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Drew Pardoll (Johns Hopkins Oncology Center) examines prospects for therapeutic cancer vaccines. Considering how it is that the immune system fails to recognize and destroy cancer cells, Pardoll discusses contemporary vaccine approaches aimed at exposing cancer antigens to the cellular arm of the immune system, and the first, promising steps toward therapeutic cancer vaccines.

Cancer vaccines

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Since the turn of the century, scientists have studied the interactions between the immune system and cancer cells so that antitumor immunity could be amplified as a means of cancer therapy. In the 1890s, William Coley began to treat cancer patients with bacterial extracts (Coley's toxins) to activate general systemic immunity, a portion of which might be directed against the tumor[1]. One hundred years later, the molecular understanding of immune recognition and immune regulation provides opportunities that Coley never had to create cancer vaccines with much greater potency and specificity for tumor cells and diminished toxicity for normal tissues. In contrast to prophylactic vaccines against infectious agents, in which the generation of neutralizing humoral immunity is the most important feature, the major focus in cancer vaccine development has been on the generation of antigen specific T-cell responses.

Principles of cancer immunity

Unlike most vaccines for infectious agents, cancer vaccination is therapeutic, involving attempts to activate immune responses against antigens in the tumor to which the immune system has already been exposed. In contrast to the original proposals of the Immune Surveillance Hypothesis[2,3], effective immune responses against tumor neoantigens arising during transformation are rarely observed. A number of mechanisms have been proposed to explain the failure to develop effective endogenous immunity against cancer. Generation of antigen-loss tumor variants[4–6], loss of MHC expression[7–10], downregulation of antigen processing machinery[11] and expression of local inhibitory molecules, such as tumor necrosis factor β (ref. 12) and Fas ligand[13], are examples of acquired resistance that are likely to play an important role in some cancers. However, recent evidence supports a more fundamental mechanism of immunologic nonresponsiveness to cancer.

When the immune system encounters a new antigen in the periphery, the outcome is not necessarily activation. Numerous experiments demonstrate that encounter of antigens by mature T cells often results in the induction of tolerance because of either ignorance, anergy or physical deletion[14–16]. What determines the outcome of antigen encounter is the context in which that antigen is presented to the immune system. Thus, the outcome of inflammation or tissue destruction that occurs during viral or bacterial infection (or when antigen is mixed with the appropriate adjuvant) is typically activation. When antigen is expressed endogenously, in the absence of the danger signals that accompany tissue destruction and inflammation, the typical outcome is immunologic tolerance (Fig. 1).

Ultimately, it appears that the immune response at the T-cell level is dependent on the costimulatory signals present at the time of antigen recognition. In response to certain inflammatory cytokines, antigen presenting cells (APCs) express costimulatory molecules such as B7, which promote T-cell activation. In the absence of the appropriate costimulatory signals, engagement of the T-cell receptor itself typically leads to ignorance, anergy or apoptosis of the antigen specific T cell. It is reasonable to imagine that as tumors accumulate neoantigens during transformation, the absence of associated inflammatory or tissue destructive processes at these early stages results in tolerance to these neoantigens.

The ability of tumors to induce tolerance of T cells specific to their antigens has been recently demonstrated[17]. In other murine tumor systems, antigenic tumor cells have been found to grow progressively in immunocompetent hosts without inducing either acute or memory T cell responses[18,19]. A common theme of these studies is that tumors are poor stimulators of immune responses and may be capable of actively inducing tolerance. Thus, in order to be effective, cancer vaccines must either break down tolerance or activate a cryptic population of T cells that escaped tolerance by virtue of their low affinity for antigens expressed by the tumor[20].

One of the primary goals of all cancer vaccines is to target the immunizing antigen(s) to appropriate bone marrow derived APCs. Depending on the type of vaccine, this can be accomplished through a number of different pathways. One of the most important is the exogenous pathway of

