Vaccine Trials for the Clinician: Prospects for Tumor Antigens

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ABSTRACT

Recent insights in antigen presentation, the identification of human tumor antigens, and the demonstration of MHC class-I-restricted cytotoxic T lymphocyte (CTL) recognition of peptides encoded by tumor antigen have renewed the interest and enthusiasm for the development of cancer vaccines. Melanoma serves as a paradigm of an immunogenic human tumor, and several tumor antigens, including MAGE, MART-1/Melan-A and gp100, recognized by CTLs, have now been isolated. Candidate antigens for novel vaccine trials may include HLA class-I-binding tumor peptides that serve as CTL epitopes, whole tumor protein, or DNA-based vaccines. Requirements for the use of peptides are that the patient's tumor presents the relevant CTL epitopes as used in the vaccine and expresses the appropriate MHC class-I-restricting molecule. Immunological monitoring may be facilitated when using peptide-based vaccines. Because optimal presentation of tumor antigens may depend on provision of appropriate costimulatory signals, it may be more advantageous to administer professional antigen-presenting cells (APCs), such as dendritic cells (DCs) pulsed with tumor peptide or protein, to cancer patients.

Developments in molecular genetics have led to a new approach in vaccines consisting of cancer cells genetically engineered to express immunomodulatory molecules. This may result in increased antitumor responses to both gene-modified as well as unmodified tumor cells.

The therapeutic approach is extended to vaccination trials with recombinant viruses containing the genes encoding tumor antigens, minigenes containing multiple CTL epitopes, or double recombinant vectors engineered to express both the tumor antigen and immunostimulatory molecules.

Clinical peptide, protein, and DNA-based vaccine trials have recently been initiated. Thus far, exciting clinical remissions were obtained in melanoma patients following vaccination with HLA-A1-binding MAGE-3 peptide and in B-cell lymphoma patients immunized with autologous DCs pulsed with anti-idiotypic protein, i.e., the individual patient's unique tumor antigen. Also, following injection of foreign HLA-B7 DNA into cutaneous melanoma metastases, T-cell migration into treated lesions and enhanced cellular immunity at the site of the tumor were shown in some patients. These encouraging results suggest that effective new vaccines in cancer will be identified. The Oncologist 1997;2:284-299

INTRODUCTION

With the maturing of our insights into the biology of cancer and basic immunological mechanisms, we now have opportunities to rationally develop vaccine approaches against cancer. The idea of developing a cancer vaccine has been a dream of immunologists for years and is based on the concept that tumors possess distinct antigens that should be recognized by the immune system.

Different mechanisms may be used to amplify tumor-specific immune responses. In the absence of well-defined tumor regression antigens, attempts to develop cancer vaccines have made use of live or irradiated allogeneic or autologous tumor cells or tumor cell lysates, either alone or mixed with adjuvants such as bacillus Calmette-Guérin (BCG) and Corynebacterium parvum [1-13]. These vaccination studies have been largely unsuccessful, although in some reports, the remissions associated with disease-free survival were shown to correlate with the return of delayed-type hypersensitivity (DTH) responses to recall antigens and the development of a DTH response to autologous tumor cells. Also, well-defined immunogenic molecules such as gangliosides (glycosphingolipids anchored in the lipid bilayer of plasma membranes and overexpressed in melanomas and neuroblastomas) that are derived from melanoma cells or other sources [14-17] and anti-idiotypic
antibodies carrying the “mirror image” of the antigen [18-20] have been administered to cancer patients as vaccine.

More recently, vaccination trials have been initiated using tumor cells that were genetically modified by DNA sequences encoding a variety of immunomodulatory molecules to increase their immunogenicity. Unraveling of the cellular basis of antigen recognition has fueled the current interest in exploration of tumor peptides for vaccination against cancer, particularly in melanoma. Rational vaccine protocols may include tumor peptide or (protein) antigen, administered alone or pulsed onto dendritic cells (DCs). The following overview will present the background and current state of immunobiology-driven vaccine development for cancer aiming at T cell-mediated antitumor responses.

**Antigen Recognition by T Cells**

The immune system has over time generated two arms to defend the body: the humoral and the cellular immune response. T lymphocytes play a central role in the cellular immune response. Antibodies recognize antigens as native, folded protein at the cell surface, whereas T cells recognize antigen as a fragment of protein (peptide) complexed with a major histocompatibility complex (MHC) molecule on the surface of cells (Fig. 1) [21, 22]. The MHC molecules are highly polymorphic, and the different alleles have distinct peptide-binding specificity. Sequencing of peptides eluted from MHC molecules resulted in the discovery of allele-specific motifs which correspond to critical anchor residues; these residues fit into specific pockets of MHC molecules [23, 24].

Intracellular proteins in the cytosol are cleaved by proteasomes into short peptides comprising 8 to 10 amino acids. These peptides are transported via the specialized transporter associated with antigen processing (TAP) into the endoplasmic reticulum where they bind to newly synthesized MHC class I molecules. After binding, the complex is transported through the Golgi apparatus to the cell surface, where it can be recognized by cytotoxic T cells (CD8+) (Fig. 1). Since all endogenous intracellular proteins can be presented to the immune system in this way, any tumor-specific structure may function as a potential tumor-specific antigen and be recognized by T cells.

