

Cancer Immunotherapy With Peptide-Based Vaccines: What Have We Achieved? Where Are We Going?

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Many human tumor-associated antigens (TAAs) have recently been identified and molecularly characterized. When bound to major histocompatibility complex molecules, TAA peptides are recognized by T cells. Clinical studies have therefore been initiated to assess the therapeutic potential of active immunization or vaccination with TAA peptides in patients with metastatic cancer. So far, only a limited number of TAA peptides, mostly those recognized by CD8⁺ T cells in melanoma patients, have been clinically tested. In some clinical trials, partial or complete tumor regression was observed in approximately 10%–30% of patients. No serious side effects have been reported. The clinical responses, however, were often not associated with a detectable T-cell-specific antitumor immune response when patients' T cells were evaluated in *ex vivo* assays. In this review, we analyze the available human TAA peptides, the potential immunogenicity (i.e., the ability to trigger a tumor-specific T-cell response) of TAA peptides *in vitro* and *ex vivo*, and the potential to construct slightly modified forms of TAA peptides that have increased T-cell stimulatory activity. We discuss the available data from clinical trials of TAA peptide-based vaccination (including those that used dendritic cells to present TAA peptides), identify possible reasons for the limited clinical efficacy of these vaccines, and suggest ways to improve the clinical outcome of TAA peptide-based vaccination for cancer patients. [J Natl Cancer Inst 2002;94:805–18]

Tumor cells express antigens that can be recognized by the host's immune system. These tumor-associated antigens (TAAs) can be injected into cancer patients in an attempt to induce a systemic immune response that may result in the destruction of the cancer growing in different body tissues. This procedure is defined as active immunotherapy or vaccination inasmuch as the host's immune system is either activated *de novo* or restimulated to mount an effective, tumor-specific immune reaction that may ultimately lead to tumor regression. However, until now, the vaccination approach for cancer has been carried out in the presence of the disease (i.e., in immunocompromised subjects) and not, as it occurs in prophylactic vaccination against infectious diseases, in healthy individuals. Moreover, although in infectious disease vaccination, the antibody response is of major importance, in anticancer vaccination, the focus is on the induction of T-lymphocyte responses. In fact, a considerable body of data from animal models and with human cells *in vitro* indicates that T cells are the major factor for the immunologic control of tumor growth when neoplastic cells express TAA. Although vaccination against cancer has a long history, clinical results of studies of cellular vaccines from the last few decades have been

inconclusive because of the lack of knowledge of the molecular nature and tissue distribution of TAAs used to immunize individuals and the limited availability of sensitive *ex vivo* assays for evaluating the T-cell immune response to the vaccine.

It has been known for some time that T cells recognize antigens in the form of short peptides bound to major histocompatibility complex (MHC) molecules (1). However, it was not until 1991 that the first report describing the cloning of a gene encoding a human TAA, the melanoma antigen-1 (MAGE-1) was published (2). The identification of its nonamer peptide, which is recognized by human leukocyte antigen (HLA)-A1-restricted cytotoxic T lymphocytes (CTLs), was published the following year (3).

Identification of TAA peptides expressed by different human tumors [see (4)] provided the basis for antigen-specific active immunotherapy or vaccination and facilitated the design of new vaccination clinical trials. However, it soon became clear that TAA peptides differ in their *in vivo* immunogenicity, and that antigenicity depends on many factors. Over the last several years, the results of clinical trials aimed at testing toxicity and clinical and immunologic responses of cancer patients given peptide-based vaccines to elicit a T-cell response have been difficult to interpret for several reasons, including heterogeneity of the peptides used and of the HLA-A alleles that recognize them, different vaccine formulations, different clinical conditions of immunized patients, and problems with *in vivo* and/or *ex vivo* evaluation of the vaccine-specific T-cell response.

In this review, we evaluate some of the features of TAA peptide-based vaccination, identify the current obstacles, and delineate potential solutions to increase the clinical efficacy of peptide-based vaccination for cancer patients.

PEPTIDES AS TUMOR ANTIGENS

TAA Peptides and Their T-Cell Immunogenicity

Proteins may contain one or more peptides or epitopes recognizable by T cells. All cells express on their surface class I and class II MHC molecules bound to short peptides of 8–10 and

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13–20 amino acids, respectively, that are derived by specific intracellular processing of proteins. Amino acid sequencing of the naturally processed peptides eluted from a given MHC allele revealed that each MHC allelic variant efficiently binds only a subset of peptides that share conserved amino acid residues at fixed positions (5). The position and chemical nature of the amino acids that directly affect the ability of the peptide to bind the appropriate MHC allele are defined as the peptide binding motif. The peptide–MHC complex is recognized by the T-cell receptor (TCR) on the surface of T lymphocytes. In addition to directly contacting the MHC molecule, other peptide residues are available to establish a direct contact with the TCR (6). Thus, whether a TAA peptide elicits a T-cell response is governed both by the ability of the peptide to bind the presenting MHC allele and by the resulting affinity of the peptide–MHC complex for the TCR.

Several approaches have led to the identification of TAAs and their peptides that are recognized by tumor-specific T cells (4). On the basis of their tissue distribution, T-cell defined TAAs now include a) differentiation antigens, which are expressed in a lineage-related manner and are detected in the normal counterpart of neoplastic tissue, and b) tumor-restricted antigens, which are expressed only on neoplastic cells. Tumor-restricted antigens encompass both shared antigens of different origin and unique antigens (Table 1). For unique TAAs (7–10), the immunogenic peptide includes a mutated amino acid sequence that confers immunogenicity through the exposure of an altered nonself epitope. Some tumor-restricted TAA epitopes are encoded by intron sequences (11,12). These TAAs appear to be highly immunogenic, because a high frequency of specific CTLs directed against them has been detected in cancer patients with a favorable prognosis after therapy (13,14).

The majority of known TAA peptides are presented in association with class I MHC molecules and are recognized by patients' tumor-specific CD8⁺ T cells, whereas a small number of TAA epitopes are presented in association with class II MHC molecules and are recognized by CD4⁺ T cells (4,15–19). Although tens of T-cell-defined TAA epitopes are now available for potential clinical application as vaccines, they are still of limited clinical use for the majority of cancers. Moreover, most of the available TAAs are expressed by melanoma, whereas relatively few TAA epitopes have been characterized in other tumors. In addition, most of the already known TAA epitopes are recognized by only a few HLA alleles that are widely represented in the Caucasian population, leaving few epitopes avail-

able for recognition by T cells of subjects with less frequent HLA alleles. Moreover, the majority of TAA epitopes are derived from normal proteins for which immune tolerance may prevent immunogenicity.