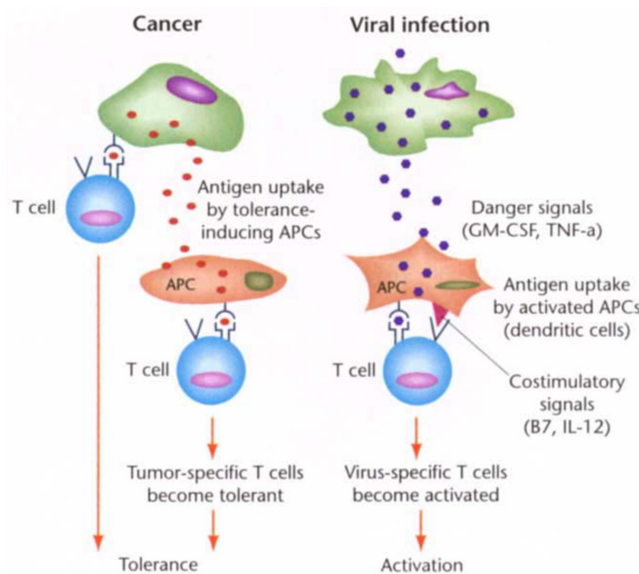

Fig. 1 Different immune responses to tumor antigens and viral antigens. During the inflammation and tissue destruction that accompanies viral infection (or injection of antigen mixed with adjuvant), antigen is targeted to activated antigen presenting cells (APCs) that express costimulatory molecules such as B7 and the outcome is usually activation (right). When antigen is expressed endogenously, as is the case with tumor cells, there are no danger signals such as during inflammation and tissue destruction. Antigen is either presented directly by the tumor or by APCs that do not express costimulatory signals and the typical outcome is immunologic tolerance (left).

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antigen uptake. This is operative in most whole cell and protein subunit vaccines and depends largely upon the adjuvant with which the tumor cell or tumor antigen(s) is formulated. Recent studies have demonstrated that tumor antigens endocytosed by bone marrow derived APCs are not only introduced into the MHC class II processing pathway but are also introduced into the MHC class I processing pathway (termed crosspriming)[21–24]. A second pathway for introduction of antigen into appropriate bone marrow derived APCs is via direct transduction. This pathway is likely to be operative in certain recombinant viral and bacterial vaccines. A third pathway of targeting antigens to APCs is putatively operative in peptide vaccines. In this case, peptide loads empty MHC molecules on the surface of APCs, thereby bypassing the processing steps. For all of these pathways, the two interrelated variables that determine immunologic outcome are the density of peptide/MHC complexes displayed on APCs and the particular costimulatory molecules (membrane and secreted) expressed by APCs.

Tumor antigens recognized by the immune system

In addition to principles of antigen presentation, the rational development of cancer vaccines also depends upon the molecular definition of tumor antigens capable of being targeted by the immune system. In accordance with the shift of emphasis from humoral antitumor immunity to T-cell dependent immunity, recent efforts in tumor antigen identification have shifted from antigens recognized by antibodies to those recognized by T cells.

Most investigations into tumor antigens recognized by T cells have focused on $CD8^+$ T cells and MHC class I-restricted antigens. However, $CD4^+$ T cell responses, which are MHC class II-restricted, are equally important in antitumor immunity[25–28]. For virtually all antitumor vaccines, $CD4^+$ T cells are critical in priming both cytotoxic T lymphocytes (CTLs) and antigen nonspecific effector immune responses including generation of reactive oxygen intermediates by macrophages and Th_2 dependent eosinophil activation[25,29].

The most informative approach to the identification of tumor antigens recognized by T cells begins with the establishment of tumor-specific T-cell lines or clones from individuals with cancer (predominantly melanoma because among human cancers, tumor-reactive T cells are most readily cultured from melanoma patients). For both murine and human cancers, antigens identified in this fashion fall into three categories based on their pattern of expression: (1) unique tumor antigens expressed exclusively in the tumor from which they were identified; (2) shared tumor-specific antigens expressed in many tumors but not in normal adult tissues; (3) tissue-specific differentiation antigens expressed by the normal tissue from which the tumor arose. In addition, oncogene and tumor suppressor gene products and viral antigens in virus-associated tumors are candidate tumor antigens (Table).

Unique Tumor Antigens Recognized by T Cells. Antigens expressed uniquely by an individual tumor are generally the products of a mutation or rearrangement. Although they are not suitable for incorporation into generic cancer vaccines, it is critical to understand which components of antitumor immunity are directed against unique versus shared tumor antigens. If the major tumor rejection responses are directed against unique antigens, cancer vaccines must use the patient's own tumor cells to be successful.

A number of MHC class I-restricted melanoma antigens derived from the products of a mutated gene have been identified. Some of them represent mutations that are involved in transformation. For example, one peptide is derived from a mutation in the cyclin dependent kinase 4 gene that interferes with binding of $p16^{INK4a}$ (ref. 30). Another is derived from a mutation in the *β-catenin* gene, which has been implicated in tumorigenesis based on its altered binding to E-cadherins[31]. A recently identified unique squamous cell carcinoma antigen represents an inactivating point mutation in the *caspase-8* gene, a critical regulator of apoptosis[32].