Antigen-presenting cells (APCs) express high levels of MHC class I and II and accessory/costimulatory molecules [25, 26], and migrate to central lymphoid organs where optimal priming of T cells can occur and immune responses are initiated. DCs are the most potent APCs in the body that play a major role in initiating immune responses such as activation of MHC-restricted T cell responses and the formation of T cell-dependent antibodies. APCs take up extracellular proteins by either endocytosis or phagocytosis. Foreign proteins are degraded into peptides in acidified endosomes, where they bind to newly synthesized MHC class II molecules, which are specifically targeted to this compartment. The MHC-peptide complex is then brought to the cell surface where it can be recognized by CD4+ helper T cells. Depending on the helper T cell that binds to the complex, B cells are stimulated and antibody production augmented. Like most cells, APCs use their MHC class I molecules to present peptides from endogenous proteins. Evidence has now emerged that in addition to presenting endogenous peptides, a subset of APCs can also acquire and present exogenous proteins on MHC class I molecules [27-31]. The discovery of this second antigen-presenting pathway provides an opportunity to develop new kinds of protein-based vaccines that will result in initiation of cytotoxic T lymphocyte (CTL) responses to class-I-binding peptides derived from exogenously administered proteins. While intact proteins need to be processed to generate antigenic peptides, soluble peptides can bind directly to a small fraction of empty class I or class II molecules present on the cell surface.
Human Tumor Antigens Recognized by T Cells

Melanoma is the most striking example of a non-virus-induced immunogenic tumor in man that is able to elicit T cell-mediated antitumor immunity in vivo. The majority of human tumor antigens defined by T cells have been identified utilizing patients’ T lymphocytes as effector cells and tumor cells obtained from autologous (metastatic) tumor deposits as targets. Several investigators have isolated cross-reactive tumor-specific CTLs from peripheral blood, lymphocytes, or tumor-infiltrating lymphocytes of melanoma patients, and these CTLs are able to recognize common tumor antigen expressed in melanomas that share the restricting HLA class I allele [32-35]. Since 1991, a number of genes encoding human melanoma antigens recognized by T cells have been cloned utilizing melanoma-reactive CTLs [36-44].

Three types of T cell-defined antigens encoding for several HLA class-I- or class-II-binding peptides have now been identified in melanoma (Table 1) and these antigens are targets for vaccination (Table 2). The first group of antigens is expressed in melanoma but also in other cancers (e.g., melanoma antigen [MAGE], BAGE, GAGE). The second group of antigens is specific for melanocytic differentiation, and these antigens are shared by melanoma and melanocytes (e.g., tyrosinase, MART-1/Melan-A, and gp100). The third group of antigens is unique, resulting from point mutations expressed by the individual patient’s tumor (e.g., MUM-1, CDK4).

The first melanoma antigen that was identified is MAGE-1. MAGE-1, -2, and -3 were the original family of human melanoma-specific antigens that were molecularly identified using a DNA library to clone the gene [36, 43]. MAGE-1 antigen is restricted by HLA-A1 or Cw16 [45, 46], while MAGE-3 yields peptides recognized by HLA-A1- or HLA-A2-restricted CTLs [47, 48] (Table 1). The MAGE genes are not expressed in normal adult tissues except testis, but are also expressed in carcinomas of breast and lung carcinomas [49] (Table 2). The function of MAGE has not been elucidated yet. More recently, other genes called BAGE [37] and GAGE-1 and -2 [38] were identified in melanoma and shown to be expressed in other cancers, but not in adult tissue except the testis. BAGE and GAGE were shown to be restricted by HLA-Cw16 and HLA-Cw6, respectively (Table 1).

The melanoma antigens MART-1/Melan-A, gp100, tyrosinase, and tyrosinase-related protein (TRP1 or gp75) represent differentiation antigens expressed by normal melanocytes. Tyrosinase is a key enzyme in the melanin synthesis pathway in pigmented cells; the functions of the other genes are not known. MART-1/Melan-A, gp100, and tyrosinase each yield several HLA-A2-binding CTL epitopes, but the four differentiation antigens are also recognized by HLA-A24, -A31, and -B44-restricted CTLs [40-42, 44, 50-60] (Table 1). HLA-A2 is the most frequent MHC class I allele in