To overcome such limitations, the search for new, possibly cancer-specific TAAs from histologically different tumors and containing peptide epitopes recognized by T cells in the context of less frequent HLA alleles remains of crucial importance. Now that the human genome has been sequenced, many novel proteins have been identified, some of which may be potential targets for an immunologic reaction against tumor cells. Thus, research to identify cancer-specific TAA will undoubtedly increase.

Modified and Optimized Peptides

The lack of a sufficient immune response to control cancer growth *in vivo* stems from, among other reasons, the poor immunogenicity of natural epitopes expressed by tumor cells. In fact, with the exception of the immunodominant melanoma Melan-A/MART-1_{27–35} and gp100 peptides, which readily activate specific T cells *in vitro* (20) and *in vivo* (21,22), most T-cell responses require repeated *in vitro* stimulation with TAA epitopes (20,22) and show limited immunogenicity when used as vaccines for cancer patients (23,24). A new strategy for increasing *in vivo* immunogenicity consists of modifying the peptide sequence at amino acid residues that are crucial for the interaction with the HLA or with the specific TCR.

Multiple approaches have been devised to increase peptide immunogenicity (25–31) (Table 2). For example, we have described (32) the superagonist variant Melan-A/MART-1_{27–35}/27L, in which an alanine at position 27 is replaced with a leucine. This modification induces a qualitatively and quantitatively improved anti-Melan-A/MART-1 T-cell response without changing the HLA binding affinity or stability. This variant presumably improves immunogenicity through a more efficient interaction with the TCR (32). Specific T cells generated by *in vitro* exposure to the Melan-A/MART-1_{27–35}/27L superagonist, but not to the native peptide, were also found to release high amounts of interleukin 2 (IL-2), a marker of full T-cell activation (32). A similar effect has been described for the decamer Melan-A/MART-1_{26–35}/27L and the modified peptide epitope gp100_{209–217} (2M) (25,26).

The strategy of modifying TAA peptides to increase and potentiate antitumor T-cell responses is a new tool for the preparation of more effective cancer vaccines. However, several find-

Table 1. Classification of tumor-associated antigens recognized by T cells*

Antigen	Subgroup	Tissue distribution	Mechanism or expression
Differentiation antigens	Specific tissue lineage (e.g., melanocytes)	Normal and neoplastic tissues	Normal differentiation
Tumor-restricted, shared	Encoded by germ line but not by somatic cell genes	Different tumors; normal testis and placenta (e.g., MAGE)	Demethylation
	Lineage-related	Melanomas (e.g., TRP-2/INT-2, GnT-V)	Alteration in splicing or transcription
	Virus-induced (e.g., HPV, HBV, EBV)	Cervix, head and neck, anus, penis, and liver cancers; Burkitt's lymphoma	Encoded by viral genes
Tumor-restricted, unique	Oncogene, oncosuppressor or fusion proteins†	Different tumors	Mutation, translocation
		Single tumor only	Point mutation
Ubiquitous		Many normal and neoplastic tissues	Normal expression

*For a more detailed description of class I and class II HLA-restricted epitopes of TAA, see (4). MAGE = melanoma antigen; TRP-2/INT-2 = tyrosinase-related protein 2/intron 2 antigen; GnT-V = N-acetylglucosaminyltransferase V; HPV = human papillomavirus; HBV = hepatitis B virus; EBV = Epstein-Barr virus.

†Fusion protein = protein encoded by fused genes containing a new sequence resulting from chromosome translocation.

Table 2. Approaches to increase immunogenicity of human tumor-associated antigen (TAA) peptides*

Approach	Mechanism	Tumor antigens involved (examples)	Reference
Substitution at anchor position	Improved peptide–HLA binding	Melan-A/MART-1 _{26–35} 2L	(25)
Introduction of aromatic amino acid at P1, P4, and P5†	Improved peptide–HLA binding	gp100 _{209–217} 2M	(26)
	Increased HLA binding and stability	Her-2/neu, folate-binding protein	(27,28)
Terminal modification (e.g., acetylation, amidation)	Inhibition of proteolytic degradation	Melan-A/MART-1 _{27–35}	(29)
Substitution of cystein residues	Reduction of dimerization	NY-ESO-1	(30)
Modification of TCR interacting amino acid residues	Increase triggering of TCR	CAP1-6D (CEA)	(31)
Modification of individual amino acid residues	Increase triggering of TCR	Melan-A/MART-1 _{27–35} 1L	(32)

*TAA = tumor-associated antigen; HLA = human leukocyte antigen; TCR = T-cell receptor; CAP1-6D = CEA agonistic peptide 4-6D; CEA = carcinoembryonic antigen; NY-ESO-1 = New York-esophagus-1 antigen.

†P1, P4, and P5 = positions 1, 4, and 5 in the peptide sequence.

ings (30,33,34) suggest caution in the use of modified TAA epitopes because, in some cases, they induce responses *in vivo* that reduce the ability of the immune system to control tumor growth.

T-CELL RESPONSES TO TUMOR PEPTIDES

TAA Peptide-Specific T Cells Can Be Isolated From Healthy Donors and Cancer Patients

The immune repertoire of healthy individuals and cancer patients contains a pool of T cells directed against TAA epitopes, although the frequency of such T cells is usually higher in cancer patients (35). However, cancer patients' CD4⁺ and/or CD8⁺ T cells that have been successfully activated against peptides from the candidate TAA presented by specialized antigen-presenting cells may fail to recognize the same TAA peptide on neoplastic cells (36,37). Several factors may explain the lack of T-cell recognition of tumor cells, including, most importantly, the low abundance of peptide–HLA epitope complexes on the tumor cell surface (38).

Recently, expression of TAA epitopes has been shown to be modulated by intracellular degradation pathways involving the proteasome (39). A mutation at position 273 (a mutational hotspot) in the p53 protein inhibits proteasome-mediated proteolytic cleavage between amino acid residues 272 and 273, preventing the production of the wild-type peptide (residues 264–272) that is recognized by HLA-A2.1-restricted T cells (40). This result indicates that mutations in candidate TAAs may not necessarily generate new T-cell epitopes and may, in some instances, even prevent recognition of normal sequences. Knowledge of the molecular mechanisms of peptide production by the proteasome and the modulation of such processes by cytokines will allow investigators to design and/or choose peptides that can be presented efficiently to patients' CTLs (41).