Shared tumor-specific antigens. Although crossprotection experiments with independently arising murine tumors suggest that the most immunogenic tumor antigens are unique[33], lower levels of crossimmunization are indeed observed in a number of instances, suggesting the existence of shared tumor antigens. The majority of shared tumor antigens isolated from both murine and human tumors represent reactivation of genes normally not expressed in adult tissues but transcriptionally activated in some tumors[34,35].

In humans, the best characterized example is the *mage* gene family. Family members are expressed in melanoma and less frequently in other tumor types. This family is a prototype for a number of tumor antigens with expression that is restricted to tumor and testis[36, 37]. MAGE-1 and MAGE-3 donate peptides recognized by melanoma-specific $CD8^+$ T cells. Because of their pattern of shared and selective expression in tumors, these antigens represent promising candidates for antigen-specific cancer vaccines.

Tissue-specific differentiation antigens. One of the surprises to fall out of the identification of melanoma-specific antigens is that the majority of T cells derived from melanoma patients recognize nonmutated peptides derived from melanocyte-specific differentiation antigens. This was somewhat unexpected because T cells specific for self-antigens are expected to be deleted or rendered functionally tolerant. In melanoma patients, the most commonly recognized MHC class I-restricted tissue-specific antigens are derived from proteins of melanosomes (the pigment granules in melanocytes) many of which are involved in pigment biosynthesis[38]. HLA-A2-restricted peptides from two of these melanosomal antigens, Mart-1/melan-A and gp100, appear to be recognized by T cells from a large proportion of patients and represent dominant reactivities within melanoma-specific

Potential sources of tumor antigens

Category of antigen	*Example*
Oncogene product	Mutations in Ras codon 12 (pancreatic cancer)
	bcr/abl protein (chronic myeloid leukemia)
Embryonic proteins	MAGE family (melanoma, breast cancer)
Viral proteins	Epstein-Barr virus (Burkitt's lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma)
	Human papillomavirus (cervical cancer)
	Hepatitis B virus (hepatocellular cancer)
Tissue specific antigens	Tyrosinase (melanoma)
Mutated tumor suppressor proteins	p53 (many cancers)
Idiotypic epitopes	Idiotypic immunoglobulin (B-cell lymphoma)
	T-cell receptor idiotypes (T-cell lymphoma)

CTL lines[39–41]. Another melanosomal protein, tyrosinase, is also a shared MHC class II-restricted human melanoma antigen recognized by CD4+ T cells[28].

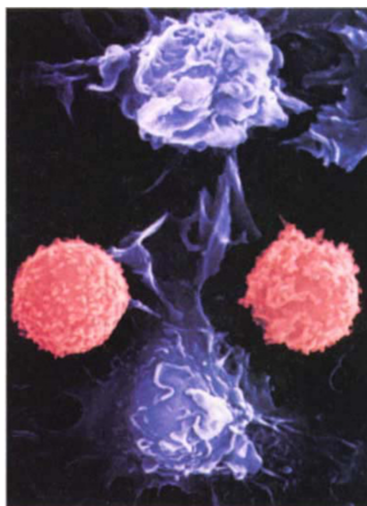
A common finding among both MHC class I and class II-restricted tissue-specific peptides recognized by T cells is their low MHC affinity, suggesting that they are inefficiently presented. These low reactivity T cells may be a functionally cryptic population that escapes immune tolerance whereas T-cell populations that recognize high affinity tissue-specific self peptides may have been actively rendered tolerant *in vivo* and cannot be established as long-term cell lines or clones *in vitro*.

Oncogene and tumor suppressor gene products as tumor antigens. Another approach to the study of tumor antigens involves the analysis of immune responses against candidate antigens that are the products of genes commonly associated with particular cancers. Because of their central role in tumorigenesis, commonly altered oncogene and tumor suppressor gene products[42,43] are particularly tantalizing targets for antitumor immunity. They elicit both antibody and T-cell responses.

The most commonly altered tumor suppressor gene in cancer, p53, has been intensively studied as a candidate tumor antigen[44]. Cancer vaccines aimed specifically at p53 mutations would be somewhat impractical because any single p53 mutation is present in a very small fraction of human cancers. Therefore, the idea of wild-type p53 as a potential target for T-cell immunotherapy is gaining momentum. At first glance, p53 might seem to be a poor target because of its ubiquitous expression in normal tissues. However, many of the p53 mutations found in cancer result in a protein which, although inactive, is expressed at significantly higher levels than in normal tissues. Indeed, Melief and colleagues demonstrated that CTLs specific for a wild-type p53 peptide can indeed create a therapeutic window when adoptively transferred into animals bearing a tumor that overexpresses p53 (ref. 45).