<table>
<thead>
<tr>
<th>Tumor antigens</th>
<th>MHC-restriction</th>
<th>Peptide sequence</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Melanoma antigens</strong></td>
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<tr>
<td>Melanoma differentiation antigens shared in melanoma and melanocytes</td>
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<tr>
<td>MART-1/Melan-A</td>
<td>HLA-A2</td>
<td>AAGIGILTV</td>
<td>[50]</td>
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<td>gp100</td>
<td>HLA-A2</td>
<td>ITDQVPFSV</td>
<td>[51]</td>
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<tr>
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<td>VLYRYFSFSV</td>
<td>[51]</td>
<td></td>
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<tr>
<td>gp100</td>
<td>HLA-A2</td>
<td>FLWGPRALV</td>
<td>[48]</td>
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<tr>
<td>gp100</td>
<td>HLA-A2</td>
<td>YLEPGPVTA</td>
<td>[51, 64]</td>
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<tr>
<td>Tyrosinase</td>
<td>HLA-A2</td>
<td>MLLAVLYCL</td>
<td>[56]</td>
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<tr>
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<td>YMNGTMQSV</td>
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<td>gp100</td>
<td>HLA-A2</td>
<td>APLWHRLF</td>
<td>[57]</td>
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<td>HLA-B44</td>
<td>AFLPWHRLF</td>
<td>[57]</td>
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<tr>
<td>gp100</td>
<td>HLA-DR4</td>
<td>QNILSNAPGQFP</td>
<td>[62]</td>
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<td>gp100</td>
<td>HLA-DR4</td>
<td>SYLQSDPSDFQD</td>
<td>[62]</td>
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<td>TRP-1 (gp 75)</td>
<td>HLA-A31</td>
<td>MSLQRQFLR</td>
<td>[59]</td>
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<td>Tumor-specific antigens also expressed in other cancers</td>
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<tr>
<td>MAGE-1</td>
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<td>EADPTGHSY</td>
<td>[45]</td>
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<tr>
<td>HLA-Cw16</td>
<td>SAYGEPRK</td>
<td>[46]</td>
<td></td>
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<tr>
<td>MAGE-3</td>
<td>HLA-A1</td>
<td>EVDPGHLY</td>
<td>[47]</td>
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<tr>
<td>HLA-A2</td>
<td>FLWGRPRLV</td>
<td>[48]</td>
<td></td>
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<tr>
<td>BAGE</td>
<td>HLA-Cw16</td>
<td>AARAVFLA</td>
<td>[37]</td>
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<tr>
<td>GAGE-1, -2</td>
<td>HLA-Cw6</td>
<td>YRPPRRYY</td>
<td>[38]</td>
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<td><strong>Tumor-specific, mutated gene products</strong></td>
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<tr>
<td>MUM-1</td>
<td>HLA-B44</td>
<td>EEKLTVVLF*</td>
<td>[66]</td>
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<tr>
<td>CDK4</td>
<td>HLA-A2</td>
<td>ACDFHGHGFE*</td>
<td>[67]</td>
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<tr>
<td>β-catenin</td>
<td>HLA-A24</td>
<td>SYLDSGIHE*</td>
<td>[68]</td>
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<td><strong>Antigens in epithelial cancers</strong></td>
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<tr>
<td>HER-2/neu</td>
<td>HLA-A2</td>
<td>KIFGSLAF</td>
<td>[79]</td>
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<tr>
<td>HER-2/neu</td>
<td>HLA-A2</td>
<td>IISAVGILL</td>
<td>[80]</td>
</tr>
<tr>
<td>CEA</td>
<td>HLA-A2</td>
<td>YLSGANLNL</td>
<td>[102]</td>
</tr>
<tr>
<td>HPV-E6, -E7</td>
<td>HLA-A2</td>
<td>YMLDLQPETT</td>
<td>[88]</td>
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</table>

* Mutations are underlined.
Caucasians and appears to be the predominant restriction element for an antimelanoma-directed immune response. MART-1/Melan-A, and gp100 appear to be recognized by a high percentage of tumor-infiltrating lymphocytes (TILs) and tumor-reactive CTL lines obtained from HLA-A2 patients [40, 41, 50-53]. Tyrosinase has also been identified as recognized by CD4+ T cells in a class II-restricted manner [61, 62] (Table 1). Some of the peptides derived from the differentiation antigens were isolated by elution of peptides from the MHC molecules at the cell surface of melanoma cells [63, 64]. The immunogenicity of these melanocyte differentiation antigens demonstrates that an immune mechanism against nonmutated self-antigens with limited tissue distribution can be mounted in cancer patients and that it is indeed possible to alter the state of tolerance to self-antigens [65].

The third group of antigens comprises mutated gene products, namely mutated MUM-1, CDK4, and β-catenin, recognized by autologous lymphocytes from individual patients [65-68] (Table 1). A CTL-epitope encoded by a mutated intron was recently identified and the gene product resulting from incomplete mRNA splicing was named MUM-1. Mutated cyclin-dependent kinase 4 (CDK4, a protein involved in cell cycling) encodes for another CTL epitope. β-catenin is involved in cell-cell adhesion.

T-cell mediated antitumor reactivity has also been found in other types of malignancies including breast carcinoma, ovarian and renal cell carcinoma [69-83]. Targets for vaccination include viral products in virus-induced malignancies, fusion proteins derived from chromosomal breakpoints, and the products of oncogenes that are either mutated or overexpressed in malignant tumors [84-101] (Table 2).

Human papilloma virus (HPV) type 16 (HPV16) is strongly associated with cervical carcinogenesis. The HPV16 E6 and E7 oncoproteins are constitutively expressed in the majority of cervical tumor cells and are, therefore, attractive targets for CTL-mediated immunotherapy. CTL reactivity against HPV proteins has also been found in cervical cancer patients. Although a number of HLA-A2-binding peptides encoded by the viral oncogenes E6 and E7 from HPV type 16 are identified [84, 86, 87], only a small number of patients with HPV16-associated cervical lesions were shown to have a natural CTL response to these peptides [88]. This suggests that in many cervical cancer patients a CTL response against such proteins can be induced and that HPV16 E6 or E7 peptide-based vaccination strategies may be useful.

The predominance of oncogene activation in human cancer makes the mutated oncogene products attractive candidates for immunotherapy [89, 90]. Somatic point mutations in ras oncogenes are frequently found in pancreatic (90%) and other gastro-intestinal adenocarcinomas. Mutant ras peptides are therefore a candidate vaccine for specific immunotherapy in pancreatic and colon carcinoma patients [90]. Both CD4+ and CD8+ T cell clones recognizing mutant ras have been identified in patients with colorectal cancer [91-93]. Furthermore, human HLA class-I-restricted CTL responses against mutant ras could be induced in vitro [94]. However, the CTLs generated in vitro did not show lytic activity against tumor cells expressing mutant ras. The p53 protein represents another potential target as p53 mutations, leading to enhanced expression of p53, are common in several cancers, e.g., colon, lung, and breast carcinoma [95-97].

Chromosomal translocations may result in the generation of fusion genes such as the bcr-abl gene in chronic myelogenous leukemia. CTLs specific for bcr-abl peptides that bind to HLA class I molecules can be generated [98, 100, 101]. Fusion proteins are common in human malignancy and are candidate antigens for vaccine trials.

