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Educational Review

# Vaccine Therapy for Cancer

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**Background:** Tumor-specific cytotoxic T-lymphocytes (CTLs) can be isolated from the solid tumors, draining lymph nodes, metastatic effusions, and peripheral blood of cancer patients. Despite this evidence for a cell-mediated immune response to cancer, attempts at active specific immunotherapy using cancer vaccines have met with little success in clinical trials.

**Methods:** We have reviewed the immunobiology of the cell-mediated immune response to cancer by focusing on what is known about the major histocompatibility complex (MHC)-restricted interaction between tumor cells and $CD8^+$ or $CD4^+$ T-cells. In addition, we review the recent advances in the identification of tumor-associated antigens (TAAs) that are recognized by tumor-specific CTLs in melanoma and other cancers. In discussing these antigens, we highlight the recent identification of several MHC-restricted antigenic peptides that are recognized by CTLs from patients with melanoma and those with ovarian and breast cancer. We examine the implications that the discovery of these TAAs and peptides will have on the development of new anticancer vaccines. We review the most recent vaccine trials in melanoma and other cancers and focus on current concepts aimed at improving the therapeutic efficacy of future vaccines, including genetically engineered tumor cell vaccines.

**Conclusions:** With the recent identification of several TAAs and antigenic peptide epitopes in melanoma and other cancers, immunotherapy researchers are now focusing on new strategies for the development of anticancer vaccines. As the repertoire of known TAAs increases and our understanding of the immunobiology of cell-mediated immunity to cancer improves, immunotherapists remain cautiously optimistic in their quest for effective cancer vaccines.

**Key Words:** Tumor vaccines—Tumor-associated antigen—Cytokines—T-lymphocytes—Immunotherapy.

Active specific immunotherapy for cancer has been an area of intense research by surgical oncologists for over a century. Many murine studies have shown that immunization with intact tumor protects the animal against subsequent tumor challenges (1,2). Despite the multitude of clinical trials of vaccine therapy in humans, statistically significant improvement in disease-free survival has evaded researchers. In an exhaustive review on the history of immunotherapy, Oettgen and Old chronicled all the vaccine trials published from 1902 to 1989. Of the >100 studies performed, the vast majority showed no clinical response to vaccine therapy. Of those that did show a statistically significant increase in disease-free survival, only one was randomized with appropriate controls, and the increase in survival was modest (3). Similarly, in the past few years, despite the identification of several tumor-associated antigens (TAAs) in melanoma and other cancers, the translation of these findings into clinical benefit has not yet been realized.

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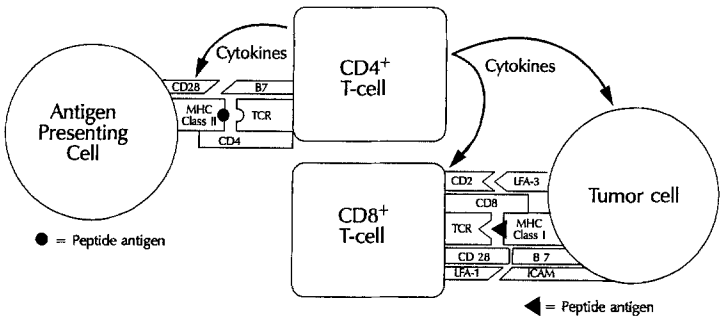


FIG. 1. Schematic representation of the cell-mediated immune response to cancer cells.

## BACKGROUND

Tumor-specific, class I major histocompatibility complex (MHC)-restricted cytotoxic T-lymphocytes (CTLs) can be isolated from the tumor tissue, tumor-draining lymph nodes, malignant effusions, and peripheral blood of cancer patients, confirming the existence of a cell-mediated immune response to certain cancers. The immune response, although demonstrable in several histologically distinct tumor systems, is inadequate as evidenced by the progression of disease in a large proportion of these patients who go on to develop widespread metastasis. For decades, researchers have attempted, with little success, to characterize and manipulate this immune response in order to improve clinical outcome. Recent advances in our understanding of the antitumor immune response, as well as powerful new methods of isolating and identifying TAAs, has renewed interest and enthusiasm in the quest for an effective anticancer vaccine therapy.

The identification of many different TAAs in melanoma and in several other cancers has been an encouraging development in the field of tumor immunology. As the focus of research in antitumor immunity has shifted from the humoral response to the cell-mediated response, the repertoire of potential TAAs has dramatically increased. The absolute requirement of cell surface expression necessary for an antibody-mediated immune response is no longer a restriction. CTLs recognize antigenic peptides processed by antigen-presenting cells (APCs) or by tumor cells themselves and presented on the cell surface by MHC. These antigens can be derived from any cell compartment and need not be expressed in intact form on the tumor cell surface to be recognized by immune effectors. To understand the recent developments in the field of tumor vaccine therapy, it is crucial to appreciate the specific nature of this MHC-restricted T-cell/tumor antigen interaction.