The most extensively studied oncogene with potential as a candidate tumor antigen is Ras. Mutations in K-Ras are less complex than those in p53 (most activating mutations fall at residues 12,13 or 61 (ref. 43). Indeed, antibodies and T cells specific for mutant Ras have been identified in patients with gastrointestinal cancer[46]. One additional oncogene that is being evaluated as a target for antitumor immunity is her-2/neu, a membrane tyrosine kinase receptor that is expressed at low levels in mammary, ovarian and other epithelia. Though not mutated, its expression is significantly increased in a number of carcinomas, particularly those of breast and ovary[47]. In addition to classical cell-mediated immunity[48], the membrane localization of her-2/neu makes it a tumor- associated antigen against which antibody based immunity may provide clinical benefit[49].

Virus-associated tumor antigens. As an increasing number of human cancers have been associated with specific viruses, viral antigens have re-emerged as important tumor-associated antigens. A number of important cancers that develop in immunocompetent individuals have been demonstrated to be virus-associated.



Cancer cells (blue) and lymphocytes

The two most common cancers worldwide, hepatoma and cervical cancer, are clearly associated with viral infection. In the case of hepatoma, hepatitis B virus has been implicated as the etiologic agent[50]. Although expression of viral gene products within hepatomas is variable and inconsistent, 80–90 percent of cervical cancers express the E6 and E7 antigens from one of four 'high risk' human papillomavirus (HPV) types: HPV-16, HPV-18, HPV-31 and HPV-45 (refs. 51, 52).

For virus-associated cancers in immunocompetent individuals, the HPV E6 and E7 antigens provide the most promising targets because of their ubiquitous expression in cervical cancers. Critical questions about virus-associated cancers in immunocompetent individuals include how the virus avoids immunologic elimination at the time of initial infection and the mechanism of immune tolerance to viral antigens that are persistently expressed in the tumor. Despite reports of generation of CTL responses *in vitro* against E7, including specific peptides presented by common HLA types such as HLA-A2 (refs. 53, 54), it is currently unclear whether clearance of virus or progression to cancer correlates with activation or tolerance induction among HPV-specific T cells, respectively.

Although virus-associated tumor antigens represent important targets for therapeutic cancer vaccines, they also provide an ideal opportunity for prophylactic vaccines. Indeed, the introduction of prophylactic hepatitis B virus vaccines in Taiwan has already decreased the incidence of hepatomas among vaccinees[55]—a major achievement in cancer prevention.

Cell-based cancer vaccines

Despite ongoing efforts to define immunologically relevant cancer antigens, we currently have little idea about the most important tumor rejection antigens for the majority of human cancers. For this reason, most cancer vaccine approaches thus far use tumor cells themselves as a source of antigen. The validity of cell-based cancer vaccine approaches depends upon their capacity to induce stronger immunity against tumor-specific or tumor-selective antigens than against ubiquitously expressed self antigens within the tumor. Early generations of cell-based cancer vaccines have consisted of killed tumor cells or tumor cell lysates mixed with adjuvants such as *Bacillus Calmette Guerin* (BCG) and *Corynebacterium parvum*, in an attempt to amplify tumor- specific immune responses[56,57].

Subsequently, genetically modified tumor vaccines have begun to replace the complex and inconsistent mixtures of tumor cells and bacteria. The forerunner of these studies was the work of Lindenman and Klein[58], who showed that vaccination with influenza virus-infected tumor cell lysates generated enhanced systemic immune responses following challenge with the original tumor cells. A more recent version of this approach is the transduction of tumor cells with specific viral genes[59] and allogeneic MHC genes[60,61] in order to enhance their immunogenicity. In some cases, animals which rejected the tumor transfectants became immune to challenge with nontransfected tumor cells.

Currently, the most popular genetically modified cell-based

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vaccines take advantage of the large set of cloned genes encoding cytokines and costimulatory molecules[62]. An important principle emphasized by all of these studies is that the sustained local release of cytokines produces dramatic local inflammation without any systemic effects or toxicity. Although the pharmacokinetics of different cytokines vary tremendously, it is rare to detect significant amounts of cytokines in the serum, even after injection of large numbers of cells transduced with cytokine genes.