Evidence for Natural *In Vivo* TAA Immunogenicity

The isolation of peptide-specific T cells from cancer patients has been used to gain information regarding the *in vivo* immunogenicity of cancer cells. For example, after *in vitro* stimulation with the peptide, T cells specific for the Melan-A/MART-1_{27–35} peptide could be generated from 13 of 13 subjects with melanoma but from only five of nine donors without melanoma, which leads to the conclusion that the blood of patients with cancer contains a higher frequency of anti-Melan-A/MART-1_{27–35} CTL precursors than does the blood of patients without cancer. The higher frequency in melanoma patients was the result of a “priming” or expansion of patients' T cells caused by the *in vivo*

“natural” recognition of Melan-A/MART-1_{27–35}-positive neoplastic cells (35). Similar differences in the frequency of anti-TAA peptide CTLs in patients versus healthy donors have since been shown for other human tumors and different TAA epitopes such as survivin, an apoptosis inhibitory protein (42) in patients with melanoma (43), and epithelial cell adhesion molecule (Ep-CAM), Her-2/neu, and carcinoembryonic antigen (CEA) in patients with colorectal cancer (44).

These findings suggest that TAA-driven expansion of the immune repertoire, leading to a state of systemic immunity, takes place in at least some cancer patients. Evidence consistent with this possibility has been obtained by investigating the frequency, phenotype, and function of Melan-A/MART-1_{27–35}-specific T-cell precursors (45). A subset of patients had a high frequency (>1/2000 peripheral blood lymphocytes [PBLs]) of peptide-specific CTL precursors (45). These precursors were predominately in the CD45RO⁺ memory T-cell subset and were associated with an *in vitro* induction of Melan-A/MART-1_{27–35}-specific effectors after antigen-presenting cell (APC)-mediated presentation of the peptide. By contrast, patients with a low frequency (<1/40 000 PBLs) of peptide-specific T cells had CTL precursors only in the CD45RA⁺ naive T-cell subset, which were associated with a limited or delayed *in vitro* expansion of Melan-A/MART-1_{27–35}-specific T cells. In agreement with these data, circulating CD45RO⁺ memory CTL precursors specific for Melan-A/MART-1_{27–35} have been reported in melanoma patients (46).

However, cancer patients and healthy donors may differ not only in the absolute number of circulating T cells directed against a TAA but also in the functional activity of such effector cells (47). The association between phenotype and function of peptide-specific T cells has been recently corroborated by the identification of new markers of naive and memory T cells (48). One such marker is the chemokine (C-C motif) receptor 7 (CCR7). T cells identified in the blood of melanoma patients and of healthy donors by staining with HLA-A2/Melan-A/MART-1_{27–35} peptide tetramers did not respond to the Melan-A/MART-1_{27–35} peptide *ex vivo* if they had a CCR7⁺, CD45RA⁺ phenotype but did respond if they had a CCR7⁻, CD45RO⁺ phenotype (49). In addition, the frequency of peptide-specific T cells was higher in tumor-invaded lymph nodes than in peripheral blood. These T cells had a CD45RO⁺ phenotype and had the ability to proliferate in culture to become TAA peptide-specific effectors (50). A shift in the phenotype associated with the loss of both CD45RO and CD28 molecules was recently shown to occur in the memory effector T cells isolated from colon cancer patients (44).

The immunogenicity of TAA peptides was initially defined by the response elicited by class I HLA-restricted epitopes. However, the recent identification of class II HLA-restricted epitopes in known TAA peptides and the definition of a new class of TAA peptides recognized by patients' autologous sera through Serex—the serological analysis of TAA by recombinant cDNA expression cloning (51)—has allowed a more detailed and integrated view of the immune response to cancer. The emerging picture is that, during natural tumor history, at least some TAA peptides (e.g., New York Esophagus [NY-ESO]-1 or Her-2/neu) expressed by neoplastic cells elicit both cell- and antibody-mediated immune responses (52). Because CD4⁺ T-helper lymphocytes are crucial for the activation of the B-cell-dependent antibody responses, the identification of antibodies to TAA suggests the presence of a CD4⁺ T-helper arm of the immune system that may contribute to both antibody production by B lymphocytes and CD8⁺ CTL reactivity against the same tumor protein (52–54).

Thus, it is likely that an increasing number of human TAA peptides will be shown to encompass determinants recognized by B cells and by CD4⁺ and CD8⁺ T cells, with the attractive possibility that a CD4-mediated T-helper arm will allow the patient to mount an effective antitumor immune response. Whether most TAA peptides elicit similar T-cell responses *in vivo* in patients and whether the *in vivo* T cell–TAA interactions are similar in patients with different tumors and in different clinical conditions remains to be determined.

VACCINATION WITH PEPTIDES: LESSONS FROM ANIMAL MODELS

Most studies of antitumor vaccination in animal models have been performed with single TAA epitopes administered with a variety of adjuvants. Studies of both prophylactic and therapeutic vaccinations have been conducted. For viral diseases and virally induced tumors, prophylactic vaccination with synthetic peptides recognized by T cells was effective in animal models (55,56). For tumors that were not virally induced, prophylactic vaccination was less effective, although clear examples of antitumor efficacy exist (57,58). However, although they are effective in some selected examples, prophylactic vaccination with peptides, either alone or emulsified in conventional immunologic adjuvants (e.g., complete or incomplete Freund's adjuvants), often failed to induce detectable immune responses against TAA in mice, especially when vaccination was attempted against antigens that most closely correspond to known human TAA. When used for therapy, peptide vaccination with incomplete Freund's adjuvant was effective in only a few animal models (55).

Single Epitope Versus Polyepitope Vaccines

Given that both animal and human tumors express multiple TAA epitopes that are recognized by T cells (59) and that some of the TAA epitopes can be lost or expressed at different times during tumor growth, a vaccine against multiple TAA epitopes could be more effective than a vaccine against a single epitope. To determine whether there is any possible immunodominance of one or more TAA epitopes that may prevent T-cell recognition of other TAA epitopes, the immunogenic activity of polyepitope vaccines has been tested in two different murine models: one in which the mice were vaccinated with a recombinant ad-

enovirus encoding multiple epitopes against virally induced tumors (60), and one in which HLA-A2 transgenic mice were vaccinated with a poxvirus vaccine encoding multiple HLA-A2-restricted epitopes derived from melanoma antigens (61). CTL responses were generated against all the epitopes included in the vaccines. However, the clinical efficacy of such polypeptide vaccines has not been directly compared against that of each single epitope. Thus, TAA polypeptide vaccines may substantially increase the possibility of targeting tumor cells that have lost one or more TAA epitopes but have retained at least one of the original epitopes.

Tolerizing Versus Immunizing Potential of Peptides

The immune system may become specifically unresponsive, or tolerant, to antigens for several reasons (62). Under certain circumstances, vaccination with peptides may induce epitope-specific T-cell tolerization rather than activation. This phenomenon has been reported in mouse models of infectious diseases and of adenovirus-transformed tumor cells (63,64). In some circumstances, the same peptide can activate or tolerize epitope-specific T cells, depending on the dose and route of administration. Peptides emulsified in incomplete Freund's adjuvant can activate CTLs when administered once subcutaneously and can induce a functionally effective tolerance when administered repeatedly intraperitoneally (64). In addition, some peptides, despite being well-known tumor rejection epitopes when expressed by irradiated tumor cells, can exert tolerogenic activity, even when administered subcutaneously in incomplete Freund's adjuvant (64). For adenovirus-transformed mouse tumor cells, peptide-induced T-cell tolerization has substantial functional consequences: enhanced tumor growth and reduced survival (64).

