoma	MART-1 + gp100 peptides	GM-CSF Plasmid
	Melanoma	gp100 peptides	Montanide ISA-51 + anti-CTLA4
	Melanoma	MART-1 + gp100 + tyrosinase peptides	Montanide ISA-51 + G-CSF-Ftl3L
	Melanoma	MART-1 + gp100 + tyrosinase peptides	Montanide ISA-51 + anti-CTLA4
	NSCLC	Ras peptide	GM-CSF
	NSCLC	Cyclophilin B peptides	IFA
	NSCLC	NY-ESO peptide	GM-CSF
	Ras-positive cancer	Ras peptide	Detox B
	Ras-positive cancer	Ras peptide	Detox PC + IL-2 + GM-CSF

Table 2. Ongoing Phase I or II Nonrandomized Trials of Cancer Vaccines (Cont'd)

Type of Vaccine	Type of Cancer	Vaccine Composition	Adjuvant
	Prostate	PSA peptide	GM-CSF + IL-2
	Prostate	MUC-1 peptide + globo H + Lewys-y	KLH + QS21
	Her2/neu-positive	MVF-Her-2-CRL peptide	
	Ovarian	p53 peptide	Montanide ISA-51 + GM-CSF
	Ovarian	p53 peptide	Montanide ISA-51 + GM-CSF + IL-2
	Cervical	HPV E6 + E7 peptides	GM-CSF
	Pancreas	Telomerase peptide	GM-CSF
	Hepatocellular Carcinoma	AFP peptides	Montanide ISA-51
Proteins	NHL	Idiotype protein-KLH	GM-CSF
	NHL	Fab idiotype protein	GM-CSF
	Melanoma	MAGE-3 protein	CpG
	NY-ESO–positive	NY-ESO protein	
Dendritic cells/APC	Advanced	DC + p53 peptide	
	Advanced	DC + Ras peptide	IL-2
	CNS	DC + autologous tumor lysate	
	Myeloma	DC + M protein	
	Melanoma	DC + MART-1 peptide	
	Melanoma	DC + gp100 + tyrosinase peptide	
	Melanoma	DC + MART-1 + gp100 peptides	IL-2
	Melanoma	DC + MART-1 + gp100 + tyrosinase peptides	CD40-L + IFNγ + IL-2
	Melanoma	DC + irradiated tumor cells	GM-CSF
	Melanoma	DC + allogeneic tumor lysates	
	Melanoma	DC + allogeneic tumor lysate-vaccinia IL-2	
	Melanoma	DC + apoptotic bodies	
	Melanoma	DC exosomes + MART-1 peptide	
	Melanoma	PBMC + MART-1 peptide	IL-12
	NSCLC	DC + autologous tumor lysate	
	NSCLC	DC + p53 peptide	
	Breast	DC + p53 peptide	IL-2
	Breast	DC + Her2/neu peptide	
	Ovary	DC + p53 peptide	IL-2
	Cervical	DC + HPV-16 E7 peptide	
	Cervical	PBMC + HPV-16 E6 or E7 peptide	
	Gynecologic	DC + tumor lysates	KLH
	RCC	DC + allogeneic tumor lysate	GM-CSF
	Prostate	DC + PSMA peptide	BCG
	Prostate	DC + allogeneic tumor lysate	KLH
	Prostate	PBMC + PSMA peptide	IL-12
	Sarcoma	DC + Ewing's fusion peptide	
	Hepatocellular	DC + AFP peptides	
	Hepatocellular	DC + allogeneic tumor lysate	
	p53- or Ras-positive	APC + p53 or Ras-mutated peptides	
	MUC-1–positive	DC + MUC-1 protein	
Gene modified dendritic cells	Advanced	DC + Autologous tumor RNA	
	GI	DC + CEA RNA	
	GI	DC + Autologous tumor RNA	
	Melanoma	DC + Adenovirus-MART-1	
	Melanoma	DC + Adenovirus-gp100	
	Melanoma	DC + Adenovirus-MART-1 + adenovirus-gp100	IL-2
	Breast	DC + Autologous tumor RNA	
	RCC	DC + Autologous tumor RNA	
	Prostate	DC + PSA RNA	
	CEA-positive	DC + Fowlpox-CEA-B7-ICAM-LFA	
	CEA-positive	DC + Avipox-CEA-B7-ICAM-LFA	