Among the different cytokine genes used to modify tumor immunogenicity, granulocyte-macrophage colony stimulating factor (GM-CSF) appears to be the most potent[26]. The uniquely enhanced immunologic effect of paracrine GM-CSF in multiple tumor vaccine models relates specifically to it's role in promoting local dendritic cell (DC) differentiation at the vaccine site. GM-CSF has been identified as a critical factor for inducing the differentiation of primitive hematopoietic precursors into DCs, a type of APC that is distinct from granulocytes and macrophages and that initiates the most potent T cell responses[63]. Because DCs are felt to be the primary cell necessary for activating virgin T cells, their role in priming immunologic responses is now considered central.

A large number of clinical trials testing the efficacy of genetically modified whole-cell tumor vaccines using both B7 and cytokine genes are currently underway. A recent report of a Phase I trial in patients with advanced renal cancer provides preliminary evidence of the immunologic activity of autologous GM-CSF gene transduced vaccines[64]. This trial, which compared GM-CSF gene transduced and nontransduced vaccines, demonstrated enhanced immunogenicity of the former as measured by induction of delayed type hypersensitivity reactions against autologous tumor.

Because direct transduction of autologous tumors is highly individualized, expensive and labor intensive, simpler approaches that maintain the immunologic activity of paracrine cytokine elaboration are currently being developed (Fig. 2). One approach, which takes advantage of the fact that the cytokine does not need to be produced by the tumor itself, involves admixing tumor cells with either a generic transduced bystander cell or biopolymer microspheres containing cytokines[65]. This approach obviates the need for culture or transduction of each patient's tumor cells. Another approach currently under clinical investigation uses standardized gene-transduced tumor cell lines as vaccines. This strategy is based on the idea that some tumor rejection antigens are shared rather than unique. This type of vaccine is often referred to as an allogeneic vaccine because the vaccinating cell line expresses MHC alleles foreign (allogeneic) to the vaccinated patient. Because it is now established that tumor antigens are presented by host bone marrow derived APCs rather than the vaccinating tumor itself[21], MHC compatibility between patient and tumor is not required for this type of vaccine[66, 67].

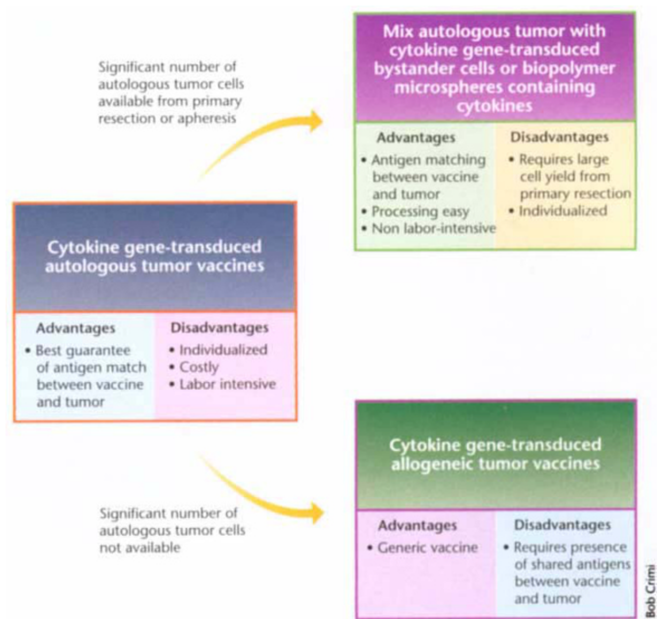


Fig. 2 Cytokine cancer vaccines. For some tumors (cancers of colon, kidney, ovary; leukemias and lymphomas) from which large numbers of tumor cells can be obtained, vaccines are produced by admixture of patient cells with either bystander cells that have been transduced with cytokine genes or with biopolymer microspheres containing cytokines. When autologous tumor is not available, patients can be immunized with allogeneic tumor cells (derived from cell lines of the same type as the patient's tumor) that have been transduced with cytokine genes. This strategy depends on the existence of appropriate antigens shared by the patient's tumor and the tumor cells in the vaccine.

Antigen-specific cancer vaccines

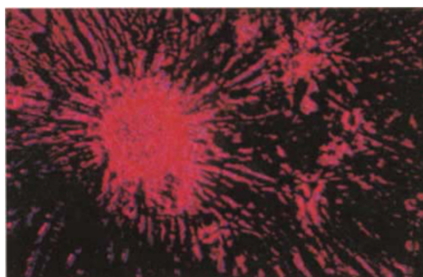
Although cell-based vaccines are currently the major form of cancer vaccine tested clinically, innovative approaches to antigen-specific vaccination are under way. The ability to activate immune responses against selected immunodominant tumor antigens (when identified) provides for a much greater degree of control in targeting antitumor immune responses. There are already a few human tumors, such as melanoma and virus-associated cancers, where tumor-associated antigens have been identified and candidate antigen-specific vaccines are being tested clinically. Their optimal development requires identification of the most potent tumor rejection antigens and the appropriate route or vehicle (adjuvant) by which the antigen is delivered to the immune system. As the investment in clinical testing of antigen-specific vaccines increases, it will be critical to identify general rules for the definition of immunogenicity so that vaccine optimization is rational rather than empirical.