$CD8^+$ CTLs can recognize endogenous 9-10 amino acid antigenic peptides when presented on the cell surface bound to an MHC molecule. (Fig. 1). The nature of this interaction is well characterized and known to be antigen specific and MHC restricted, as evidenced by the vast repertoire of unique T-cell receptors (TCRs), which are capable of recognizing a specific antigen only when presented in the context of the MHC. Class I–restricted antigen presentation involves a complex series of reactions whereby endogenous antigenic foreign and/or self proteins are degraded by the tumor cell into small peptides and transported to the cell surface bound to a specific class I MHC allele. As depicted in Fig. 1, the TCR/MHC/peptide interaction is further stabilized by several accessory or costimulatory molecules (e.g., CD4 or CD8, B7, intercellular adhesion molecule [ICAM-1], and leukocyte function-associated antigen [LFA-3]), all of which bind to specific receptors on the T-cell surface.

Class II–restricted antigen presentation usually requires the presence of professional APCs distinct from the tumor cell. Exogenous, soluble proteins are degraded and processed in the lysosome compartment of professional APCs and are presented on the cell surface bound to class II MHC. This antigen/MHC complex is then recognized by class II restricted, antigen-specific helper $CD4^+$ T-cells. Because most tumor cells express class I on their cell surface, they are capable of acting as class I–restricted APCs by presenting their TAAs in a class I–restricted manner to elicit an antitumor $CD8^+$ T-cell response (4). In general, CTLs specific for the particular antigen bind to the MHC/peptide complex and exert their cytotoxic effect when provided with support in the form of cytokines from class II–restricted $CD4^+$ T cells.

$CD4^+$ T cells, or T-helper cells, are known to be necessary in an antitumor immune response (5,6).

TABLE 1. *Tumor-associated antigens recognized by cytotoxic T-lymphocytes*

| Antigen (reference) | MHC restriction | Tissue distribution | Peptide | Mutated? |
|---|---|---|---|---|
| Melanoma | | | | |
| MAGE-1 (14,15) | A1 | Melanocyte, testis | EADPTGHSY | No |
| | Cw1601 | | SAYGEPRKL | No |
| MAGE-3 (16) | A1 | Melanocyte, testis | EVDPIGHLY | No |
| Tyrosinase (17) | A2 | Melanocyte | MLLAVLYCL | No |
| | | | YMNGTMSQV | No |
| gp100/pMel-17 (18,19) | A2 | Melanocyte | YLEPGPVTA | No |
| | | | LLDGTATLRL | No |
| MART-1/Melan-A (20) | A2 | Melanocyte | AAGIGILTV | No |
| Breast/ovarian | | | | |
| HER2/neu (21) | A2 | All epithelial cells | IISAVVGIL | No |
| Mucin (22) | none | All mucin-producing epithelial cells | (PDTR) repeat | No |

Despite the evidence that a certain subset of $CD4^+$ T cells can be cytotoxic, the classical role of $CD4^+$ cells is understood to be supportive. Research over the past 10 years has resulted in the identification of different functional subsets of $CD4^+$ T cells that can be characterized by the profile of cytokines that they secrete. $CD4^+$ T cells differentiate into subsets that either principally secrete interleukin (IL)-2, interferon (IFN)-$\gamma$, and tumor necrosis factor (TNF)-$\beta$ (Th1 cells) or IL-4, IL-5, IL-6, and IL-10 (Th2 cells). In general, by secreting these cytokines, Th1 $CD4^+$ cells can enhance the $CD8^+$ cellular CTL response, whereas Th2 cells exert their effect on humoral immunity by helping B cells differentiate into antibody-producing cells. Several studies have shown the crucial role that such a helper function plays in antitumor immunity. $CD4^+$ T cells can recognize TAAs when presented on the cell surface of APCs in the context of MHC class II, provide paracrine cytokine stimulation or inhibition to $CD8^+$ CTLs, and direct the predominant response to the cellular arm or the humoral arm of the immune system.

Although the vast majority of TAAs have been identified by their ability to be recognized by class I–restricted $CD8^+$ CTLs, recent studies also have demonstrated class II TAAs recognized by $CD4^+$ cells (7–9). An effective tumor vaccine would likely require activation of both arms of the cell-mediated immune response, i.e., the class I–restricted cytotoxic response and the class II–restricted Th1 helper response.

## TUMOR-ASSOCIATED ANTIGENS

Recent advances in the identification of TAAs that are recognized by CTLs has renewed interest in the feasibility of developing an effective anticancer vaccine. The tumor-specific, human leukocyte antigen (HLA)-restricted CTL response to melanoma, ovarian cancer, and renal cell carcinoma has been well documented (10–13). The isolation of melanoma and ovarian TAAs recognized by CTLs has led to the identification of several 9-10 amino acid peptide epitopes that are presented on the cell surface of tumor cells and recognized by a specific TCR. Table 1 summarizes the list of recently discovered melanoma antigens that were identified based on their recognition by tumor-specific CTLs (14–22).