One possible explanation for the limited therapeutic efficacy of TAA peptide vaccination lies in the fact that activation of peptide-specific CTL responses requires the delivery of inflammatory signals from monocytes, lymphocytes, or granulocytes recruited at the site of vaccination; such signals may or may not be provided by standard adjuvants like incomplete Freund's adjuvant. An efficient activation signal, however, may be provided by bacterial DNA or synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides (CpG-ODN). Such signals can stimulate B cells, natural killer (NK) cells, T cells, monocytes, and antigen-presenting cells; more importantly, such signals can promote maturation of DCs, a step that will result in the activation of the antibody and cell-mediated immune responses (65–68). More recently, CpG-ODN have been shown to improve the antitumor activity of antigen-presenting cells loaded with TAA peptides and promote a 10-fold to 100-fold increase in the induction of CTL responses to peptide immunization (67). Results of vaccination trials that use CpG-ODN as immunologic adjuvants in humans are eagerly awaited.

Thus, irrespective of the mechanism involved, the data suggest that caution should be exercised when designing peptide vaccination strategies. More information is needed on the fate and kinetics of peptides after their *in vivo* administration to better understand whether peptide immunogenicity can be modulated either by modifying their sequence or by using appropriate adjuvants.

Immunotherapy With Peptide-Loaded DCs

DCs are antigen-presenting cells that have the crucial function of presenting antigens, including TAAs, to naive T cells in

lymph nodes. DCs are present in most tissues and can be recruited to the site of tumor cell growth by cytokines. At the tumor site, DCs can internalize and process TAAs and then travel to draining lymph nodes where they present, with high efficiency, peptide–MHC complexes to T cells (69). In fact, the generation of tumor-specific T cells against TAA peptides appears to require a phase of “antigen presentation” *in vivo* by cells expressing MHC class II molecules (i.e., the antigen-presenting cells), of which the most efficient appear to be DCs (70). This principle was suggested first by Bevan in 1976 (71) and termed “cross-priming.” In 1989, Romani et al. (72) showed that murine DCs could take up proteins, process them, and present peptide–MHC complexes to T-cell clones. A similar observation was reported in a tumor model by Huang et al. (73).

These findings (70–73) provided the rationale for using antigen-loaded DCs as potential activators of antitumor responses. An early study (74) showed that mice injected with antigen-loaded DCs were protected against subsequent challenge with the same tumor. Even in a therapeutic setting, such a vaccination was effective because tumors regressed in treated animals. It should be noted that the *in vivo* rejection of tumors expressing certain TAA (e.g., the tyrosinase-related protein 2 [TRP-2] or Muc-1) may be better achieved by TAA-loaded DCs rather than by other strategies (e.g., naked DNA or peptide plus adjuvants), although all strategies may induce CTL and/or antibody responses (75,76).

The efficacy of peptide-loaded DC vaccination in terms of CTL induction and antitumor activity depends on additional critical factors, such as the route of DC administration and the origin of the DCs. Recent results with TAA peptide-loaded murine bone marrow-derived DCs indicated that subcutaneously injected DCs had greater antitumor activity than intravenously injected DCs and that subcutaneously injected DCs home to T-cell areas of the draining lymph nodes, whereas intravenously injected DCs home to the spleen (77). Although in mice the induced immunity was influenced by the route of administration of the vaccine, in cancer patients the induced immunity was independent of the route of administration (78). Murine models have also provided evidence that antigen-presenting cells from tumor-bearing animals may be less effective in inducing antitumor responses than are DCs from cancer-free normal mice (79).

An early study revealed that the antitumor mechanisms activated by DCs requires cooperation between T-cell subsets and the expression of co-stimulatory molecules (such as B7–1), and TH1 cytokines (such as IL-2), interferon γ (IFN- γ), and tumor necrosis factor- α (80). Furthermore, the efficacy of DC-based vaccination against tumor growth can be substantially improved by combining the injection of TAA-loaded DCs with the administration of cytokines such as IL-12 (81).

The mechanisms that allow DCs to promote effective antitumor immunity or to convert a tolerogenic peptide into a CTL-priming one are being studied (82). Such mechanisms include the CD40L–CD40 T-cell co-stimulatory pathway, because the *in vivo* administration of an activating antibody to CD40 could convert a peptide vaccine from an inducer of CTL tolerance to an activator of tumor-specific immunity (83). Such activity is further increased by the *in vivo* administration of antibodies that block the cytotoxic T lymphocyte antigen 4 (CTLA-4) molecule, which is known to deliver an inhibitory signal to antigen-activated T cells (84).

Thus, DCs appear to be powerful adjuvants for peptide-based immunization strategies in animal models. Further improvement might be obtained by pulsing DCs with a mixture of CD8⁺ and CD4⁺ T-cell epitopes (52, 53) to induce both helper and effector T-cell responses.

PEPTIDE-BASED VACCINES IN THE CLINICAL SETTING

Melanoma peptides were the first to be tested in phase I and phase II studies for active immunization of metastatic melanoma patients (85,86). Clinical and immunologic results of several of these trials are summarized in Table 3. In general, clinical responses were observed in 10%–30% of the treated patients. Rosenberg et al. and Cormier et al. (87–89) injected patients who had metastatic melanoma with peptides derived from differentiation TAA mixed with incomplete Freund’s adjuvant. Fifteen of 16 patients receiving Melan-A/MART-1_{27–35} with incomplete Freund’s adjuvant developed a CTL-specific response in their blood but showed no concomitant clinical effect (88). By contrast, 42% of patients receiving a modified gp100 peptide (gp100–209.2M) and a high systemic dose of IL-2 showed a clinical response. Patients who received the peptide alone showed no response (87). Ten of 22 patients with resected stage III and IV melanoma who were treated with Melan-A/MART-1_{27–35} peptide with incomplete Freund’s adjuvant developed immune responses that were associated with a prolonged time to relapse (90), a finding that suggests a clinical benefit. In patients with resected melanoma, vaccination with both gp100–210M and tyrosinase in incomplete Freund’s adjuvant, with and without administering IL-12, resulted in a tumor-specific immune response of 90%; however, a subset of patients receiving IL-12 showed an *in vitro* release of cytokines by T cells higher than that of patients not given IL-12 (91). Relapse-free and overall survival of these vaccinated subjects compared favorably with historical groups (91). In patients with metastatic melanoma, vaccination with the MAGE-3.A1 peptide without adjuvant resulted in a 27% objective response rate in the absence of any detectable CTL induction (92). Similar trials involving multiple TAA peptides given with incomplete Freund’s adjuvant or cytokine adjuvants to melanoma patients are ongoing in the United States and Europe.