Peptide vaccines. The first antigen-specific cancer vaccines to be tested clinically have used specific peptides presented by common HLA alleles. Almost all peptide-based vaccines thus far have used MHC class I-restricted antigenic peptides. Protective CTL responses induced by vaccination with MHC class I binding peptides were first reported for cytomegalovirus and Sendai virus mixed with Freund's incomplete adjuvant[68,69]. Vaccination of tumor-bearing mice with MHC class I binding immunogenic peptides has been reported to result in the induction of protective immunity[70].

Peptide vaccination depends on the loading of empty MHC molecules on APCs *in vivo*. However, simple administration of peptide without a means of targeting activating APCs can potentially lead to loading of MHC class I molecules on nonprofessional APCs, which could result in tolerance. Indeed, administration of some peptides at high doses by an intraperitoneal route induced immunologic tolerance rather than protective immunity[71]. Similarly, in an adenoviral- induced murine tumor model, vaccination with an adenoviral peptide in Freund's incomplete adjuvant caused an enhanced out-

growth of tumors. The increased tumor growth was paralleled by specific tolerance induction for the injected peptide epitope[72]. It has been proposed that certain peptides of peptide/adjuvant vaccines induce tolerance by 'leaking out' of the Freund's incomplete adjuvant into serum and binding to APCs that do not express costimulatory signals.

Peptide vaccines for a number of cancers are being tested clinically. A Phase I trial using an HLA-A2-restricted MAGE-3 peptide in Freund's incomplete adjuvant reported promising preliminary clinical results in patients with advanced melanoma[73]. Surprisingly, induction of MAGE-3 specific CTLs could not be detected by standard *in vitro* chromium release assays, even in patients who underwent complete remission. A more extensive peptide vaccine trial carried out in melanoma patients evaluated a gp100 peptide analogue modified at the MHC anchor residue to produce higher affinity for HLA-A2 molecules[74]. A large proportion of T cells stimulated by the anchor-modified peptide recognized the wild-type gp100 peptide[75]. Immunization with the anchor-modified gp100 peptide in Freund's incomplete adjuvant induced much greater gp100, melanoma-reactive CTL activity (as assayed by *in vitro* chromium release) than vaccination with wild-type gp100 peptide. However, neither wild-type nor anchor-modified gp100 vaccines produce clinical responses. In contrast, 41 percent of melanoma patients receiving a combination of high dose interleukin (IL)-2 plus the anchor-modified gp100 vaccine had a clinical response, though larger randomized trials will be required to determine whether this combination is superior to high dose IL-2 alone. Interestingly, peripheral blood lymphocytes from patients treated treated with the combination of high dose IL-2 plus the anchor-modified gp100 vaccine demonstrated relatively little gp100-specific CTL activity as determined by *in vitro* chromium release assays. The complete lack of correlation between CTL induction and clinical response in these two vaccine trials using defined MHC class I peptides calls into serious question the relevance of classic chromium release assays as a surrogate immunologic measurement of antitumor activity.



Recombinant viral vaccines. The intrinsic immunogenicity of viruses together with the development of standard techniques to engineer recombinant viruses has engendered broad interest in recombinant viral vaccines. Based on the early work of Moss and Paoletti[76,77], recombinant vaccinia and other poxviruses have been the most popular components of such cancer vaccines[78–80]. More recently, adenoviral and other viral vectors have been selected for cancer immunotherapy[81–83]. The common denominator for all recombinant viral vaccines is the introduction of the gene(s) encoding the antigen into the viral genome using standard recombination and selection approaches adapted to the virus of interest.

Two mechanisms underlie the capacity of recombinant viral vaccines to initiate immune responses. First, the cellular damage induced by viral infection elicits danger signals that attract and activate bone marrow derived APCs that present antigens in the context of costimulatory molecules. A second mechanism for some recombinant viral vaccines involves direct infection of bone marrow derived APCs, which allows for efficient processing of endogenously synthesized antigens in the MHC class I pathway.