The first tumor-associated, class I–restricted antigen was identified in melanoma patients by Boon et al. and was given the name melanoma antigen (MAGE-1) (14). This TAA was found to be HLA-A1 restricted, which limited the potential clinical usefulness of this antigen because only a small proportion of the general population carries the HLA-A1 allele. Nevertheless, the MAGE system provided the first example of isolation and identification of a TAA recognized by class I–restricted CTLs in cancer patients. A subsequent search of the MAGE-1 protein sequence and testing of MAGE-derived peptide fragments led to the isolation of a nine amino acid antigenic peptide, which when presented on the cell surface of an $HLA\text{-}A1^+$ cell could be recognized by these tumor-specific CTLs (15).

Using a complementary DNA (cDNA) expression cloning method, Kawakami et al. recently identified the gene encoding a melanocyte-specific melanoma antigen melanoma antigen recognized by T-cells (MART-1). Identification and subsequent cloning of transiently transfected melanoma-derived cDNA fragments that were recognized by tumor-specific CTLs resulted in the identification of the MART-1 gene (23). Subsequent sequencing of

the MART-1 gene product led to the identification of the 10–amino acid peptide that these CTLs were recognizing in an HLA-A2–restricted manner (20). Because the HLA-A2 allele is expressed by a much larger proportion of the general population than HLA-A1 (~50% vs. 10%), the clinical implications of an HLA-A2–restricted TAA would have a potentially broader clinical application. The function of the MART-1 gene remains unknown, but it is believed to play a role in melanocyte differentiation. MART-1 is present in both melanoma cells and normal melanocytes, making it a melanocyte-specific but not a tumor-specific TAA.

Another important method of TAA isolation and identification was successfully demonstrated by Slingluff et al. Direct acid elution of peptide fragments from HLA class I alleles (particularly HLA-A2) followed by fractionation of the eluant using reverse phase (RP) high-performance liquid chromatography (HPLC) has resulted in the isolation and sequencing of the melanoma antigen pMel-17 (18). Given the large number of peptides represented on the cell surface bound to MHC molecules (up to 2,000 by HLA class I alone) (19), isolation of distinct peptides in the large quantities necessary for sequencing is a technically demanding undertaking. Because each fraction can contain >50 peptides, subfractionation is necessary to obtain purified peptide. Each fraction and subfraction must be tested for recognition by tumor-specific CTLs in order to purify the correct peptide antigen. When the correct subfraction is identified, massive numbers of tumor cells must be acid washed to obtain enough peptide in the eluant for purification and sequencing.

Using this acid elution method, the recent identification by our laboratory of a common tumor-associated peptide antigen recognized by CTLs from several different epithelial-based tumors has renewed interest in the possibility of developing a more widely applicable tumor vaccine. Using a purified peptide eluted from HLA-A2$^+$ ovarian cancer cell lines and fractionated by RP-HPLC, we showed that the ovarian cancer derived antigenic peptide could be recognized by tumor-specific CTLs from patients with ovarian, breast, or non–small cell lung carcinoma (21).

Interestingly, of the several CTL-recognized TAAs identified recently, the vast majority were not tumor specific. All the melanoma antigens are expressed in normal melanocytes, and MAGE-1 and MAGE-3 are also expressed in normal testes (Table 1). None have been shown to contain tumor-specific mutations that would explain CTL recognition of a nonself antigen. Because the perfect TAA would be present in all tumor cells, but not in normal cells, the lack of tumor specificity presents a potential problem for those attempting to develop safe, active-specific immunotherapies for cancer.

The identification of TAAs has not been limited to melanoma. Tumor-specific CTLs in ovarian cancer recognize a normal protooncogene-derived peptide antigen that is overexpressed in these cells (24). HER2/neu is a protooncogene that encodes a transmembrane protein homologous to the epidermal growth factor receptor and is expressed ubiquitously, but at low levels in normal epithelial tissues. The fact that HER2/neu is overexpressed in many epithelial-based tumors may play an important role in CTL recognition of HER2/neu-derived peptide epitopes. The lack of tumor specificity (i.e., recognition by CTLs of an overexpressed normal protein) highlights the fact that active specific immunotherapy against antigens expressed in both normal and cancerous cells has the potential to result in undesirable autoimmune complications. Nevertheless, with the exception of tumor-specific mucin–derived epitopes in ovarian and breast cancer (22) and mutated ras epitopes in certain pancreatic and colon cancers (25), the search for a tumor-specific antigen that is consistently expressed in all nonvirally induced human tumors has not been fruitful.