CTL reactivity against HER-2/neu has been found in patients [79, 80]. HER-2/neu is a growth factor receptor homologous to an epidermal growth factor receptor that is overexpressed in about one-third of breast carcinomas and also in ovarian, lung, and colon adenocarcinomas. In breast cancer, HER-2/neu overexpression has been correlated with

### Table 2. Human tumor antigens that are potential targets for vaccination in cancer patients

<table>
<thead>
<tr>
<th>Type of antigen</th>
<th>Type of cancer</th>
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<tbody>
<tr>
<td><strong>Tumor-specific antigens</strong></td>
<td></td>
</tr>
<tr>
<td>MAGE-1,-2,-3</td>
<td>Melanoma, breast, lung cancer</td>
</tr>
<tr>
<td>BAGE</td>
<td>Melanoma, breast cancer</td>
</tr>
<tr>
<td>GAGE-1,-2</td>
<td>Melanoma, breast, lung, bladder cancer</td>
</tr>
<tr>
<td>Idiotypic antibody</td>
<td>B-cell malignancy</td>
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<tr>
<td>Mucin-1</td>
<td>Breast, ovarian, pancreatic cancer</td>
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<tr>
<td><strong>Differentiation antigens</strong></td>
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<tr>
<td>Tyrosinase</td>
<td>Melanoma</td>
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<td>TRP-1</td>
<td>Melanoma</td>
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<tr>
<td>MART-1/Melan-A</td>
<td>Melanoma</td>
</tr>
<tr>
<td>gp100</td>
<td>Melanoma</td>
</tr>
<tr>
<td><strong>Prostate-specific antigen</strong></td>
<td>Prostate cancer</td>
</tr>
<tr>
<td><strong>Mutated oncogenic or fusion protein</strong></td>
<td></td>
</tr>
<tr>
<td>ras</td>
<td>Gastrointestinal, lung cancer</td>
</tr>
<tr>
<td>p53</td>
<td>Colorectal, breast, lung cancer</td>
</tr>
<tr>
<td>bcr-abl</td>
<td>Chronic myelogenous leukemia</td>
</tr>
<tr>
<td><strong>Overexpressed proteins</strong></td>
<td></td>
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<tr>
<td>HER-2/neu</td>
<td>Breast, ovarian, lung cancer</td>
</tr>
<tr>
<td><strong>Viral proteins</strong></td>
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<tr>
<td>HPV E6, E7</td>
<td>Cervical cancer</td>
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<tr>
<td>Epstein-Barr virus</td>
<td>Burkitt’s lymphoma, nasopharyngeal cancer</td>
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HPV = human papilloma virus
poorer prognosis. Also, carcinoembryonic antigen (CEA) [102] may potentially be a good candidate for vaccination in patients with colorectal cancer. In patients with overexpression of HER-2/neu, both T cells and antibodies reactive to HER-2/neu could be demonstrated [103]. Animal studies indicate that vaccines consisting of subdominant epitopes derived from these self-proteins may elicit an effective immune response [104].

MUC-1 (Mucin 1, PEM) is a large, molecular, tumor-specific carbohydrate antigen on the cell surface that is aberrantly glycosylated and upregulated in breast, ovarian, colon, and pancreatic carcinoma. MHC-unrestricted MUC-1 specific CTLs have been isolated from breast, ovarian, and pancreatic cancer patients [69, 71].

The identification of common tumor antigens implies that we do not depend solely on the use of autologous tumor cells as a vaccine in cancer patients. It justifies the use of both autologous as well as allogeneic HLA-matched tumor cells and tumor antigens as vaccines. Such vaccines may be applicable in a considerable number of cancer patients.

**GENE-MODIFIED TUMOR CELL VACCINES**

In the early 1990s, various investigators reported enhanced immunogenicity of tumor cells following genetic modification with cytokine cDNA. In several models, interleukin 2 (IL-2) gene-modified tumor cells as well as the wild-type, unmodified parental tumor cells were rejected [105-108]. Similar results have been obtained following the use of tumor cells engineered to secrete other cytokines, e.g., IL-4, IL-7, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), G-CSF, and GM-CSF [109-116]. Most reports indicate that this approach is highly effective in protecting animals from subsequent tumorigenic doses of non-modified tumor cells, but few animal studies indicate that this approach is also effective in eradication of already-established tumors. Similar strong antitumor responses were obtained following vaccination with tumor cells engineered to express foreign MHC genes, class I or II, or the B7 (costimulatory molecule) cDNA [117-125].

The effector cells that mediate the observed reduced tumor regression may differ depending on the model studied. Since the first human gene marking was approved and conducted in the U.S. [126], several gene therapy protocols have been initiated there and in Europe. The advantage of a genetically modified autologous cell vaccine is that it contains the whole collection of tumor proteins and therefore has the greatest chance of inducing an immune response against relevant tumor antigens. However, growing autologous tumor cells in vitro to establish tumor cell lines is time-consuming and often unsuccessful. Following administration, allogeneic tumor cells that share an HLA class I allele may in vivo either directly present shared immunodominant tumor peptides to class-I-restricted CTLs or first be degraded and processed by professional APCs (Fig. 2A and Fig. 2B). These APCs will process and select the appropriate epitopes, which will enter the class II or even the class I route to stimulate the patient’s CD4+ or CD8+ T cells.