Abbreviations: NHL, non-Hodgkin's lymphoma; ALL, acute lymphoblastic leukemia; GI, gastrointestinal cancer; NSCLC, non–small-cell lung cancer; RCC, renal cell carcinoma; DC, dendritic cells; APC, antigen-presenting cells; PBMC, peripheral-blood mononuclear cells; PAL, peptide-activated lymphocytes; EBV, Epstein-Barr virus; HPV, human papilloma virus; IFN, interferon; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; BCG, bacillus Calmette-Guérin; DNP, dinitrophenyl; KLH, keyhole limpet hemocyanin; IFA, incomplete Freund's adjuvant; SEA, Staphylococcus superantigen; HSV, herpes simplex virus; TCR, T-cell receptor; CEA, carcinoembryonic antigen; PSA, prostate-specific antigen; AFP, alpha-fetoprotein; CYP1B1, cytochrome P450 1B1; GnT-V, *N*-acetylglucosaminyltransferase-V; CD40-L, CD40 ligand; CY, cyclophosphamide; CLL, chronic lymphocytic leukemia; Hsp, heat shock protein; GD2, disialoganglioside antigen; MART-1, melanoma antigen recognized by T-cells; MAGE, melanoma antigen; NY-ESO, New York branch of Ludwig Institute esophageal antigen number 1; MHC, major histocompatibility complex; PSMA, prostate specific membrane antigen; CTLA4, cytotoxic T lymphocyte antigen-4; G-CSF, granulocyte colony-stimulating factor.

Table 3. Ongoing Phase I, II, or III Randomized Trials of Cancer Vaccines

Type of Vaccine	Phase	Type of Cancer	Vaccine Composition	Comparison
Whole-cell or lysate cancer vaccines	II	Advanced	Autologous cells	± IFNγ or GM-CSF
	II	Melanoma	Autologous cell lysates	± Cyclophosphamide
	II	RCC	Autologous cell lysates	± Cyclophosphamide
	III	Melanoma	Allogeneic cells + BCG	BCG
	III	Melanoma	Allogeneic cells + Detox	Observation
Viral vectors	I	Advanced	Fowlpox-B7.1	Fowlpox-B7-ICAM-LFA
	II	GI	Vaccinia-CEA + avipox-CEA	ID versus IM
	II	GI	Avipox-CEA-B7 + chemotherapy	Chemotherapy
	II	GI	Avipox-CEA-B7-ICAM-LFA	Recombinant GM-CSF versus GM-CSF Transduction
	II	Melanoma	Fowlpox-tyrosinase + vaccinia-tyrosinase	± IL-2
	II	Prostate	XRT + vaccinia-PSA + vaccinia-B7.1 + fowlpox-PSA	XRT
	II	Prostate	Fowlpox-PSA → vaccinia-PSA	Vaccinia-PSA → fowlpox-PSA
	II	Prostate	Vaccinia-PSA + rV-B7.1 + fowlpox-PSA + GM-CSF + IL-2	Nilutamide
	II	Prostate	Vaccinia-PSA + rV-B7.1 + fowlpox-PSA + XRT	XRT
	II	Prostate	Vaccinia-MUC-1-IL-2	Different schedules
Naked DNA	I	Prostate	PSMA plasmid	Human versus murine
	III	Melanoma	IL-2 plasmid + chemotherapy	Chemotherapy
	III	Melanoma	IFN plasmid	Different schedules
Peptides	I	MAGE-12–positive	MAGE-12 + montanide ISA-51	Different schedules
	I	CML, AML, MDS	PR1 peptide + montanide ISA-51	Different doses
	I	Melanoma	gp100 + MART-1 + tyrosinase + montanide ISA-51	± GM-CSF
	I	Melanoma	MART-1 + tyrosinase + MAGE-10 + NY-ESO	± GM-CSF
	I	p53-positive	p53 peptide + IL-2	Different routes
	II	Advanced	Telomerase peptide + montanide ISA-51	Different schedules
	II	Melanoma	gp100 + MART-1 peptides + montanide ISA-51	± IL-2
	II	Melanoma	gp100 + tyrosinase peptides + montanide ISA-51 + GM-CSF	± GM-CSF
	II	Melanoma	gp100 peptide + montanide ISA-51	± tyrosinase + TRP-1 peptides
	II	Melanoma	gp100 + tyrosinase + MART-1 peptides + montanide ISA-51	± GM-CSF or + IFNα or + GM-CSF and IFNα
	II	Melanoma	gp100 + tyrosinase + MART-1 peptides + IL-12 + montanide ISA-51	Alum adjuvant or GM-CSF
	II	Melanoma	Tyrosinase + MART-1 + NY-ESO + flu peptides + montanide ISA-51	Flt3-L
	II	Melanoma	gp100 peptide + montanide ISA-51	± IL-2
	II	Melanoma	gp100 + tyrosinase + TRP-1 peptides	Different schedules
	II	Melanoma	ESO-1 peptide + montanide ISA-51	Different schedules
	II	Melanoma	MART-1 peptide	Montanide ISA-51 or GM-CSF
	II	Melanoma	4× gp100 + 3× tyrosinase + 4× MAGE + 1× NY-ESO (total 12)	Four peptides total + GM-CSF + montanide ISA-51
		Peptides + GM-CSF + montanide ISA-51		
	II	GI	Ras peptide + GM-CSF	Different doses
	II	GI	CEA peptide + montanide ISA-51	CEA peptides + GM-CSF
	II	RCC	von Hippel-Lindau peptide montanide ISA-51	± GM-CSF + IL-2
	II	Prostate	Her-2/neu E75	± GM-CSF or Flt3-L
	III	Melanoma	IL-2 + gp100 peptide + montanide ISA-51	IL-2
	III	Melanoma	IL-2	± gp100 peptide
	III	Melanoma	gp100 + tyrosinase + MART-1 Peptides + montanide ISA-51 + GM-CSF	GM-CSF
	III	Ocular melanoma	NA17 + gp100 + tyrosinase + MART-1 peptides	Observation
Protein	III	NHL	Idiotype peptide + KLH	± GM-CSF
	III	NHL	Idiotype peptide + KLH	KLH + GM-CSF
Heat shock proteins	III	RCC	Autologous Hsp-96	Observation
	III	Melanoma	Autologous Hsp-96	Standard therapy
Dendritic cells-APC	I	Melanoma	DC + gp100 + MART-1 peptides	Different routes
	I	Melanoma	DC + gp100 + MART-1 peptides	± IL-2
	II	Advanced	PBMC + GM-CSF + p53 + Ras peptides + PAL	PBMC + GM-CSF + p53 + Ras peptides
	II	Melanoma	Monocyte-derived DC + gp100 + MART-1	CD34-derived DC + gp100 + MART-1
	II	Melanoma	DC + autologous cancer lysates	Different routes
	III	Prostate	DC + PAP + GM-CSF	DC