In addition to the discovery of several TAAs recognized by CD8$^+$ CTLs, the recent characterization of antigens specific for CD4$^+$ T cells should further advance the possible development of an effective tumor vaccine. The crucial role of CD4$^+$ helper T cells is well known (5,6). Theoretically, a polyvalent vaccine containing CD4$^+$ and CD8$^+$ specific epitopes should stimulate a more potent and effective immune response, as demonstrated by Berzofsky et al. in an animal model (26). Because the focus of immunotherapy research has been dominated by the CD8$^+$ cytotoxic T-cell response to cancer cells, recent work examining the role of CD4$^+$ cells in the anticancer immune response should enhance our understanding. Several groups have demonstrated a tumor-specific CD4$^+$ T cell–mediated immune response in melanoma patients. Because most human tumors lack class II MHC molecules on their cell surface after in vitro culture, an Epstein-Barr virus–transformed B-cell system has been used for antigen presentation to patient-derived CD4$^+$ cells. Using this method, Topalian et

TABLE 2. *Review of recent clinical trials of vaccine therapy for melanoma*

| Vaccine (reference) | Adjuvant | Patients | Results | Comments |
|---|---|---|---|---|
| Polyvalent allogeneic melanoma cells (29) | Randomized to one of three biologic response modifiers: cimetidine, indomethacin, cyclophosphamide | n = 136, stage II–III | Of 40 patients with evaluable disease, 23% had regression: three CR, six PR | Increased DFS using historical controls |
| Allogeneic tumor cell lysate (30) | DETOX | n = 25, stage II–III | One CR, three PR, one stable disease, two mixed response | No controls; phase III randomized trial ongoing |
| Autologous melanoma cell (31) | BGC, cyclophosphamide | n = 64, stage II–III, metastatic lesion available for resection | Four CR, one PR | Nonrandomized; antitumor responses associated with DTH response |
| Vaccinia melanoma oncolysate (32) | Vaccinia virus | n = 39, stage II–III | Improved DFS | Historical controls; phase III randomized study ongoing |
| Polyvalent shed melanoma antigens (33) | Xenogenic proteins | n = 81, stage II–III | Improved DFS | Historical controls; clinical response correlates with DTH response |
| Ganglioside antigen (GM2) (34) | KLH | n = 122, stage II–III | No improvement in DFS in randomized controlled study | Prognosis correlates with presence of anti-GM2 antibody |

al. showed $CD4^+$ T-cell reactivity against a broadly expressed melanoma-associated antigen derived from the normal tyrosinase gene (8). In addition, Boon et al. recently reported the discovery of a separate tyrosinase-derived antigen recognized by $CD8^+$ effectors in a class I–restricted manner and by $CD4^+$ effectors in a class II–restricted manner (27).

The application of these powerful new methods of identifying TAAs at a molecular level should result in the isolation and cloning of the genes for several important antigens in the near future, not only in melanoma, but in many distinct tumor types. Understanding these antigens and their interaction with the different arms of the cell-mediated immune response can only serve to enhance the possibility of developing an effective tumor vaccine for humans.

## NEW STRATEGIES OF VACCINE DEVELOPMENT

Successful vaccination depends on several factors. One requirement is the activation of APCs with uptake of antigen and presentation in association with class I and/or class II MHC. This is necessary for the initiation and propagation of a cell-mediated immune response. A second requirement is the stimulation of both cytotoxic and helper T cells that recognize a variety of different epitopes, in order to avoid tolerance due to antigenic drift or altered antigen presentation by tumor cells. A third requirement is the stimulation of the appropriate subpopulation of $CD4^+$ helper T cells, which should provide adequate cytokine support in the local microenvironment to support the antitumor immune response (28). In addition, antigens should be coupled with effective adjuvants that provide nonspecific stimulation and, as a result, augmentation of the specific immune response.

Before the identification of TAA antigens in melanoma and other cancers, the majority of tumor vaccines used in clinical and preclinical studies were derived from either intact autologous or allogeneic tumor cells or from tumor lysates. The advantage of using tumor cells as immunogens lies in the fact that the entire repertoire of TAAs should be represented in intact tumor or tumor lysates, which allows for a polyvalent immune response. These tumor cells and lysates are coupled with nonspecific adjuvants to augment the immune response to tumor antigens. Table 2 lists several recent clinical trials that tested the efficacy of tumor cell–based or antigen-based vaccines in patients with melanoma. Although responses in a minority of patients can be observed, a statistically significant improvement in disease-free survival has not been shown in a well-controlled, randomized trial of melanoma vaccine therapy (29–34).

Although all patients treated had either regional or distant metastases at the time of vaccine therapy, the trials cited in Table 2 differ in several aspects. Vaccines consisted of either allogeneic tumor cells or lysates (29,30,32), autologous tumor cells (31), or isolated antigen (33,34). In addition, each vaccine trial was conducted using a different adjuvant or biologic response modifier. A recent review of mechanisms, benefits, and side effects of the many different adjuvants in clinical use attests to the lack of consensus on the best means of adjuvant-mediated immunopotentiation (35).