We have initiated a clinical study in metastatic melanoma patients to evaluate the toxicity and antitumor efficacy of weekly s.c. injections of IL-2-secreting, allogeneic melanoma cells that share one or more HLA class I alleles with the patient [127]. We have observed inflammatory reactions and regression of distant metastases, and, in some cases, significant increases in antimelanoma CTLs frequencies. There are now several ongoing human gene therapy trials employing a similar approach with tumor cells engineered to express costimulatory molecules or secrete cytokines for the treatment of patients with cancer [128, 129]. In one study, a limited antitumor T cell response was found following vaccination [130]. Now that the feasibility of these strategies has been demonstrated, they should be applied in the adjuvant setting in high-risk patients because the approach of gene-modified tumor cell vaccines will only be successful when minimal tumor burden exists.

**PEPTIDE-BASED CANCER VACCINES**

The advantages of synthetic peptides are that the preparations show chemical consistency from batch to batch and that immunological monitoring of defined T cell epitope is easier. Another advantage is the relatively simple and inexpensive production of large quantities and the possibilities of constructing multi-epitope vaccines by combining CTL epitopes derived from different tumor antigens.

A disadvantage of peptide vaccines is the restriction of each peptide to one HLA molecule [131]. For a peptide-based vaccine to be widely applicable, it will be necessary to identify multiple peptide epitopes that are presented by all the major MHC class alleles. Persistence of peptide antigen
in vivo will be limited by clearance and degradation. The presence of serum peptidases may alter the antigenicity of peptides or rapidly inactivate peptides. To raise the immunogenicity, peptides can be injected with adjuvants, in liposomes, or by direct attachment of lipids [132]. Immunization with DCs, the most powerful professional APCs that are able to prime naive CTLs in vitro and in vivo, may represent a cancer vaccine that is superior over a vaccine containing peptide alone [133-136].

Peptide-based vaccine therapies in melanoma patients have recently been initiated in a few centers in Europe and the U.S. [137]. Marchand et al. reported significant tumor regressions (including one complete remission) in 3 out of 12 HLA-A1-positive tumor-bearing melanoma patients who were immunized with s.c. injections of the synthetic HLA-A1-binding MAGE-3 peptide. These results are remarkable, as the peptide was injected without adjuvant, and tumor responses in the absence of adjuvant had not been anticipated. Jaeger et al. [138] vaccinated six HLA-A+ metastatic melanoma patients intradermally with multiple CTL epitopes, i.e., peptides derived from MART-1/Melan-A, tyrosinase, and gp100/Pmel 17. In addition, the influenza matrix peptide was administered as a control. DTH reactions were observed in five out of six patients. Generation of peptide-specific CTLs was documented against MART-1/Melan-A-derived peptide epitopes, the tyrosinase signal peptide, and the influenza matrix peptide after vaccination. No tumor regressions were observed.

A number of clinical studies under the guidance of Rosenberg employing synthetic HLA-A2-binding peptides derived from the melanoma differentiation antigens MART-1/Melan-A and gp100 are in progress. In a phase I study, 28 melanoma patients were immunized with escalating doses of the immunodominant gp100 nonapeptides gp100aa154-162, gpaa209-217, and gp100aa280-289 administered s.c. in incomplete Freund’s adjuvant [139]. Administration of more than two immunizations with the gp100aa209-217 and gp 100aa280-289 peptides appeared to further enhance the patient’s immune reactivity against the 209 and 280 epitopes. No 154 peptide-specific activity could be demonstrated in the patients immunized with gp100aa154-162. Because the immunodominant gp100 peptides have relatively low binding affinity to HLA-A2, peptides modified at the HLA-A2-binding anchor positions, but not at T cell receptor (TCR) contact residues, were selected based on MHC binding affinity [140]. Two of these peptides, one containing an amino acid substitution at position 2 of gp100aa209-217, and one containing an amino acid substitution at position 9 of gp 100aa280-289, are high-affinity binding peptides and seem to be more immunogenic than the native epitopes. A clinical study with these modified high-affinity binding peptides is now in progress.

**Figure 2.**
In another study, three patients with advanced metastatic melanoma were vaccinated with autologous cultured DCs pulsed with an HLA-A1-binding MAGE-1 nonapeptide. MAGE-1 peptide-specific CTLs were demonstrable after, but not prior to, vaccination, and these CTLs were capable of lysing HLA-A1 MAGE-1-positive melanoma cells in vitro [141]. No major therapeutic responses were noted, possibly because of the advanced stage of the disease.

Others investigated the potential of vaccinating cancer patients with peptides derived from mutant ras and showed that vaccination of end-stage pancreatic carcinoma patients with mutant ras peptide-pulsed APCs from peripheral blood resulted in a transient ras-specific proliferative T cell response in some of these patients [142, 143].

**Protein Vaccines**

Several lines of evidence may support the delivery of entire proteins rather than the use of a peptide-based vaccine. Use of a peptide vaccine is limited to single epitopes. The use of whole protein vaccines may be advantageous over peptide vaccines in that it provides a wider range of multiple MHC class I (and class II) epitopes, several T cell epitopes binding a single MHC allele, and also several T cell epitopes binding to different MHC class I alleles. Therefore, whole proteins may provide T cell epitopes that have not been identified in the context of other common HLA alleles.

B cell malignancies are unique in that they express abundant tumor-specific cell-surface antigen (immunoglobulin) which is not shed from the neoplastic cells. As these malignancies are monoclonal, all the cells of a given tumor express identical immunoglobulin receptors, making them a suitable tumor-specific target for immunotherapy. However, each lymphoma has a unique idiotypic immunoglobulin, and anti-idiotypic strategies must therefore be tailored to individual patients.