Abbreviations: NHL, non-Hodgkin's lymphoma; GI, gastrointestinal cancer; RCC, renal cell carcinoma; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; Flt3-L, Flit-3 ligand; BCG, bacillus Calmette-Guérin; KLH, keyhole limpet hemocyanin; XRT, x-ray therapy; PAL, peptide-activated lymphocytes; DC, dendritic cells; APC, antigen-presenting cells; PBMC, peripheral blood mononuclear cells; CEA, carcinoembryonic antigen; PSA, prostate-specific antigen; PAP, prostatic acid phosphatase; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; IFNγ, interferon gamma; MART-1, Melanoma Antigen Recognized by T-cells; MAGE, Melanoma Antigen; NY-ESO, New York branch of Ludwig Institute esophageal antigen number 1.

with autologous tumor cells, or the use of viral vectors with enhanced transduction efficiency.[69] These strategies decrease vaccine production time. Tumor cells are usually not good antigen presenters per se, and these whole-cell–based vaccines have additional problems when immunosuppressive molecules are produced by the tumor cells.

Heat shock proteins. Heat shock proteins are intracellular molecules that act as chaperones for antigens.[70,71] When a cell is subjected to temperature changes, heat shock proteins bind to intracellular peptides. These complexes can be isolated, which provides an efficient method of obtaining purified, tumor-derived, nondefined antigens. Tumor peptide–heat shock protein complexes can be administered as vaccines to humans, and these peptide complexes will require cross-presentation by host APCs to generate a cellular immune response (Fig 3). Dendritic cells have a specific receptor for heat shock proteins (CD91) and its engagement leads to maturation of the dendritic cells.[72] Therefore, heat shock protein complexes released by necrotic cells function as endogenous danger signals as well as a method to cross-present tumor antigens by dendritic cells. Heat shock proteins explored for clinical immunotherapy may contain a defined antigen (E7 antigen derived from the human papilloma virus, MAGE tumor antigen) or nondefined tumor antigens, which involve the individualized production of heat shock proteins from fresh tumor samples. Procurement of individualized heat shock protein–tumor antigen vaccines has been successfully demonstrated in a multi-institutional trial setting with a centralized processing facility, which assesses the feasibility of this approach for large-scale clinical testing.