Strategies range from simple carrier adjuvants that enhance an immunoglobulin response (33) to macrophage stimulators such as DETOX (29), which links a lipid A moiety (for enhanced macrophage uptake) with a mycobacterial cell wall skeleton. Even the DNA alkylating agent cyclophosphamide, generally used for immunosuppression, has been used as a vaccine adjuvant because of its ability to inhibit the proliferation of suppressor T cells (30). Given the array of different adjuvants and the numerous strategies for their development, it is clear that the most effective adjuvant-mediated method of immunopotentiation remains unknown.

It is clear that tumor-specific and HLA-restricted CTLs can be isolated from cancer patients with many different cancers. If that is the case, then why is the antitumor immune response incapable of eradicating disease, especially in melanoma, which we know to be an immunogenic tumor that elicits a massive T-cell infiltration? Because the evidence suggests that tumor cells themselves are capable of presenting antigen on their cell surface in association with MHC class I, many have theorized that the inadequacy of the immune response to cancer is due to poor class II–restricted antigen presentation and therefore a weak helper T-cell response (4,36). Because tumor cells do not usually express class II, the possibility of class II–restricted antigen presentation of exogenous TAAs is delayed until lysed tumor cells release soluble intracellular antigens. According to this theory, the paucity of proinflammatory helper cytokines promotes anergy to TAAs by their failure to support antitumor, cell-mediated immunity. With this concept in mind, several investigators have attempted to circumvent the lack of a class II–restricted T-helper response by providing cytokine support artificially.

Initial attempts to provide cytokines for the propagation of cell-mediated immunity focused on the systemic administration of IL-2 and IFN-$\gamma$ (37,38). Because of the toxicity and variability in effectiveness of systemic administration, recent work has focused on local delivery systems that provide cytokines in the microenvironment of the tumor vaccine innoculum. This has been accomplished by the genetic modification of tumor cells or fibroblasts into which the genes for certain cytokines have been transfected or transduced (39–45). This genetically altered cytokine-expressing cell is then injected as a vaccine to induce antitumor immunity. In murine models the results have been encouraging. Vaccinations with tumor cells transfected with IL-2, IL-4, IFN-$\gamma$, TNF-$\alpha$, granulocyte-macrophage colony-stimulating factor, IL-6, or IL-12 have all imparted immunity to subsequent challenge with wild-type tumors in mice. Table 3 summarizes these murine genetically modified tumor vaccine experiments. Several vaccine trials with cytokine-transfected tumor cells or fibroblasts are currently underway in human patients with metastatic melanoma and other malignancies. Table 4 lists the ongoing, National Institutes of Health (NIH)-approved human trials involving therapy with genetically modified tumor cell vaccines. Their efficacy remains to be seen.

In addition to intact and tumor lysate vaccines, the recent identification of several TAAs has prompted the study of peptide antigen-based vaccines. Peptide vaccine trials using MHC-restricted melanoma TAAs are ongoing. Rosenberg et al. at the Surgery Branch of the NIH are using a MART-1 peptide vaccine in HLA-A2$^+$ melanoma patients. As one would expect, the difficulty with MHC-restricted peptide vaccines is the heterogeneity of HLA alleles in the general population. An HLA-A2–restricted peptide antigen recognized by CTLs in an HLA-A2$^+$ patient might not be presented by MHC in another patient who does not carry the A2 allele.

Because of the heterogeneity of MHC-restricted tumor-associated peptide antigens, which limits their broad-based usefulness in the general population, others have examined the possibility of using non-MHC–restricted methods of antigen presentation in vaccine therapy. Finn et al. have shown that the abnormal architecture of nonmutated mucin proteins on the surface of breast, ovarian, and other mucin-producing cancer cells allows for the exposure of unique, tumor-specific epitopes that can be recognized by CTLs. This antigen, MUC-1, has been shown to be derived from the tandem repeat portion of the mucin molecule, which allows for a

TABLE 3. *Review of genetically modified murine tumor vaccines*

| Gene transfected | Model | Results | Comments |
|---|---|---|---|
| IL-2 (38,39) | Mouse fibrosarcoma transfected with human IL-2 gene | Abrogated tumorigenicity, induced long-lasting immunity against challenge with parental wild type tumor | Retroviral vectors used for gene transfer |
| | Poorly immunogenic murine colon carcinoma transfected with murine IL-2 cDNA | Tumor-specific CTL isolated from splenocytes of vaccinated animals, protection against subsequent challenge | Bovine papilloma virus vector; antitumor response independent of host $CD4^+$ T-cells |
| IL-4 (40) | Murine plasmacytoma and murine mammary adenocarcinoma transfected with murine IL-4 gene | Abrogated tumorigenicity, reversed by IL-4 mAb | Macrophages and eosinophils predominate cellular infiltrate seen within tumors; effect not $CD8^+$ CTL mediated |
| IL-6 (41) | Lewis lung carcinoma cells transfected with human IL-6 cDNA | Two of three IL-6 transfectant clones show growth inhibition; decreased pulmonary metastasis | T cell–dependent mechanism |
| IL-12 (42) | Coinjection of murine fibroblasts transfected with murine IL-12 gene and murine melanoma cell line | Delayed emergence of detectable tumor | Protection against subsequent tumor challenge not tested because of development of palpable tumor in all immunized mice; peritumoral accumulation of macrophages seen histologically |
| TNF-α (43) | Murine plasmacytoma transfected with murine TNF-α cDNA | Abrogated tumorigenicity or delayed tumor growth | Protective immunity against recallenge not tested; peritumoral infiltration of macrophages seen histologically |
| GM-CSF (44) | Murine melanoma transfected with murine GM-CSF | Protection against subsequent challenge with parental tumor | Immunoprotection requires both $CD4^+$ and $CD8^+$ host T cells |