A clinical trial was initiated to evaluate the efficacy of tumor-specific idiotype protein-pulsed autologous DCs in the treatment of B cell lymphoma [144]. Tumor biopsies were obtained from patients with B cell lymphoma, and the immunoglobulin (idiotype protein) produced by each tumor was obtained by cell fusion techniques. DCs were isolated from the peripheral blood of patients with lymphoma by leukapheresis and density-gradient centrifugation. Four patients with follicular B cell lymphoma received a series of three or four infusions of antigen-pulsed DCs followed by s.c. injections of soluble antigen two weeks later. All patients developed measurable antitumor cellular immune responses. In addition, three of the four patients experienced clinical remissions of the disease (two complete remissions and one partial remission). This study has demonstrated the ability of antigen-pulsed DCs to stimulate clinically relevant immune responses in humans.

**Recombinant Viral Vectors**

In contrast with tumor cells that are often poorly immunogenic, viruses or viral extracts can elicit a strong specific and lifelong immunity. In animal models, it has been shown that model tumor antigens presented by viruses are highly immunogenic, whereas the same model antigen presented by tumor cells is not [145]. Several viruses, including recombinant vaccinia virus, fowlpox virus, and adenovirus encoding model tumor antigens, have been shown to express antigens within the cytoplasm of infected cells, resulting in the induction of murine immunity. However, immunization with live attenuated or recombinant viruses may also pose safety problems due to their infectious nature. Furthermore, the delivery of whole genes encoding tumor antigens that are involved in carcinogenesis may lead to malignant transformation of recombinant-virus-infected cells. For instance, viral vector vaccines containing the functional HPV E6 and E7 oncogenes, mutated oncogenes, aberrant fusion proteins, or tumor antigens (such as MAGE, GAGE, and BAGE, whose functions are still unknown), should be considered unsafe. By introducing only the CTL epitopes derived from such tumor antigens into viral vectors, T cell immunity may be induced without introducing potential hazards. Nonviral delivery systems are also being developed as an alternative to current delivery strategies employing viral-mediated gene therapy approaches.

Recombinant viral vectors can now be constructed in such a way that the presentation of the antigens to the T cells is optimized. For instance, recombinant viruses were constructed containing minigenes encoding antigenic peptides with an amino-terminal endoplasmic reticulum insertion sequence (circumventing requirements for proteolysis and transport) and were shown to greatly enhance the CD8+ CTL immune response [146-148]. Furthermore, vaccinia viruses carrying string-of-beads constructs containing the genetic code for multiple CTL epitopes are constructed, and each epitope within the polypeptide protein has been shown to be processed and presented for CTL-mediated lysis. Such polypeptide CTL vaccines have been shown to be effective in eliciting the desired immune response [149-151]. In addition, molecular technology now enables us to construct viral vectors that allow specific targeting of an antigen to the endosomal and lysosomal compartments, resulting in enhanced presentation via the MHC class II pathway and subsequent recognition of the target antigen by CD4+ T cells [152, 153]. Moreover, viral vectors can be constructed which encode not only the tumor antigen of interest but also for cytokines or other costimulatory molecules that can facilitate the activation of a powerful cellular immune response [154-157].
In one of the first clinical vaccination studies against a (self) tumor antigen employing a vaccinia viral construct containing the carcinoembryonic antigen, Tsang et al. [102] were able to show that tolerance can be broken by vaccination. CTL responses to a specific CEA epitope were induced and the CTLs were able to lyse tumor cells expressing CEA. In this study, in spite of the enhanced immunoreactivity, no clinical benefit was obtained, possibly because of the advanced stage of the disease in the patients. A trial using the same recombinant vaccinia-CEA vector is now performed in gastrointestinal cancer patients with minimal disease.

Recently, Borysiewicz et al. [158] reported a first clinical trial in late-stage cervical cancer patients in which patients were immunized by dermal scarification with a recombinant vaccinia viral construct encoding modified HPV 16 and 18 E6 and E7 protein sequences. All patients mounted an antivaccinia antibody response. Three of eight patients developed an HPV-specific antibody response. HPV-specific cytotoxic T lymphocytes were detected in one of three evaluable patients.

**DNA Vaccination**

DNA delivering methods have been shown in animals to be efficient enough to raise immune responses [159-164]. With naked DNA vaccines, the host cell manufactures the protein and CTL epitope. Plasmids are easy to manipulate and can accommodate large sequences of foreign DNA. They can be produced at a high level of purity and are associated with low immunogenicity. DNA-based vaccinations with naked DNA encoding tumor antigen may supersede the more complex technology of other gene therapy protocols.

The gene gun gene delivery system [165-167] is an effective means of introducing antigen-encoding expression vectors into the epidermis. The immunization of the skin results in temporal presence of DNA and expression of antigen, but elicits humoral and cellular immune responses and protective immunity. The skin is rich in DCs and ballistic cutaneous genetic immunization may result in in vivo transfection of skin-derived DCs. Endogenously synthesized antigen can access the MHC class-I-restricted pathway of transfected DCs. Following migration to regional lymphoid organs, the DCs can present the tumor antigen to T cells with appropriate costimulatory signals for T cell activation.

DNA immunization can produce long-term humoral and cellular immune responses qualitatively similar to that of live attenuated vaccines [161-164]. Priming of MHC class-I-restricted CD8+ T cells may require ingestion, processing, and presentation of peptides derived from the expressed protein on MHC class I molecules by host APCs (cross-priming) in vivo [168-170].

Although treatment of B cell lymphoma patients with anti-idiotypic antibody has been demonstrated to be successful [171], analysis of lymphoma cells at the time of relapse pointed to somatic mutation in the idiotypic V genes (idiotype escape) as an important mechanism by which the neoplastic clones survive exposure to an anti-idiotype monoclonal antibody. Importantly, loss of surface immunoglobulin does not appear to occur. A phase I trial has been initiated to test the safety of the genetic approach to personalized idiotypic vaccination with DNA encoding the idiotypic V gene [172]. Variable region gene sequences coding for the lymphoma idotype were isolated directly from biopsy material by polymerase chain reaction (PCR) amplification, cloning, and DNA sequence analysis of the cloned PCR product.