TAA peptides have been administered to cancer patients in conjunction with granulocyte-macrophage colony-stimulating factor (GM-CSF), which induces the recruitment of DCs at the site of vaccination and promotes their differentiation, a process that favors TAA processing and presentation. Jager et al. (93) treated three patients who had metastatic melanoma with a mixture of Melan-A/MART-1_{27–35}/gp100/tyrosinase peptides and GM-CSF, with the cytokine given first locally and then at distant sites. Induction of CTLs against one or more of the peptides and evidence of transient tumor regression was observed in all three patients (93). In a larger study of 51 melanoma patients who received the above treatment (except for a subgroup given IL-12 instead of GM-CSF), the same group (Jager E, Knuth A: personal communication) found 11 clinical responses and 23 patients with stable disease (Table 3). The results of a trial conducted by Scheibenbogen et al. (94) in which metastatic melanoma patients were vaccinated with tyrosinase peptides and GM-CSF, however, showed only one mixed clinical response (Table 3), with four of five tested patients displaying increased anti-peptide-specific T-cell reactivity. Three different peptides from NY-ESO-1 were given to 12 HLA-A2 cancer patients

Table 3. Results of peptide-based vaccination trials in cancer patients*

Tumor	Peptide vaccine	Adjuvant	Trial phase	No. of patients	Clinical response	% of patients showing a response	Principal investigator (location)	Reference
Melanoma	gp100(gp209)-2M	IFA + IL-2	II	31	1 CR, 12 PR	42	S. Rosenberg (Bethesda)	(87)
	MART-1 ₂₇₋₃₅	IFA	I	18	1 PR	5	S. Rosenberg (Bethesda)	(88)
	gp100 + MART-1 ₂₇₋₃₅	GM-CSF or IL-12	II	51	5 CR, 6 PR	21	A. Knuth (Frankfurt)	Personal communication (89)
	+ tyrosinase							
	gp100	IFA + IL-12 or GM-CSF	I/II	27	0	0	S. Rosenberg (Bethesda)	(89)
	MART-1 ₂₇₋₃₅	IFA	I/II	25	Increased DFS		J. Weber (Los Angeles)	(90)
	gp100 (210M)	IFA ± IL-12	II	48	>DFS		J. Weber (Los Angeles)	(91)
+ tyrosinase								
Melanoma and others	MAGE-3.A1	None	I/II	25	3 CR, 4 PR	28	T. Boon (Brussels)	(92)
	Tyrosinase	GM-CSF	II	18	0†	0	U. Keilholz (Berlin)	(94)
	NY-ESO-1	± GM-CSF	I/II	12	3 SD	25	A. Knuth (Frankfurt)	(95)
Pancreatic cancer	K-Ras/12	GM-CSF	I/II	48	Increased OS		G. Gaudernack (Oslo)	(97)
CIN	HPV-16/E7	IFA	I/II	18	3 CR, 6 PR	50	J. Weber (Los Angeles)	(115)
	+ KSS/PADRE							

*IFA = incomplete Freund's adjuvant; IL = interleukin; CR = complete response; PR = partial response; MART-1 = melanoma antigen recognized by T cells; GM-CSF = granulocyte-macrophage colony-stimulating factor; DFS, disease-free survival; MAGE-3.A1 = melanoma antigen-3 peptide restricted by HLA-A1; NY-ESO-1 = New York-esophagus antigen-1; SD = stable disease, K-Ras/12 = K-Ras mutated at position 12; OS = overall survival; CIN = cervical intraepithelial neoplasia; HPV = human papilloma virus; E7 = early protein 7; KSS = amino acid sequence of the linker peptide; PADRE = pan-DR epitope. †0 = None of the treated subjects showed a partial or complete clinical response, but one patient had a mixed response.

with progressing NY-ESO-1-expressing tumors of different histologies (95). First the patients received the peptides without GM-CSF. After 50 days, patients with no evidence of disease progression then received the peptides with GM-CSF. Of those who could be tested, an induction of peptide-specific CTL responses (observed in four of seven patients) was associated with disease stabilization and objective regression of some but not other metastases (mixed response).

Peptides have also been used to immunize patients with cancers of the colon, ovary, breast, pancreas, and cervix. Because approximately 90% of pancreatic adenocarcinomas have a specific mutation in the 12th, 13th, or 61st codon of the K-Ras oncogene, because peptides containing such mutations can generate both class I and class II MHC-restricted T-cell responses (96), Gjertsen et al. (97) intradermally vaccinated patients with pancreatic adenocarcinomas with K-Ras peptides and GM-CSF. A peptide-specific T-cell reaction was elicited in more than 50% of these subjects. Moreover, patients with advanced cancer who demonstrated an immune response to the peptide survived longer than those without a notable immune response (Table 3). This finding suggests a potential clinical benefit that should be confirmed by phase III trials.

Patients with Her-2/neu-positive breast and ovarian cancers were vaccinated with class II HLA-restricted Her-2/neu peptides together with GM-CSF. All patients developed Her-2/neu peptide-specific T-cell responses that were detectable even by delayed type hypersensitivity reactions to the given peptide (98). Final clinical response rates from this study are not yet available. Trials of TAA peptide-based vaccinations are ongoing for hematologic tumors. T cells can recognize peptides of fusion proteins characteristic of certain forms of leukemia (e.g., acute promyelocytic leukemia and chronic myeloid leukemia) (99). Vaccination trials exploiting these peptides have been initiated; in one trial, three of six patients who were treated developed vaccine-specific T-cell proliferative responses—but not CTL responses—with some evidence of clinical benefit (100).

The reasons for the limited clinical response in all these studies are not clear, but they may be related to the nature of the TAA used (weakly immunogenic peptides from normal proteins), the vaccination schedule, the lack of CD4⁺ helper-T-cell activation, the host's immune cell dysfunction, escape mechanisms of the tumor cells, and/or high tumor burden. At least some of these drawbacks have been overcome with the use of DCs.

Vaccination With Peptides Presented by DCs

The clinical use of DCs became possible in humans because of new methods and technologies that allow the easy generation and collection of a large number of either monocyte- or CD34-derived autologous DCs from cancer patients (101,102). After it was shown *in vitro* that, as in animal models, autologous DCs can effectively present human TAA as loaded proteins or peptides to naive T cells (103), several clinical studies were initiated to test the potential therapeutic efficacy of such a vaccination approach in cancer patients. These trials are summarized in Table 4 and briefly described hereafter.