strong, tumor-specific antigenic stimulus because of the abundance of the repeated antigenic segment. Phase I trials of a mucin-derived peptide-based vaccine have demonstated a classic delayed-type hypersensitivity reaction in patients with advanced breast cancer characterized by local infiltration by activated, mucin-specific $CD8^+$ and $CD4^+$ T cells. Despite the evidence of a local response to the antigen, only a modest increase in peripherally circulating mucin peptide–specific CTLs was observed (46). Interestingly, the mucin-specific CTLs derived from these patients are not MHC restricted and therefore do not recognize antigen in the classical class I–restricted manner. This, in part, could be a possible explanation for the lack of activation of a systemic antimucin immune response.

Srivastava et al. have shown that immunization with heat shock protein (HSP)/peptide complexes elicits protective immunity in mice. According to this paradigm, HSPs are not antigenic per se, but rather, they act similarly to MHC in that they are carriers of immunogenic peptides. In this regard, HSP/peptide complexes are tumor specific and immunogenic. It is theorized that HSP/peptide complexes are recognized by γδ T cells (a small subset of T cells distinct from the classical αβ T cell), the function of which is not well understood. Macrophages play an important role as depletion of these cells abrogates the protective immunity, suggesting reprocessing of HSP-derived tumor antigens by these class II–restricted APCs. Because HSPs can be rapidly and reproducibly purified from tumor cells, Srivastava and Udono asserted that patient-specific HSP/peptide vaccines could be clinically effective (47).

## SUMMARY

Despite the recent vast improvement in our knowledge and understanding of tumor-associated antigens and the CTL response to certain cancers, a clinically effective cancer vaccine has not been realized. The presence of TAAs capable of CTL recognition is no longer questioned and has been well documented in several tumor models. Because T cells that can recognize and lyse tumor cells are present in the immune repertoire of cancer patients, the task remains to find the best tumor antigens and manipulate the immune system in order to elicit an

**TABLE 4.** *Current NIH-approved human gene transfer vaccine protocols*

| Principal investigator | Institution | Vaccine | Gene transfected |
|---|---|---|---|
| Melanoma | | | |
| Gansbacher B | Memorial Sloan Kettering Cancer Center, New York, NY | Allogeneic HLA-A2–matched tumor cells | IL-2 |
| Lotze M | University of Pittsburgh, PA | Autologous fibroblasts mixed with tumor | IL-4 (2) or IL-12 (3) |
| Siegler HF | Duke University Medical Center, Durham, NC | Autologous tumor cells | IFN-γ |
| Das Gupta TK | University of Illinois, Chicago, IL | Allogeneic tumor cells | IL-2 |
| Sznol M | National Institutes of Health, Frederick, MD | Allogeneic HLA-A2 or HLA-A1 tumor cells | B7 |
| Economou JS | UCLA Medical Center, Los Angeles, CA | Autologous tumor cells | IL-2 |
| Dranoff G | Dana-Farber Cancer Institute, Boston, MA | Autologous tumor cells | GM-CSF |
| Renal cell carcinoma | | | |
| Gansbacher B | Memorial Sloan Kettering Cancer Center, New York, NY | Allogeneic HLA-A2–matched tumor cells | IL-2 |
| Simons J | Johns Hopkins Oncology Center, Baltimore, MD | Autologous tumor cells | GM-CSF |
| CNS malignancy | | | |
| Sobol R | San Diego Regional Cancer Center, CA | Allogeneic tumor cells or fibroblasts | IL-2 |
| Small cell lung cancer | | | |
| Cassileth P | Miami Veterans Administration Hospital, FL | Allogeneic tumor cells | IL-2 |
| Colon cancer | | | |
| Sobol R | San Diego Regional Cancer Center, CA | Allogeneic tumor cells or fibroblasts | IL-2 |
| Breast cancer | | | |
| Lyerly HK | Duke University medical Center, Durham, NC | Autologous tumor cells | IL-2 |
| Prostate cancer | | | |
| Simons J | Johns Hopkins Oncology Center, Baltimore, MD | Autologous tumor cells | GM-CSF |

From the office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD.
I1-4 protocol includes breast, colon and renal cell carcinoma patients.
IL-12 protocol includes lymphoma, breast cancer and head and neck cancer patients.

effective antitumor response in vivo. Current strategies using genetically modified tumor cell vaccines transfected with genes expressing cytokines and costimulatory molecules aim to alleviate the inadequacy of tumor-specific T-cell help.