The capacity of some viruses to directly infect APCs allows for modifications of recombinant viral vaccines to enhance the processing and presentation of encoded antigens and incorporation of genes encoding costimulatory molecules. For example, infection with recombinant vaccinia virus that expresses genes encoding minimal MHC class I-restricted peptides results in enhanced MHC class I presentation of antigen *in vivo*[84]. Grafting of endosomal/lysosomal sorting signals onto the gene encoding antigen enhances MHC class II processing and CD4+ T-cell activation by recombinant poxviruses[85,86]. Incorporation of genes for the B7 costimulatory protein and for cytokines into recombinant poxviruses also enhances vaccine potency[87].

Preliminary results of clinical trials with recombinant viral cancer vaccines are just now beginning to be evaluated[88]. One of the major barriers to effective vaccination with viruses such as vaccinia and adenovirus is inhibition of vaccine 'take' by preexisting neutralizing antibodies. These antibodies are the result of previous exposure to crossreacting viruses (adenovirus) or previous immunization (vaccinia). Ultimately, realization of the full clinical value of recombinant viral vaccines will require development of methods to transiently eliminate neutralizing antibodies and/or to design viral vectors to which most individuals have not been exposed.

Recombinant bacterial vaccines. One of the most interesting recombinant vaccine approaches involves the use of engineered bacteria. A number of bacterial strains including *Salmonella*[89–91], BCG (refs. 92, 93) and *Listeria monocytogenes*[94,95] display two characteristics that make them promising vaccine vectors. First, they possess enteric routes of infection, providing the possibility of oral vaccine delivery. Second, they infect monocytes and macrophages and can therefore target antigens to professional APCs.

Recombinant *Listeria* vaccines have been tested in animal models of macroscopic cancer with promising results[96,97]. This organism makes a particular interesting vector because of its two-phase intracellular life cycle[98]. Upon infecting monocytes or macrophages, *L. monocytogenes* occupies phagolysosomes and then secretes listeriolysin O, which destabilizes the phagolysosomal membrane and allows transit of bacteria into the cytoplasm. The dual phagolysosomal-cytoplasmic life cycle of *L. monocytogenes* allows for efficient processing of secreted antigens in the MHC class II pathway during the phagolysosomal phase and in the MHC class I pathway during the cytosolic phase. In addition to antigen-specific T cells, activation of components of the innate immune response by recombinant *L. monocytogenes*[99, 100] most likely contributes to the effective access of antigen-specific T cells to tumor beds.

Nucleic acid vaccines. Vaccines composed of naked DNA have engendered tremendous interest ever since the original report by Liu and colleagues that naked DNA encoding influenza nucleoprotein could protect animals from influenza challenge[101] (see Liu, page 515). In addition to infectious diseases, naked DNA vaccines encoding model tumor antigens provide some degree of systemic tumor protection[102].

In general, the potency of naked DNA vaccines is less than that of recombinant viral vaccines. This decreased potency is probably attributable to the fact that naked DNA does not undergo a replicative amplification as occurs with live recombinant viral and bacterial vaccines, thereby limiting the amount of antigen

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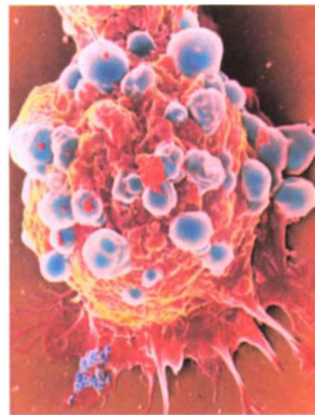
ultimately presented to the immune system. Also, naked DNA vaccines generate a much smaller inflammatory or danger response than a live viral infection. Nonetheless, injection of nucleic acid does induce local inflammation and some activation of bone marrow derived APCs (ref. 103). Recent experiments demonstrate that it is indeed these cells that present the antigens encoded by naked DNA (ref. 104).


Breast cancer cell.

An important feature of nucleic acid vaccines is that unmethylated CpG tracts found in the DNA of bacterial vectors activate both macrophages and other bone marrow derived APCs (refs. 105, 106). Through mechanisms yet to be elucidated, unmethylated CpG tracts directly stimulate macrophages to produce proinflammatory cytokines such as IL-12 (ref. 107). The proinflammatory effects of unmethylated CpG tracts in nucleic acid vaccines are likely to be critical for their capacity to induce immunity.

Dendritic cell vaccines

Based on the emerging concept of the central role of APCs in the initiation of immune responses, DC-based vaccines are under active investigation. Many factors appear to be responsible for the unique potency of DCs in activating T cells. These cells express 50-fold higher levels of MHC molecules than macrophages, providing more peptide/MHC ligand for T-cell receptor engagement. Also, they express extremely high levels of important adhesion and costimulatory molecules critical for T-cell activation. Other DC-specific genes, such as one encoding a T-cell specific chemokine[108], add to the list of features that give DCs their unique prowess in initiating T-cell responses.