Peptide-based vaccines. Tumor-derived peptide epitopes that contain the appropriate HLA-restricted amino acid sequence[6] can be synthetically manufactured and administered together with an immunologic adjuvant (an agent used to augment the immune response to an antigen; Tables 2 and 3). This allows the attraction of host APCs to the immunization site and epitope cross-presentation (Fig 3).[73] Many immunodominant epitopes from tumor antigens have a low to intermediate binding affinity for the MHC molecule and are subdominant epitopes recognized by low-affinity T cells that have evaded central tolerance selection in the thymus. This low binding affinity usually is due to lack of optimal amino acids at the peptide anchoring sites, usually at positions 2 and 9 for MHC class I–binding peptides.[74] Preclinical studies and studies in humans have demonstrated that tumor-derived peptides engineered to have an enhanced ability to bind to MHC molecules by substitution of amino acids at anchor positions (known as fixed-anchor analogues) lead to enhanced immune responses.[73] Their main advantage is the ease of manufacture and storage. Peptide-based vaccines require knowledge and matching of the exact HLA haplotype and antigen to provide the appropriate peptide epitope for each individual. For example, the most common HLA in the general population is HLA-A*0201 (or HLA-A2.1), which accounts for 30% to 40% of the major ethnicities. An HLA-A*0201 peptide-based approach would therefore only be suitable for slightly more than one third of the patients whose tumors express a certain tumor antigen for which the immunodominant HLA-binding epitope has been defined, which is currently limited to few cancers.[4] Another limitation includes the number of antigens that can be administered as a vaccine; it is possible that the tumor cells simply stop expressing that antigen or antigens (antigen-variant escape mutants).

Naked DNA. Intramuscular injection of naked DNA sequences results in gene expression and the generation of immune responses.[75,76] These DNA plasmids, which consist of an antigen gene regulated by a promoter with constitutive activity (which is always on, like the cytomegalovirus early enhancer-promoter), can also be conjugated with gold particles and propelled into the skin using a helium gas gene gun. The protein antigen produced by the target cells (usually myocytes or fibroblasts, depending on the injection route) is taken up by host APCs, processed, and cross-presented to the immune system in the draining lymph nodes (Fig 3), although direct transfection of rare APCs residing at the injection site has also been demonstrated. In addition to the limitations of its restricted use in the few tumors with molecularly defined antigens (a limitation common to all antigen-specific vaccines), naked DNA plasmids have low immunologic potency for generation of antitumor responses to self antigens in humans.[4] However, naked DNA immunization may have a future as vaccines for xenoantigens of infectious diseases or cancer (such as epitopes from the human papilloma virus), for which more promising data have been obtained.[77-79] Avenues to increase the immunologic potency of DNA-based genetic immunization include increasing the plasmid dose (primates and humans seem to require much higher doses than smaller animals to generate detectable immune responses); facilitating the coexpression of immunostimulatory molecules such as cytokines, chemokines, or costimulatory molecules; and inserting an alpha virus replicon or herpes virus intracellular transport proteins.

Viral vectors. A variety of gene therapy vectors have been adapted to cancer immunotherapy. Tumor antigen DNA sequences can be inserted into attenuated pox viruses that are unable to replicate in mammalian hosts (such as modified vaccinia Ankara, fowlpox, or canarypox). Other vectors include recombinant replication-incompetent viral vectors (adenovirus, retrovirus, lentivirus), which are modified viruses that have been specifically mutated to be incapable of self-replication into infectious progeny virions after infection of a single target cell, but that efficiently express the foreign gene inserted in the vector (see reviews in[4,80]). This form of genetic immunization has also resulted in weak immunologic responses in humans,[81] although pox vectors have demonstrated a clear ability to stimulate antigen-specific T cells.[82] The low immunogenicity to the tumor antigen may be due, in part, to the presence of pre-existing neutralizing antibodies to the vector (which is common for adenoviruses), low intrinsic ability to stimulate an immune response to the transgene as opposed to the viral antigens, viral epitope dominance that decreases the host immune response to the tumor antigen epitopes, or the skewing of the response to a humoral instead of a cytotoxic response. Enhancing the immune potency of viral vector immunization can be achieved by the coexpression of cytokines or costimulatory molecules in the viral vector because these viral vectors usually have a large capacity