Patients with metastatic melanoma were vaccinated with DCs loaded *ex vivo* either with class I HLA-restricted melanoma antigen peptides (i.e., Melan-A/MART-1₂₇₋₃₅, tyrosinase, gp100, MAGE-3) or with tumor cell lysates and directly injected into the lymph node (104). A clinical response rate of 25% (including three complete and durable responses) was observed in eight of 32 patients (104). In a vaccination trial of metastatic melanoma subjects, a high frequency of anti-MAGE-3 CTL responses was elicited by administering MAGE-3, peptide-loaded, autologous DCs (105) (Table 4). However, although some individual skin metastases disappeared, no clinically significant regression of metastatic lesions was observed (105). Lotze et al. (106) obtained a response in five of 28 patients with metastatic melanoma who were injected with autologous DCs loaded with three different melanoma peptides. A recent study by Banchereau et al. (107) showed that when HLA-A2-positive

Table 4. Results of peptide-loaded autologous dendritic cell vaccination in patients with metastatic disease*

Tumor	Peptide vaccine	No. of patients	Clinical response (No.)	% of response	Principal investigator (location)	Reference
Melanoma	MART-1, gp100, tyrosinase, MAGE-3	32	8 (3CR, 5PR)	25	D. Schadendorf (Heidelberg)	(104)
	MAGE-3.A1	8	0	0	G. Schuler (Erlangen)	(105)
	MART-1 + tyrosinase + gp100	28	3 (2CR, 1PR)	17	M. T. Lotze (Pittsburgh)	(106)
	Melan-A/MART-1 + tyrosinase + gp100 + MAGE-3.A2	18	3CR	17	J. Banchereau (Dallas)	(107)
Prostate carcinoma	PAP/GM-CSF	31	0†	0	F. Valone (Seattle)	(108)
	PSMA/A2	33	8 (2CR, 6PR)	22	G. Murphy (Seattle)	(109)
Bladder cancer	MAGE-3.A24	4	1PR	25	M. Murai (Tokyo)	(111)
Follicular B lymphoma	Idiotypic/KLH conjugate	4	3 (2CR, 1PR)	75	R. Levy (Stanford)	(112)

*MART-1 = melanoma antigen recognized by T cells-1; MAGE-3 = melanoma antigen-3; CR = complete response; PR = partial response; MAGE-3.A1 = melanoma antigen-3 peptide restricted by HLA-A1; MAGE-3.A2 = melanoma antigen-3 peptide restricted by HLA-A2; PAP = prostate acid phosphatase; GM-CSF = granulocyte-macrophage colony-stimulating factor; PSMA/A2 = prostate surface membrane antigen peptide recognized by HLA-A2; MAGE-3.A24 = melanoma antigen-3 peptide restricted by HLA-A24; KLH = keyhole limpet hemocyanin.

†More than 50% reduction of serum level of prostate-specific antigen (PSA) was noted in three patients.

patients with metastatic melanoma were vaccinated with autologous DCs loaded with four different melanoma antigen peptides, T-cell responses to at least one peptide were induced in 16 of 18 subjects; the clinical responses included three complete responses and three more patients who regressed one or more lesions without reaching a partial response (at least 50% regression of the whole tumor mass) (Table 4).

Studies have also been performed in patients with prostate cancer who received DCs loaded with peptides from prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), or prostate acid phosphatase (PAP) (Table 4). These antigens are prostate-specific proteins that are usually overexpressed in prostate cancers and released in the blood. In one study (108), some patients vaccinated with PAP/GM-CSF fusion protein-loaded autologous DCs showed a dose-response effect between the number of DCs injected and T-cell proliferation to PAP; other subjects showed a reduction in serum PSA levels but no overall clinical tumor regression. In a trial with 33 HLA-A2-positive patients with hormone-refractory prostate cancer who received PSMA peptide-loaded DCs (109), only two patients showed a peptide-specific T-cell response, which was not accompanied by a clinically significant response. However, circulating PSA levels were reduced by at least 50% in approximately 30% of the patients. This response was associated with a general nonspecific immune reactivity measured before vaccination (110). A pilot study in which four subjects with bladder cancer were vaccinated with MAGE-3 peptide-loaded DCs has also shown promising results (111).

Vaccination with DCs has been clinically successful in the treatment of follicular B-cell lymphomas (112) (Table 4). Several reasons may account for the favorable response to immunotherapy in these tumors: 1) nearly all the tumor cells express the same TAA epitope in the form of the immunoglobulin idiotype determinant (i.e., the immunoglobulin fragment associated with its antigen recognition site); 2) the tumor burden can be considerably reduced by chemotherapy before vaccination; and 3) both antibody and T-lymphocyte responses are easily induced by administering autologous DCs pulsed with the idiotype conjugated with the CD4 stimulatory agent keyhole limpet hemocyanin (KLH). The antifollicular B-cell lymphoma vaccine has now been simplified by using GM-CSF as an adjuvant instead of DCs, with the result that complete, durable clinical responses have been obtained in eight of 11 patients, along with a tumor-specific T-cell response (113). Studies are ongoing in patients with multiple myeloma.

Tumor cell lysates represent a convenient source of antigens useful for vaccinating patients because they provide DCs with multiple TAA peptides, some of which have not yet been characterized. Such an approach has been used in vaccinating patients with melanoma (99). Although the approach is being used for other tumors, more information is needed to define the repertoire of TAA peptides included in tumor lysates and their immunogenicity *in vivo*. In addition, lysates also include normal proteins that should be ignored and tolerated by the host but that, under certain conditions, may also cause autoimmunity.

All together, TAA-peptide-loaded DC vaccines appear to generate a more frequent and stronger CTL response than do vaccines composed of peptides and incomplete Freund's adjuvant; however, clinical responses, except for the response to B-cell lymphoma, remain rather low. Thus, although the use of DCs can help elicit a T-cell response in the majority of subjects, it appears that the efferent part of the immune response is often unable to target and/or destroy metastatic tumor cells.

Vaccination Against Virus-Induced Tumors

Virus-induced tumors express high levels of homogeneous TAAs represented by viral proteins directly encoded by the virus genome. Human papillomavirus 16 (HPV-16), the cause of cervical cancer, is also associated with cancers of the anus, penis, and head and neck. Several HPV peptides have been identified [see (4)] that, when presented by antigen-presenting cells, can trigger a T-cell response. Results from studies with animal models (114) suggest that injection of HPV peptides in incomplete Freund's adjuvant can elicit T-cell responses that are associated with tumor rejection in both prophylactic and therapeutic settings. In a phase I trial, 18 patients with high-grade vulvar or cervical intraepithelial neoplasia (CIN), who were positive for HPV, were given an HLA-A2-restricted peptide in incomplete Freund's adjuvant. A DC infiltrate was detected in all six CIN case patients tested; a T-cell response to the peptides was seen in 10 of 16 patients (Table 3) (115). Moreover, three complete responses and six partial responses were obtained, indicating that this HPV-16 peptide vaccine has relevant biologic and clinical effects and the potential for more extensive use in patients with cervical dysplasia (115).