Another approach is to enhance the antitumor immune response by in vivo injection of a foreign HLA class I gene, i.e., HLA-B7, by DNA liposome complexes directly into tumor deposits. Nabel and co-workers showed that the introduction of HLA-B7 DNA into cutaneous melanoma metastases by direct injection brought about a 10%-15% transduction of the tumor cells [173]. The HLA-B7 protein expression could be demonstrated to be expressed in the tumor cells near the site of injection. No systemic toxicity was observed, and regression of a distant lung metastasis in one of the five reported patients suggests that allogeneic effects may indeed enhance antitumor immune response. In a more recent update on 10 patients, Nabel et al. [174] reported that in most patients HLA-B7 gene transfer did not markedly alter the frequency of circulating tumor-specific CTL in peripheral blood, whereas T cell migration into treated lesions was enhanced in the majority of patients, and tumor-infiltrating frequency of lymphocyte reactivity was enhanced in the two patients studied. In one patient, subsequent treatment with tumor-infiltrating lymphocytes derived from gene-modified tumor resulted in a complete regression of the residual disease. These data suggest that immunological monitoring of lymphocytes from the peripheral blood compartment does not provide accurate information and, instead, T cells from metastatic sites should be followed.
GUIDELINES TO CLINICAL APPLICATIONS OF CANCER VACCINES

Melanoma serves as a paradigm for tumor immunology, and cancer vaccine trials will initially focus on melanoma. Various CTL epitopes and the encoding tumor antigens have been identified in melanoma, enabling the clinician to explore several strategies, as outlined in Table 3. Clinical trials will have to demonstrate whether a single strategy will prove to be superior. In animal studies, immunotherapeutic approaches are more likely to be successful when the tumor load is relatively small. Based on this fact, it may be important to select patients with small tumor burden, for instance, high-risk patients following excision of a thick primary melanoma or following regional lymph node dissection (AJCC stage IIB and III melanoma patients). The disadvantage of such adjuvant studies may be the long period of time and large numbers of patients required to demonstrate the efficacy of the approach. For this reason, ability to monitor effective immune responses becomes even more critical. It may be useful to determine before entry to the study whether the patient’s cellular immunity is able to react to known antigens by performing a simple delayed hypersensitivity test to recall antigens.

In order to enable an effective T cell-mediated antitumor response to be mounted in the patient, tumor cell surface expression of MHC class I molecules that are able to present tumor peptides is a prerequisite. Thus, MHC class I expression of patients’ tumor cells has to be determined (Table 4).

The choice of tumor antigen to be administered as a vaccine, whether formulated as peptide, protein, gene, or minigene, has to be based on the tumor antigen expression in the patient’s tumor cells. The frequent expression in melanoma of differentiation antigens and shared tumor antigens enables the use of tumor antigen-based vaccines. The infrequently expressed unique antigens that arise from point mutations provide candidate vaccines for individual patients. However, they require identification in individual patients and are therefore less attractive candidates for clinical trials.

All melanoma patients are candidates for vaccination with either gene-modified, whole-tumor cell vaccines, tumor protein vaccines, or vaccines consisting of DNA encoding the tumor antigen. In HLA-typed HLA-A2 or HLA-A1 melanoma patients, various peptide-based strategies are possible (Tables 1 and 4). The existence of multiple HLA-A2-binding epitopes in gp100, MART-1/Melan-A, and tyrosinase, and of HLA-A2-binding peptide derived from MAGE-3, enables the use of multivalent peptide vaccines in HLA-A2-positive melanoma patients. HLA-A1 positive patients are eligible for MAGE-1 and MAGE-3 binding peptide-based vaccines.

Until now, only a few CTL epitopes (mainly HLA-A2-binding peptides) derived from various tumor antigens have been identified. Because HLA-A2 molecules are expressed in 40% to 50% of Caucasians, HLA-A2-binding peptides may have therapeutic potential in a

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**Table 3. Cancer vaccines aimed at induction or enhancement of a T cell-mediated antitumor response**

<table>
<thead>
<tr>
<th>Whole tumor cell vaccines</th>
<th>Peptides (single or multivalent peptide vaccine)</th>
<th>Proteins</th>
<th>DNA-encoding tumor antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified</td>
<td>Alone (alone)</td>
<td>Alone</td>
<td>Gene gun for intradermal injection</td>
</tr>
<tr>
<td>Gene-modified with genes encoding cytokines, costimulatory molecules or other genes to enhance the immune response</td>
<td>With adjuvants</td>
<td>With adjuvants</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>Linked to lipids, liposomes</td>
<td>Linked to lipids</td>
<td>Linked to lipids</td>
<td>Linked to lipids</td>
</tr>
<tr>
<td>Pulsed onto APCs</td>
<td>Pulsed onto APCs</td>
<td>Pulsed onto APCs</td>
<td>APCs transduced with DNA-encoding tumor antigens or a minigene containing multiple CTL epitopes</td>
</tr>
</tbody>
</table>

**Table 4. Monitoring of vaccine trials in cancer patients**

**Prestudy screening**

<table>
<thead>
<tr>
<th>Stage of the disease and performance status</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTH test to recall antigens</td>
</tr>
<tr>
<td>Establish tumor phenotype</td>
</tr>
<tr>
<td>• MHC class I and II</td>
</tr>
<tr>
<td>• Tumor antigen expression (RT-PCR)</td>
</tr>
<tr>
<td>HLA typing (HLA-A2 or -A1 for known HLA-A2 and -A1-binding peptides)</td>
</tr>
<tr>
<td>Obtain peripheral blood lymphocytes (buffy coat or leukapheresis)</td>
</tr>
</tbody>
</table>