to carry and express multiple genes.[83] Several such vectors are in clinical trial development, and usually carry a tumor antigen gene and costimulatory, adhesion, or other immune-enhancing molecules (Tables 2 and 3).

The prime-boost strategy. The sequential administration of naked DNA and a viral vector has resulted in synergistic immune activation; it is a potent method of generating immune responses to tumor antigens in what is now known as the prime-boost strategy (see review in[84]). The initial injection of a plasmid allows the activation of infrequent T cells without other immune cells competing for the antigen because the naked DNA has a limited inflammatory potential. After a rest period, these antigen-specific high-avidity lymphocytes are boosted by the re-exposure to the same antigen, now in a more inflammatory milieu generated by the highly immunogenic viral proteins from the recombinant viral vector. Preclinical murine and primate models have shown that this heterologous prime-boost regimen induces 10- to 100-fold higher frequencies of T cells than do naked DNA or recombinant viral vectors alone.[84] A modification of this strategy is the sequential administration of two different viral vectors carrying the same tumor antigen gene, which bypasses the limitation of the development of neutralizing antibodies to the viral backbone by boosting with a different vector without shared viral epitopes.[85,86] These strategies, which avoid the need of cell culture common to the majority of highly immunologically active vaccine strategies, are rapidly undergoing clinical testing for infectious disease and cancer.[84-86]

Bacterial vectors. Tumor antigen gene segments have also been introduced into bacteria such as *Salmonella* and *Listeria*, resulting in protective immunity in animal models.[87] Advantages may include the ability to use the oral route for immunization and the strong inflammatory milieu created by bacterial products, leading to the attraction of APCs, and a preferential Th1 cytokine polarizing pattern stimulated by certain bacteria such as *Listeria*.

Augmentation of the number of APCs. As can be noted by the mechanism of action of most of the prior immunologic maneuvers, the common pathway of anticancer immune activation is the recruitment and activation of host APCs to cross-present tumor antigens to effector $CD8^+$ cytotoxic T cells (Fig 3). Cytokines such as GM-CSF have been used as vaccine adjuvants with the hope of attracting and activating dendritic cells locally at the site of vaccination. Other strategies are aimed at systemically expanding the dendritic cell pool in the hosts, which may be achieved by the administration of cytokines such as the combination of GM-CSF and IL-4,[88] or the administration of Flt-3 ligand.[89] In retrospective studies of tumor biopsies, a greater number of APCs infiltrating the cancer have been correlated with improvements in survival.[90] This increase in the availability of intratumoral APCs may allow more efficient cross-presentation of tumor antigens. This is being explored in human clinical trials.[88,91,92]

Ex Vivo APC-Based Vaccines

Dendritic cells. The description of culture procedures to generate large quantities of dendritic cells ex vivo starting from hematopoietic precursors or peripheral blood monocytes has allowed extensive testing in promising preclinical models and pilot clinical trials (see reviews in[28,29]). Different antigen loading procedures have been used for dendritic cell antigen presentation. For well-characterized antigens, synthetic HLA-binding peptide epitopes or the complete DNA sequence in a viral vector can be used to load the dendritic cell vaccines (see review in[93]). Dendritic cells pulsed with peptide epitopes and genetically-modified with recombinant viral vectors are conceptually similar to the vaccination with peptides in immunologic adjuvants or the direct administration of recombinant viruses, respectively, in which the dendritic cells should be perceived as powerful immunologic adjuvants for the tumor antigen. In addition, dendritic cells can be loaded with defined antigens to take advantage of antigen uptake surface receptors, such as FC receptors to take up immune complexes carrying a tumor antigen.[94]