In addition, the identification of more and more antigenic peptide epitopes that are recognized by tumor-specific CTLs should result in the development of more peptide-based vaccines for active specific immunotherapy trials, not only in melanoma, but in several other types of cancer. The ideal candidate for an effective peptide antigen would be one that is unique to tumor cells (by virtue of a tumor-specific mutation) and capable of binding to the particular MHC allele that presents antigen on the cell surface. Such an antigen has yet to be found. Current trials focus on tissue-specific, but not tumor-specific, antigens.

Considerable resources have been devoted to the identification of a solitary tumor-specific antigen that is consistently expressed by all tumors. Such efforts have been unsuccessful to date. In common with work in malarial and human immunodeficiency virus vaccine research, it is widely believed that a polyvalent vaccine is the only therapeutic strategy that will be effective against an antigenically heterogeneous target such as cancer. Identifying the correct peptides coupled with the most effective adjuvants remains a challenge for immunotherapy researchers.

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## REFERENCES

1. Gross L. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. *Cancer Res* 1943;3:326–33.
2. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst 1957;18:769–78.
3. Oettgen HF, Old LJ. In: DeVita VI, Hellman S, Rosenberg SA, eds. *Biologic therapy of cancer.* Philadelphia: JB Lippincott, 1991.
4. Ostrand-Rosenberg, S. Tumor immunotherapy: the tumor cell as an antigen-presenting cell. *Curr Opin Immunol* 1994; 6:722–7.
5. Greenberg PD, Cheever MA, Fefer A. Therapy of disseminated murine leukemia with cyclophosphamide and immune Lyt-1$^+$, 2$^-$ T cells. *J Exp Med* 1985;161:1122–34.
6. Wang P, Vanky F, Klein E. MHC class-I-restricted autotumor-specific CD4$^+$CD8$^-$ T-cell clones established from autologous mixed lymphocyte-tumor-cell culture. *Int J Cancer* 1992;51:962–7.
7. Topalian SL, Rivoltini L, Mancini M, Ng J, Hartzman RJ, Rosenberg SA. Melanoma-specific CD4+ T-lymphocytes recognize human melanoma antigens processed and presented by Epstein-Barr virus-transformed B-cells. *Int J Cancer* 1994;58:69–79.
8. Topalian SL, Rivoltini L, Mancini M, et al. Human CD4+ T-cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. *Proc Natl Acad Sci U S A* 1994;91:9461–5.
9. Goedegebuure PS, Harel W, LeMay LG, Kan-Mitchell J, Mitchell MS. Cytotoxic CD4$^+$ lymphocyte clones reactive with melanoma: the role of HLA and accessory molecules. *Vaccine Res* 1993;2:227–34.
10. Chen Q, Hersey P. MHC-restricted responses of CD8+ and CD4+ T-cell clones from regional lymph nodes of melanoma patients. *Int J Cancer* 1992;51:218–24.
11. Hom SS, Topalian SL, Simonis T, Mancini M, Rosenberg SA. Common expression of melanoma tumor-associated antigens recognized by human tumor-infiltrating lymphocytes: analysis by human leukocyte antigen restriction. *J Immunol* 1991;10:153–64.
12. Peoples GE, Schoof DD, Andrews VR, Goedegebuure PS, Eberlein TJ. T-cell recognition of ovarian cancer. *Surgery* 1993;114:227–34.
13. Schendel DJ, Gansbacher B, Oberneder R, et al. Tumor-specific lysis of human renal cell carcinoma by tumor infiltrating lymphocytes: HLA-A2–restricted recognition of autologous and allogeneic tumor lines. *J Immunol* 1993;151: 4209–20.
14. van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T-lymphocytes on a human melanoma. *Science* 1991;254:1643–7.
15. van der Bruggen P, Szikora J-P, Boel P, et al. Autologous cytolytic T-lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw1601. *Eur J Immunol* 1994;24:2134–40.
16. Gaugler B, Van den Eynde B, van der Bruggen P, et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytotoxic T-lymphocytes. *J Exp Med* 1994;179:921–30.
17. Wolfel T, Van Pel A, Brichard V, et al. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas. *Eur J Immunol* 1994;24:759–64.
18. Cox AL, Skipper J, Chen Y, et al. Identification of a peptide recognized by five melanoma-specific human cytotoxic T-cell lines. *Science* 1994;264:716–9.
19. Henderson RA, Cox AL, Sakaguchi K, Appella E, Shabanowitz J, Hunt DF, Engelhard VH. Direct identification of an endogenous peptide recognized by multiple HLA-A2.1-specific cytotoxic T-cells. *Proc Natl Acad Sci U S A* 1993; 90:10275–80.
20. Kawakami Y, Eliyahu S, Sakaguchi K, et al. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J Exp Med* 1994; 180:347–52.
21. Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ. Breast and ovarian cancer-specific cytotoxic T-lymphocytes recognize the same HER2/neu derived peptide. *Proc Natl Acad Sci U S A* 1995;92:432–6.
22. Kotera Y, Fontenot JD, Pecher G, Metzgar RS, Finn OJ. Humoral immunity against a tandem repeat epitope of human mucin MUC-1 in sera from breast, pancreatic and colon cancer patients. *Cancer Res* 1994;54:2856–60.
23. Kawakami Y, Eliyahu S, Delgado CH, et al. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T-cells infiltrating into tumor. *Proc Natl Acad Sci U S A* 1994;91:3515–9.
24. Yoshino I, Peoples GE, Goedegebuure PS, Schoof DD, Eberlein TJ. The sensitivity of human ovarian epithelial tumor cells to cytotoxic T-cells is associated with the expression of HER2/neu. *J Immunol* 1994;152:2393–400.
25. Peace DJ, Smith JW, Chen W, You SG, Cosand WL, Blake J, Cheever MA. Lysis of ras oncogene-transformed cells by specific cytotoxic T-lymphocytes elicited by primary in vitro immunization with mutated ras peptide. *J Exp Med* 1994;179:473–9.
26. Berzofsky JA. Epitope selection and design of synthetic vaccines: molecular approaches to enhancing immunogenicity and cross-reactivity of engineered vaccine. *Ann N Y Acad Sci* 1993;12:256–64.
27. Brichard V, Van Pel A, Wolfel T, et al. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T-lymphocytes on HLA-A2 melanomas. *J Exp Med* 1993; 178:489–95.
28. Ada GL. In: Paul WE, ed. *Fundamental immunology.* New York: Raven, 1993:1329.
29. Morton DL, Foshag LJ, Hoon DS, et al. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. *Ann Surg* 1992;216:463–82.
30. Mitchell MS, Harel W, Kempf RA, et al. Active-specific immunotherapy for melanoma. *J Clin Oncol* 1990;8:856–69.
31. Berd D, Maguire HC Jr, McCue P, Mastrangelo MJ. Treatment of metastatic melanoma with an autologous tumor-cell