Vaccination With Heat Shock Proteins

Heat shock proteins (HSPs) are considered natural adjuvants that show promise in cancer vaccination because they can bind

antigenic peptides within the tumor cell and chaperone peptides to antigen-presenting cells in lymph nodes (116). Antigen-presenting cells express a specific receptor that can bind different families of HSPs (117). In the mouse system, HSPs of the 96, 70, and 110 Kd subfamilies have been shown to function as potent vaccines in both prophylactic and therapeutic settings, with the ability to induce strong, specific, antitumor T-cell immunity that results in tumor rejection (116). That HSPs may bind tumor-associated peptides has been directly shown *in vitro* in mouse models of cancer and infectious diseases (116) and in human melanoma (118).

Clinical studies have been initiated with the aim of immunizing cancer-bearing patients with autologous HSP96, which is known to be immunogenic in mice [see (116)]. In a pilot study, Janetzki et al. (119) vaccinated 16 subjects with different types of cancer with autologous tumor-derived HSP96 preparations. No clinically significant toxicity was observed, and six of 12 patients that could be tested developed class I HLA-restricted, tumor-specific T cells (119). A similar study was performed in 39 patients with metastatic melanoma by us and by other Italian researchers. We found that vaccination with peptide-HSP96 complexes obtained from autologous tumors led to an increase in specific T-cell responses against melanoma antigens in 48% of patients and to clinical responses in 18% of patients (120). Although this approach is interesting, it needs to be validated by showing that autologous tumor-derived HSPs do contain individual TAA epitopes. Such peptide epitopes are thought to be more immunogenic than those derived from shared TAA and are responsible for tumor regression in animal models (116).

EX VIVO T-CELL RESPONSES DURING VACCINATION OF CANCER PATIENTS

The ultimate goal of any treatment is to cure a disease. When such achievement is not promptly accomplished, surrogate end points are used that may provide a conceptual bridge between the intervention and its target. With immunization, common sense would predict that the stronger the effect of the vaccine on the induction of immune responses, the greater the likelihood of clinical success. However, in the case of anticancer vaccines, such a relationship has remained elusive. Clinical studies designed to induce new or augment ongoing anticancer T-cell responses through peptide-based vaccination have shown that T-cell-directed peptide epitopes can be quite effective in inducing tumor-specific T cells that can be easily identified among circulating lymphocytes of immunized patients (121–124).

There are several reasons why T-cell responses to vaccination in cancer patients may not be associated and/or easily correlated with therapeutic outcome. These reasons may relate to the quality of the induced T-cell responses (e.g., TAA tolerance) or to the heterogeneity of tumors that might influence their susceptibility to otherwise adequate anticancer treatments (escape mechanisms) (125).

Altered T-Cell Functions

An accurate evaluation of the status of activation of circulating T cells can only be accomplished through direct *ex vivo* assessment (42–44,48,126). For instance, naturally occurring anticancer immune responses may be blunted in their effector function (127,128), although precursor T cells may be identified among peripheral blood mononuclear cells through peptide-HLA tetramers (tHLA) phenotyping (129). In fact, a variety of

functional alterations have been described in T and NK cells, including the selective loss of the ζ chain of the TCR-associated molecule CD3, of the TCR-associated kinase Ick, or of the signaling molecule ZAP-70, all of which can prevent IL-2 production and are associated with tumor burden (127,130). One of us (F. M. Marincola) addressed the status of activation of peptide vaccine-elicited T cells in circulating lymphocytes by direct evaluation of their ability to produce IFN- γ after vaccine-specific and tumor-specific stimulation using various direct *ex vivo* methods (131). The presence of peptide vaccine-elicited circulating T cells according to tHLA phenotyping was associated with vaccine-specific IFN- γ expression in response to cognate stimulation, suggesting that immune responses to vaccination are not totally blunted. However, tumor-specific CD8⁺ T cells may lack cytolytic function but maintain the ability to produce cytokines in response to stimulation (32). Moreover, functional dichotomy among various TAA-specific CD8⁺ CTLs in untreated patients, on the basis of expression of other surface markers (47), has not been observed in vaccinated subjects. Therefore, it remains unclear whether the immune responses elicited by vaccines are quantitatively and/or qualitatively sufficient for an effective anticancer response. Even the location where the T-cell response may occur is crucial because CTL responses have been detected in the draining lymph nodes in five of five melanoma patients vaccinated with melanoma-associated peptides but in the peripheral blood in only two of five of the melanoma patients (132).

Another parameter that may independently modulate the effectiveness of vaccine-elicited immune responses is whether T cells can localize and survive in the target tissue. Knowledge of the outcome of such immune responses beyond their appearance in the systemic circulation remains scant (128). We gathered indirect evidence regarding the localization of vaccine-induced CD8⁺ T cells at the tumor site by assessing the melanoma-specific T-cell repertoire in subsequent metastases of patients receiving an autologous tumor cell vaccine (133). More recently, such evidence was obtained by tracking the level of IFN- γ transcript abundance in identical lesions sampled by fine-needle aspiration before and during vaccination with melanoma peptides (134). Levels of IFN- γ transcript were found to be increased in eight of 11 melanoma lesions, and this increase was strongly associated with the tumor cell expression of the TAA targeted by the vaccine. However, because none of the lesions studied regressed in response to the treatment, this study suggested that localization may not be sufficient, although perhaps necessary, to induce tumor regression, as has also been reported in unimmunized subjects (45). Thus, although several assays are now available (135–137), the problem of how to assess the T-cell response in vaccinated patients (namely when and where) remains unsolved (Table 5).

Escape Mechanisms

A final category of variables that may affect tumor regression in response to a given effective immune response includes the complex behavior of cancer cells in relation to their genetic instability. That is, cancer cells may lose or reduce the expression of molecules targeted by effector T cells, such as TAAs, MHC molecules, and molecules associated with antigen processing and presentation (125,138). Such losses have been shown to profoundly affect the effector function of TAA-specific T cells by rendering cancer cells inadequate targets (125). However, the