Several methods of loading dendritic cells with uncharacterized tumor antigens have also been tested. Tumor lysates or apoptotic bodies containing uncharacterized tumor antigens can be fed to dendritic cells to take advantage of the superior ability of these cells to macropinocytose and endocytose foreign material. Whole sequences from unique cancer-derived proteins, such as idiotypes from the variable region of immunoglobulins produced in myelomas and B-cell lymphomas, can also be coincubated with dendritic cells to allow their endogenous processing and MHC class I and II presentation.[95] mRNA can be isolated from tumor cells and inserted into dendritic cells, which would allow the dendritic cells to produce the same proteins as the tumor cells and allow presentation of uncharacterized antigens.[96] Finally, dendritic cell–tumor cell hybrids constructed using techniques similar to those used to generate hybridomas allow the endogenous processing and presentation of the proteins produced by the tumor cells with the dendritic cell's antigen presenting machinery,[97] although this technique requires additional validation.[98]

Preclinical studies have tested the value of direct intratumoral injection of ex vivo–generated dendritic cells, thereby avoiding the need for tumor antigen loading ex vivo. Because these cells need to pick up the antigen and then move to the T-cell areas of lymph nodes, the most promising approaches have involved gene modification using immunostimulatory cytokines and chemokines such as IL-2, IL-7, IL-12, CD40-L, GM-CSF, lymphotactin, or secondary lymphoid tissue chemokine (SLC), which would improve antigen presentation or migration to lymph nodes.[93]

Dendritic cell-based strategies have been used in clinical trials with initial promising results in phase I and II studies.[29] These trials have demonstrated that immunization with antigen-loaded dendritic cells results in detectable T-cell activation to tumor antigens, even when these are self antigens with prior immune tolerance or ignorance. T-cell activation has translated into occasional responses in patients with low-grade lymphoma, myeloma, melanoma, neuroblastoma, and renal cell, bladder, prostate, and colon carcinoma. The largest limitation of dendritic cell–based strategies is the need for ex vivo culture to generate personalized vaccines, with the high cost and need for highly specialized facilities and personnel. The use of these vaccines in trials other than pilot experiences is hampered by the strict lot-release testing required by regulatory agencies, which needs

to be performed for each vaccine preparation. Procedures to obtain enriched dendritic cell populations from peripheral blood using an apheresis procedure followed by a short ex vivo culture, attracting skin dendritic cells using chemokines and entrapping them, and closed culture systems from the leukapheresis product to generate antigen-loaded dendritic cells vaccines, would allow additional clinical testing to assess the real value of these strategies. Furthermore, the nonstandardized methods of procuring dendritic cells, assessing maturation status, loading antigens, and administering product may yield opposing immune effects,[45,99] making comparisons among different trials difficult. There is a reasonable concern about the stimulation of autoimmune diseases, especially when the dendritic cells are loaded with antigens shared by normal and cancer cells. However, current clinical experience indicates that autoimmune phenomena have been limited to vitiligo when melanoma antigens are used for immunization, and occasional subclinical increases in antithyroid and antinuclear antibody titers have occurred.[100] Ultimately, if shown to be active for cancer treatment, specialized units such as those in place for processing hematopoietic stem cells may accommodate dendritic cell vaccine production.

Exosomes. Dendritic cells differentiated in vitro release nanometer vesicles derived from late endosomes, which contain most of the appropriate molecules to adequately present MHC-antigen complexes to the immune system.[101,102] These exosomes can be isolated by filtration of dendritic cell culture media and then loaded with custom antigens. Their use alone as vaccines or as vehicles to transfer back preassembled MHC-peptide complexes to dendritic cells is under clinical investigation.

Stimulation of Effector Cells

Nonspecific immunologic stimulants. The characterization of cytokines produced by immune system cells and their production by genetic recombinant techniques have allowed the systemic administration of supraphysiologic doses of cytokines such as IL-2 and IFN. Infrequent but durable clinical responses are seen in patients with melanoma or renal cell carcinoma after treatment with these cytokines; the immunologic mechanism involved is still unclear, even after years of clinical experience.[103] The significant toxicity of high-dose systemic cytokine therapy is the major drawback. Lower and better-tolerated doses of these same cytokines fail to achieve the same clinical benefits.[104,105]