vaccine: clinical and immunologic results in 64 patients. *J Clin Oncol* 1990;8:1858–67.
32. Sivanandham M, Wallack MK. Clinical trials with VMO for melanoma. *Ann N Y Acad Sci* 1993;690:178–89.
33. Bystryn JC. Immunogenicity and clinical activity of a polyvalent melanoma antigen vaccine prepared from shed antigens. *Ann N Y Acad Sci* 1993;690:190–203.
34. Livingston PO, Wong GY, Adluri S, et al. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. *J Clin Oncol* 1994;12:1036–44.
35. Allison AC, Byars NE. Immunologic adjuvants; desirable properties and side effects. *Mol Immunol* 1991;28:279–84.
36. Pardoll DM, Tumor antigens: a new look for the 1990's. *Nature* 1994;369:357–8.
37. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, et al. Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *N Engl J Med* 1988;319:1676–80.
38. Legha SS. Interferons in the treatment of malignant melanoma. A review of recent trials. *Cancer* 1986;57:1675–7.
39. Fearon ER, Pardoll DM, Itaya T, et al. Interleukin-2 production by tumor cells bypass T helper function in the generation of an antitumor response. *Cell* 1990;60:397–403.
40. Gansbacher B, Zeier K, Daniels B, Cronin K, Bennerji R, Gilboa E. Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. *J Exp Med* 1990;172:1217–24.
41. Tepper RI, Pattengall PK, Leder P. Murine interleukin-4 displays potent antitumor activity in vivo. *Cell* 1989;57:503–12.
42. Porgador A, Tzehoval E, Katz E, et al. Interleukin-6 transfection into Lewis lung carcinoma tumor cells suppresses the malignant phenotype and confers immunotherapeutic competence against parental metastatic cells. *Cancer Res* 1992;52:3679–86.
43. Tahara H, Zeh HJ, Storkus WJ, et al. Fibroblasts genetically engineered to secrete interleukin 12 can suppress tumor growth and induce antitumor immunity to a murine melanoma in vivo. *Cancer Res* 1994;54:182–9.
44. Blankenstein T, Qin Z, Uberla K, et al. Tumor suppression after tumor cell targeted tumor necrosis factor-a gene transfer. *J Exp Med* 1991;173:1047–53.
45. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony stimulating factor stimulates potent, specific and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 1993;90:3539–3543.
46. Finn OJ. Breast cancer immunotherapy. Massachusetts Department of Public Health Breast Cancer Research Lecture Series, Dana Farber Cancer Institute, Boston, MA, November 8, 1994.
47. Srivastava PK, Udono H. Heat-shock protein-peptide complexes in cancer immunotherapy. *Curr Opin Immunol* 1994; 6:728–32.