Based on these findings, a number of groups have used either directly isolated or GM-CSF-induced DCs as antigen carriers for tumor vaccination. The form of antigen loaded onto DCs ranges from minimal MHC class I-restricted peptides[109] to protein[110, 111]; antigen has even been presented by fusion of DCs with whole tumor cells[112]. Others have explored *ex vivo* transduction of DCs using either RNA (ref. 113) or replication-defective recombinant viral vectors[114, 115] to introduce genes encoding antigen.

DC vaccines are entering clinical testing. In one study, vaccination of B-cell lymphoma patients with DCs loaded with idiotypic antibody generated idiotype-specific immunity with some clinical response[116]. A recent vaccine trial in melanoma patients using autologous DCs (generated by culture of peripheral blood mononuclear cells in GM-CSF and IL-4, pulsed with either tumor lysate or MHC class I-restricted melanoma peptides together with keyhole limpet hemocyanin as a helper antigen) reported induction of delayed type hypersensitivity and some clinical response[117]. Although these early results look promising, the growing appreciation of different functional subtypes of DCs, each of which is generated in different ways, together with the growing number of different methods for loading DCs, necessitates careful comparative studies to determine which growth and loading conditions will ultimately produce maximal systemic antitumor immunity.

Heatshock proteins as carriers of antigen

Heatshock proteins are natural biologic adjuvants that display promise in cancer vaccination. Heat shock proteins gp96 (of the endoplasmic reticulum) and hsp70 (in the cytosol) act as immunologic adjuvants[118, 119]. These heatshock proteins, or chaperonins, have the capacity to bind a wide array of peptides[120]. Immunization with either native gp96 or hsp70 purified from tumor cells (which carry arrays of tumor-specific peptides) generate systemic antitumor immunity[121]. More recently, vaccination with recombinant hsp70 tethered to a model peptide antigen was shown to induce antigen-specific $CD8^+$ T-cell responses[122].

The capacity of certain heatshock proteins to act as adjuvants is based upon two features. First, peptide-loaded gp96 has been shown *in vitro* to effectively introduce antigens into the MHC class I processing pathway. Thus, gp96 and possibly other heatshock proteins may represent important molecular conduits for antigens into both the MHC class I and class II pathways of APCs. Second, there is evidence that binding of gp96 to macrophages induces them to secrete proinflammatory cytokines. Thus, heatshock proteins may augment the function of cells to which they are targeting peptides.

Amplification of vaccine potency

As more is learned about the molecular regulation of immune responses, additional strategies to block inhibitory pathways of T-cell activation *in vivo* have been explored. One of the most promising examples is the blockade of the CTLA-4 inhibitory pathway. CTLA-4 binds B7 with about a 10-fold higher affinity than does CD28. Occupancy of CTLA-4 appears to directly counter the effects of CD28 on T-cell activation and lymphokine induction[123, 124]. The importance of CTLA-4 in T-cell homeostasis *in vivo* is most dramatically exemplified in CTLA-4 knockout mice, which develop a severe lymphoproliferative disease with immune destruction of organs[125,126]. However, if CTLA-4 could be transiently blocked *in vivo*, it might be possible to enhance vaccine potency while limiting the collateral damage seen in the constitutive genetic knockouts.

Indeed, infusion of anti-CTLA-4 antibodies elicits an enhanced antitumor response in a number of murine cancer models without overt toxicity in the treated animals[127]. Recently CTLA-4 blockade was found to be most effective when given together with a cancer vaccine[128]. Thus, CTLA-4 blockade confined to the time of vaccination provides a means to selectively amplify immune responses against the vaccinating antigen while limiting induction of undesirable immune responses.

Cancer vaccines at the crossroads

There has been more scientific progress in cancer vaccine development over the past decade than in the 90 years that followed the discovery of Coley's toxins. Optimism that these new vaccines will quickly revolutionize cancer therapy must be tempered by our newfound appreciation of how tumors induce immunologic tolerance to their antigens and develop resistance to immune recognition. Nonetheless, comparison of the new generation of molecular cancer vaccines with older vaccine formulations clearly demonstrates their superiority in animal models of cancer. The challenges ahead lie in the translation of these advances into reproducible clinical benefit. This will involve careful optimization of the most promising strategies, thoughtful selection of patient populations and careful clinical trial design.

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