Table 5. *Ex vivo* assays to assess the antivaccine T-cell response

Assay	Advantages	Disadvantages
⁵¹ Cr-release or cytokine-release assay after autologous MLTC/MLPC*	The assay is quantitative The assay targets the patient's own tumor cells	Requires a tumor cell line and 2–4 weeks of time May expand T cells directed to irrelevant antigens Low sensitivity (1 : 500 PBMCs)
ELISPOT (135)	The assay is quantitative and highly sensitive (1 : 20 000–50 000 PBMCs) Does not require <i>in vitro</i> re-stimulation It is rapid and can test a large number of samples simultaneously	High background due to NK activity (when bulk PBMCs are used) Reliable kits are available only for a limited number of cytokines
Tetramer HLA/peptide epitope staining (136)	The assay provides a direct enumeration of epitope-specific T cells	Requires dedicated equipment for automated reading The assay may not assess T-cell function The assay has limited sensitivity (detection of $\geq 1 : 1000$ – $1 : 5000$ T cells) by cytofluorimetric analysis
Cytokine release by intracellular staining and phenotype (137)	The assay is quantitative and combines function (cytokine release) with phenotype Does not require <i>in vitro</i> re-stimulation	Limited by the sensitivity of the cytofluorimetric analysis
Cytokine release (real-time PCR) (134)	The assay is quantitative and sensitive	Does not provide direct evidence of cytokine production or release

*⁵¹Cr = ⁵¹chromium; MLTC = mixed lymphocyte–tumor culture; MLPC = mixed lymphocyte–peptide culture; PBMCs = peripheral blood mononuclear cells; ELISPOT = enzyme-linked immune spot; NK = natural killer cells; HLA = human leukocyte antigen; PCR = polymerase chain reaction.

rate at which such impaired expression becomes functionally relevant remains to be determined (139). In a recent study, the short-term kinetics of the expression of TAA peptides targeted and not targeted by vaccination in similar lesions was followed by serial fine-needle aspiration sampling (140). Surprisingly, the loss of TAA peptide expression targeted by the vaccine almost uniformly preceded the complete regression of immune-responsive lesions, whereas TAA peptide expression was not affected in immune-resistant lesions. These findings suggest that the main reason for a lack of effect for vaccine-elicited immune responses is not the immune selection of variant cells that have lost the target peptide–HLA complex but rather the lack of effect within the target tissue itself. Immune selection, instead, appears only after successful therapy, and it may be prevented during the primary stage of treatment with the use of a broader TAA peptide-based vaccine that allows elimination of epitope-negative variant cells.

Aside from the direct relationship between the engagement of vaccine-elicited T cells with their relevant epitopes, a set of less specific variables is likely to affect and modulate the host immune response within the tumor microenvironment. Fas/FasL interactions have been implicated as modulators of immune function (141,142), and a large number of molecules with immune and/or inflammatory properties are increasingly recognized as being secreted by tumor cells (143). Recently, using serial fine-needle aspirations, melanoma metastases were followed during immunotherapy by assessing the evolution of their transcriptional profile with cDNA-based microarray technology (144). This analysis suggested that melanoma metastases evolve rapidly during therapy and increasingly produce secreted factors that may be beneficial to their survival (145). Among these factors, some have angio-regulatory and proliferative properties, and others have chemotactic and immune modulatory activities. The overall picture suggests that individual lesions can vary among different individuals and can vary dramatically in a short period of time.

We believe, therefore, that the reasons for the paradoxical dissociation between vaccine-induced T-cell responses in circulating lymphocytes and their lack of effectiveness in inducing tumor regression should be sought by complementing the analysis of the systemic effects of vaccines with the simultaneous

study of tumor–host interactions at the tumor site. Although this task is not easy, modern technology has rendered this approach feasible, at least in the case of some tumor models, such as metastatic melanoma, which is characterized by the frequent development of subcutaneous metastases that are easily accessible with sampling devices.

CONCLUDING REMARKS

Research on TAA peptides has identified a large collection of peptide epitopes that have been and are being used for the vaccination of cancer patients. The advantages of using peptide-based vaccines include 1) easy and relatively inexpensive production of synthetic peptides; 2) the simplicity of peptide administration in a clinical setting; 3) the possibility of treating only those patients whose tumors express the cognate epitopes, thus avoiding the useless immunization of patients whose tumors are TAA-negative; and 4) the availability of *in vitro* or *ex vivo* assays that can assess patients' immune response to vaccine epitopes.

Peptide-based vaccination against neoplastic diseases has made enormous progress and remains an active and crucial area of investigation, holding promise for improving the clinical outcome in cancer patients. However, several hurdles need to be overcome. The most important one, in our opinion, is the ability of tumor cells to evade a strong tumor-specific immune response [see (125)].

There are several potential ways to avoid the escape of tumor cells. First, patients with early-stage disease could be vaccinated to cope with immune tolerance or immunosuppression caused by factors released by tumor cells. Second, multi-epitope vaccines could be used to bypass the heterogeneity in TAA expression. Third, cytokine adjuvants, such as GM-CSF, could be used to recruit DCs at the vaccination site and improve TAA presentation. IL-2 and/or IL-12 given systemically could be used to help to expand antitumor T cells. IL-2 could be used to restore the function of patients' T cells. Fourth, the expression of peptide–MHC complexes on target cells could be increased by the systemic administration of IFN- α or IFN- γ . Finally, as indicated by many studies [e.g., (146)] in animal models and in humans, class II-restricted HLA epitopes should be provided, even in the form

Table 6. Ways to improve the clinical outcome of peptide-based vaccines*

Factor	Improvement
Antigens	Use TAA-specific peptide epitopes that are strictly associated with tumor cells to increase the specificity of the antitumor immune response and to limit the occurrence of autoimmune toxicity. Use poly-epitope vaccines to maximize a broad immune response and to avoid tumor resistance due to loss of single peptide-HLA complexes by tumor cells. Add class II HLA-restricted epitopes to elicit CD4 ⁺ T-helper immune responses.
Adjuvants	Use cytokine adjuvants (e.g., GM-CSF, IL-2, IL-12) to improve immunogenicity of the vaccine and expand the antitumor T-cell pool.
Evaluation of immune responses	Use reliable, simple <i>ex vivo</i> assays to measure the T-cell response (e.g., ELISPOT, cytokine transcripts, peptide-HLA tetramers).
Clinical setting	Vaccines should be administered to patients with a limited tumor burden to avoid tumor-induced immune suppression and to those whose tumors express the TAA peptide included in the vaccine.

*TAA = tumor-associated antigen; HLA = human leukocyte antigen; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; ELISPOT = enzyme-linked immune spot.

of promiscuous determinants (147), to augment the strength and duration of the immune response (Table 6).

It may be premature to declare that cancer vaccines are an effective antitumor approach. However, we may be optimistic about the clinical use of peptide-based cancer vaccines. In fact, the many hurdles on the way to a successful clinical application are now identified and, therefore, may be overcome in the near future. However, we should not “down-regulate” our criticism on the whole issue of cancer vaccines. Unorthodox but scientifically sound views (148) should not be ignored so that we can temper our enthusiasm and avoid undue hype in the interest of cancer patients.

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NOTES

This review paper is dedicated to Richmond T. Prehn, one of the founders of modern tumor immunology, whose critical vision has accompanied our work in the last 30 years.

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