IL-12 is the key cytokine involved in the initiation of a type 1 immune response (Th1), leading to the stimulation of antigen-specific CTL. The administration of IL-12 to tumor-bearing hosts produces dramatic tumor responses, but there is controversy whether the response is immunologic or nonimmunologic. Data from murine models support several mechanisms of action, including the stimulation of a type 1 antigen-specific $CD8^+$ T cytotoxic response,[106] the activation of the NKT cells,[107] or an antiangiogenic effect.[108] Early clinical studies using recombinant IL-12 were terminated early because of toxicity, which may be related to unexpected schedule interactions.[109] Additional development has focused on schedules that produce lower toxicity and combinations with peptide vaccines, with mixed results to date.[110,111]

The interactions of the CD40 receptor on an APC and CD40-L on an activated $CD4^+$ T-helper cells are the biochemical basis of the T-helper function.[31] As with the administration of supraphysiologic doses of other soluble immune molecules, the in vivo antitumor effect of CD40-L may not be solely the immunologic T-helper role of the physiological CD40-L molecule; direct cytotoxic effects by cross-linking of the CD40 receptor present on some tumor cells is likely.[112]

Intratumoral plasmid injection. Naked DNA can be directly injected into tumor cells in vivo. Intratumoral injection of plasmids coding for cytokines such as IL-2, IL-12, or IFNα leads to a local (paracrine) release at the site of the tumor. This may allow the stimulation of an immune response while avoiding the high toxicity of systemic administration, or the need of ex vivo culture and gene modification of tumor cells.[113] However, as described for intratumoral injection of plasmids with the alloantigen HLA-B7, the transfection efficiency is low, leading to poor levels and short duration of transgene expression in the cancer cells and weak immune responses.[114]

Immunocytokines. Immunocytokines represent another means of local delivery of cytokines to tumors to provide high paracrine levels.[115] These compounds have two parts: an antibody-like segment that specifically recognizes a surface molecule in cancer cells, and a cytokine molecule. The Fc component is free to bind and activate NK cells expressing Fc receptors, which allows an initial direct cytotoxic effect on the cancer cell by antibody-dependent cellular cytotoxicity, thereby releasing tumor antigens. The presence of IL-2 is designed to activate both innate and adaptive immune cells at the site of tumor antigen release, which can then be cross-presented by host APCs (Fig 3). Constructs carrying IL-2 with an antibody fraction that targets either gangliosides in neuroendocrine tumors or epithelial cell adhesion molecules in epithelial cancers are in clinical development (Table 2).

Adoptive transfer of tumor-specific T-cell effectors. The adoptive transfer of immune effector cells from the immune system of a donor to a recipient of a hematopoietic stem-cell transplant to generate a graft-versus-tumor effect is, with great certainty, the most commonly used cellular immunotherapy strategy in current oncologic practice.[116] This transfer can be performed after high-dose chemotherapy and bone marrow or peripheral stem-cell engraftment of donor cells (allogeneic stem-cell transplant)[117] or, more recently, after lower doses of chemotherapy that are intended to clear host T cells that would facilitate engraftment of the donor's hematopoietic and immune system (minidose allogeneic transplant).[118] Either strategy can be followed by the adoptive transfer of donor lymphocytes, leading to enhanced graft-versus-tumor effects. The clinical activity of donor lymphocyte infusions provides a clear proof-of-concept of the nature of this phenomenon that is linked to a cellular immune response. The greatest limitation of this mode of cellular immunotherapy is its low specificity for tumor antigens, which results in the severe toxicity from graft-versus-host disease.

Peripheral blood lymphocytes stimulated in vitro with high concentrations of IL-2 generate lymphocyte-activated killer cells. The adoptive transfer of these cells showed promise in preclinical models, but clinical experiences were almost uni-

formly disappointing.[4] The adoptive transfer of TIL also has been widely studied in the preclinical and clinical setting. Although it was sought to be a tumor-specific adoptive immunotherapy,[119] these TIL may be anergic or incapable of homing to the tumor deposits, leading to poor clinical results.[120,121]

Antigen-specific cytotoxic cells that do specifically recognize tumor cells can be generated by cell cloning techniques ex vivo or can be genetically engineered by the stable transfection of a TCR that specifically recognizes a certain MHC-tumor antigen complex.[122,123] This has been made possible by the use of defined tumor antigens to stimulate lymphocytes in vitro, and the ability to clone lymphocytes derived from a single, antigen-specific T cell.[124] Adoptive transfer of clonally expanded lymphocytes to lymphopenic hosts after nonmyeloablative conditioning chemotherapy has resulted in cell proliferation and persistent clonal repopulation correlated with tumor regressions in patients with melanoma.[123] Ex vivo–expanded clonal populations of tumor antigen–specific lymphocytes can be derived from a natural or genetically engineered initiating cell. Moreover, the TCR of cytotoxic T cells can be substituted with an immunoglobulin-like surface molecule, which allows the binding to tumor-specific surface molecules not presented by MHC molecules.[122] These more elaborate forms of adoptive transfer of killer cells are being studied in ongoing clinical trials.