**Monitoring immunological response**

| DTH testing of peptide(s) or tumor cells used |
| Antitumor or peptide-specific CTL precursor frequency analysis in peripheral blood lymphocytes or TILs |
| Determination of CTL responses in bulk cultures or at the clonal level in peripheral blood lymphocytes or TILs |
| TCR expression in peripheral blood lymphocytes or TILs |
significant number of cancer patients. However, the arsenal of peptides may be extended to other new epitopes. Based on predicted HLA class-I-binding motifs that were first described for HLA-A2 and now for HLA-A1, -A3, -A11, -A24, and -B7, as well as other alleles [23, 24, 131, 175], we are now able to predict which peptides derived from a protein with known amino acid sequence will bind to certain class I alleles. It is thus possible to have such predicted peptides synthetically synthesized and to test for binding to the appropriate class I molecule. Such peptides could then be tested for immunogenicity in vitro or in HLA transgenic animal models, and also used in vaccine programs. The identification of a set of tumor-specific peptides that can bind to the previously mentioned HLA alleles enables us to develop peptide vaccines that are applicable in the majority of the human population. Another approach is to elute MHC class I-binding tumor peptides that will contain as yet unidentified tumor peptides from tumor cells and pulse these peptides onto autologous or MHC class-I-matched allogeneic, APCs. This approach clearly depends on the availability of sufficient, appropriate tumor material and can be applied to cancer patients regardless of the MHC class I phenotype.

Similar vaccination strategies may be applied in various other types of malignancies. For instance, vaccination with HER-2/neu derived peptides, alone, with adjuvant, or pulsed onto APCs, may be considered in HLA-A2 positive breast, ovarian, or lung cancer patients with minimal residual disease. Other attractive candidates for vaccination are HLA-A2-binding peptides derived from HPV E6 and E7 in cervical cancer patients.

Although the recognition of peptide class I complexes is sufficient to trigger target cell lysis, priming of CTL responses requires the presentation of the relevant antigen by professional APCs capable of providing costimulation. At present, vaccination of cancer patients with peptides or tumor proteins loaded onto DCs seems to be one of the most promising approaches. In the past, the use of DCs has been limited by the difficulty of obtaining large numbers of these APCs. Now, DCs can be routinely prepared from peripheral blood leukapheresis samples oruffy coats that are cultured with GM-CSF plus IL-4 [176]. After a few days of culturing with cytokines, DCs can be pulsed or otherwise manipulated in vitro before administration to the patient.

The immunological monitoring of cancer patients will be critical for understanding the nature of the immune responses to vaccination. At present, it remains to be established which of the existing assays is the most accurate in vitro correlate of clinical response. Immunological monitoring assays may include CTL precursor frequency assays, CTL cloning procedures, and TCR analysis on peripheral blood lymphocytes as well as on TILs. Overexpression of TCR may be associated with clonal expansion of antigen-specific T cells and a beneficial immune response to therapy. Analysis of such TILs may be more informative than analysis of circulating blood lymphocytes [174, 177].

**CONCLUSIONS**

Using past experiences, there are now many possibilities for constructing new-generation vaccines which are more rational in the specificity of their composition. Progress in molecular genetics enables us to explore the potential of genetically modified tumor cell vaccines, tumor peptide, protein, or DNA-based tumor antigen vaccines in cancer patients. The value of DCs needs to be established, and new adjuvants need to be explored. Major hurdles for vaccination remain the heterogeneity and variability of human cancer, and the immune escape mechanisms of cancer cells. Decisions regarding type of vaccination strategy should include assessment of tumor antigen and HLA class I expression in the individual patient’s tumor.

Results from animal studies and human trials of various vaccine types indicate that active immunization against a patient’s preexisting tumor is likely to be effective only when the tumor load is small. Unfortunately, the vast majority of clinical trials has been in patients where the cancer is widespread. Once it has been shown that a new protocol is feasible, patients who are at high risk to develop metastases but who still have minimal residual disease should be selected for vaccination strategies. Immunological monitoring of clinical vaccination trials is critical to our understanding of the complex events that happen in vivo following administration of a vaccine. Therefore, efforts should be made to further develop reliable assays for efficient monitoring of the state of immunization of cancer patients against tumor antigens.

Instead of whole tumor cells, synthetic peptides whose sequences correspond to epitopes of tumor antigens recognized by T lymphocytes can be used as prophylactic vaccines to prevent tumor occurrence in patients at high risk of developing melanoma or as a therapeutic vaccine in patients with metastatic disease.

The admittedly modest successes thus far obtained with vaccine trials in patients nevertheless offer grounds for optimism that important progress in immunotherapy of cancer will be made.
REFERENCES


84 Kast WM, Brandt RPM, Drifhout JW et al. HLA-A2.1 restricted candidate CTL epitopes of human papillomavirus type 16 E6 and E7 proteins identified by using the processing defective human cell line T2. J Immunother 1993;14:115-120.


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124 Townsend SE, Allison JP. Tumor rejection after direct co-stimulation of CD8 T-cells by B7-transfected melanoma cells. Science 1993;259:368-370.


128 Fenton RT, Sznol M, Luster DG et al. A phase I trial of B7-transfected or parental lethally irradiated allogeneic melanoma cell lines to induce cell-mediated immunity, against tumor-associated antigen presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma. Hum Gene Ther 1995;6:87-106.