Negative Regulatory Pathway Blockade

Understanding of the critical role of homeostatic mechanisms in limiting the strength and duration of an immune response allows the design of interventions that block the downregulation of antitumor immune responses. CTLA-4 is an activation-induced receptor with greater affinity for the costimulatory molecules B7.1 and B7.2 compared with their nature ligand CD28.[48] CTLA-4 recognition of costimulatory molecules by activated lymphocytes provides a so-called off switch for the immune response. Monoclonal antibodies that block CTLA-4 prevent its engagement by B7 costimulatory molecules and inhibit this negative signal. Animal models have shown that this intervention enhances antitumor responses, either alone or in combination with cancer vaccines;[48] clinical trials are ongoing.

Blockade of Tumor-Derived Immune-Suppressive Molecules

Tumors develop in an immune-suppressive environment that is mediated directly by products released from tumor cells or indirectly by inducing neighboring cells. Even when tumor antigen–specific T cells have been activated by APCs and are permitted to kill tumor cells, and the tumor cells continue to express the relevant antigens, the tumor microenvironment interferes with T cell function. Cyclo-oxygenase 2 enzyme inhibitors, such as aspirin, celecoxib, or rofecoxib, decrease the production of the immune-suppressive prostaglandin E_2 and may enhance tumor responses.[125] Downregulation of TGF-β, an immune-suppressive pleiotropic cytokine produced by the great majority of tumors, can be achieved by neutralizing antibodies or by antisense oligonucleotides.[126,127]

Non–T-Cell–Directed Cancer Vaccines

Monoclonal antibodies to surface receptors, such as trastuzumab or rituximab, have complex mechanisms of action leading to effective tumor regressions. One such mechanism is the stimulation of antibody-dependent cell-mediated cytotoxicity.[128] This immune-based effect, together with the recently recognized ability of immune complexes to allow antigen cross-presentation in dendritic cells,[129] may contribute to their antitumor effects by a coordinated humoral and cellular response. Several other cancer vaccines in different phases of clinical testing (some are advanced and are undergoing pivotal trials; Table 3) also are not formulated to directly stimulate a cellular immune response, but do so as an indirect effect. Most of these strategies rely on the activation of humoral (antibody) responses to a peptide or nonpeptide antigen. Resultant tumor cell damage and cross-presentation of antigen by host APCs may allow the transfer of the immunologic stimulus to cellular immune responses.[130]

In summary, cancer immunotherapy attempts to shift the balance of the immune system toward rejection of the cancer. There are sufficient data to indicate that this is a feasible goal, but how best to achieve the goal is not clear. Any attempt to target the immune system against an endogenously developed cancer is a perturbation of the immune homeostasis.[12] Shifting the balance toward tumor rejection will likely shift the balance to autoimmune reactions as well. The therapeutic window may be narrow between antitumor immune response and autoimmunity, but also between response and tolerance to tumor antigens. In the last several years, important advances have been made in the understanding of the regulatory mechanisms that govern the immune system. Tumor antigen characterization and optimal presentation is the milestone in modern antitumor immunity, and clinical results on the basis of this knowledge are already promising.

Advances in the understanding of the mechanisms of action of cellular antitumor immune responses have allowed the development of new generations of cancer vaccines, in which the key step is the recognition of the need for professional APCs to cross-present the antigen to the host immune system. The most immunologically active vaccines usually require costly and laborious ex vivo cellular cultures, whereas the cell-free vaccines that can be directly administered from an easily stored and transported vial are usually less immunologically active but more suitable for widespread clinical testing. New advances in the formulation of cancer vaccines brought by a more precise knowledge of the requirements for the generation of cellular immune responses to tumor antigens, together with the current ability to closely monitor cellular immune responses (see reviews in[131,132]), will likely provide powerful, nonindividualized, cell-free vaccines in the near future.

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