Review Article

A New Era in Anticancer Peptide Vaccines

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The use of synthetic peptides as vaccines aimed at the induction of therapeutic CD8-positive T-cell responses against tumor cells initially experienced great enthusiasm, mostly because of advances in vaccine technology, including design, synthesis, and delivery. However, despite impressive results in animal models, the application of such vaccines in humans has met with only limited success. The therapeutic activity of vaccine-stimulated, tumor-specific, CD8-positive T cells can be hampered through the physical burden of the tumor, tolerance mechanisms, and local factors within the tumor microenvironment. Recently, accumulating evidence has suggested that combining a peptide-based therapeutic vaccination with conventional chemotherapy can uncover the full potential of the antitumor immune response, increasing the success of immunotherapy. In addition, therapeutic vaccination in the preventive setting has been extremely effective in eliciting antitumor responses in preclinical tumor models and has demonstrated good promise clinically in patients with minimal residual disease. The rationale behind preventive vaccination is that patients with minimal tumor burden still have a fully competent immune system capable of developing robust antitumor responses. Finally, therapeutic CD8-positive T-cell peptide vaccines have been improved by coimmunization with T-helper epitopes expressed on long peptides. ***Cancer*** **2010;116:2071–80.** *© 2010 American Cancer Society.*

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Soon after cloning of the first human tumor-associated antigen (TAA) gene in the early 1990s,[1] the concept of peptide vaccination provided a promising modality for cancer treatment. However, despite noteworthy results in vaccine technology through all these years, clinical success remains elusive. Recent developments in the field of peptide-based vaccines have established new insights into strategies necessary for successful anticancer immunization. To date, synthetic or naturally occurring polypeptides (or long peptides) encompassing a variety of immunogenic cytotoxic T-lymphocyte (CTL) and T-helper (T_h) epitopes[2] have been the most attractive. Long peptides representing TAA epitopes offer an interesting opportunity for the induction of robust antitumor responses. These peptides, as large molecules, are not able to bind directly to major histocompatibility complex (MHC) class I molecules (as do peptides that are 8 to 10 amino acids long), so that their presentation to CTLs indicates that they have to be taken up and processed by specialized antigen-presenting cells (APCs) (ie, dendritic cells [DCs]).

The clinical setting in which vaccines are applied is the first parameter that should be taken into consideration. Tumor debulking through conventional treatments (ie, surgery, chemotherapy, radiotherapy) is a necessary step before vaccination. The development of vaccine-specific or vaccine-induced antitumor responses (characterized by epitope spreading and cross-presentation) is too slow and inadequate to overcome the accelerated tumor growth and redundant immunosuppression associated with advanced tumor load. Thus, the reduction of tumor burden may greatly diminish the immunosuppressive mechanisms sometimes associated with tumor growth to allow more vigorous expansion of antitumor effectors. Interference with immunosuppressive mechanisms has become feasible using gemcitabine and low-dose cyclophosphamide.[3]

Additional strategies for overcoming immunosuppression are being tested clinically and may become available in the near future.[4] Thus, the stage of minimal residual disease offers an attractive opportunity for combining peptide vaccination with the use of cytokines, or immunostimulatory monoclonal antibodies (MoAbs), or MoAbs against coinhibitory

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molecules. Clearly, it is preferable to vaccinate patients early in their disease course, when they have a fully competent immune system[5]; the endpoint here is to prevent disease recurrence.

Improved screening technologies for the identification of novel tumor biomarkers may enable the detection of tumors at earlier stages and ultimately may lead to the design of new preventive vaccines. In some patients, the administration of therapeutic vaccines has produced unexpected beneficial results on subsequent salvage chemotherapy after disease progression.[6] Thus, the concept of vaccinating first followed by chemotherapy may provide another promising paradigm for improved clinical results. This new approach is based on the hypothesis that the vaccine-induced antitumor immune response will be enhanced further through the influence of cytotoxic drugs on the immune system. This immunopotentiating "side effect" of chemotherapy can enhance the clinical response to chemotherapy. To this end, it should be stressed that, as time passes, chemotherapy certainly will diminish any vaccine-induced antitumor responses in the treated patients, as expected. Thus, the idea of this exciting new concept is based on the observation that there is a period of time early in chemotherapy when cytotoxic drugs may influence the immune system in a strictly positive manner, thereby potentiating the antitumor activity of vaccine-specific CTLs. This "window of opportunity" may be critical for achieving improved clinical responses. In this article, we review issues involved in the search for effective peptide-based cancer vaccines, including: long peptides, hybrid polypeptides, combined treatments, and vaccinating at early stages of disease.

Therapeutic Vaccines Combined With Chemotherapy

Accumulating bodies of evidence in the literature suggest that chemotherapeutic drugs can abrogate tumor-induced tolerance, thereby uncovering efficient antitumor responses. This concept of cancer chemotherapy acting as an adjuvant for antitumor immunity has provoked novel modalities for cancer immunotherapy based on combined chemoimmunotherapy strategies.[3,7] A decisive contribution to cancer therapy may be derived from an emerging concept that includes targeting multiple pathways of cytotoxic drug resistance by applying therapeutic vaccination combined with chemotherapy.[8] Remarkably high objective clinical response rates to second-line therapy with paclitaxel (62%) have been observed in patients with small cell lung cancer after completing a vaccination protocol using p53-pulsed DCs (compared with 16% to 20% response rates without previous vaccination).[9] Moreover, there was a good correlation in patients between response to vaccination, objective response to chemotherapy, and overall improved survival.

Arlen et al[10] designed a phase 1 study of patients with androgen-independent prostate cancer who were randomized to receive a prostate-specific antigen plus B7.1 vaccine either alone or with low-dose docetaxel. In that study, vaccine-specific immune responses were equal in both arms. The patients who progressed on vaccine alone crossed over to receive docetaxel at the time of disease progression. The median progression-free survival on docetaxel was 6.1 months after receiving vaccine compared with 3.7 months in patients on docetaxel alone (historic data for docetaxel alone). To our knowledge, this was the first clinical trial to demonstrate that docetaxel can be administered safely with immunotherapy without inhibiting vaccine-specific T-cell responses.

In another phase 1 study,[11] patients with different types of advanced cancer were immunized with the antigen cytochrome P450 1B1 (CYP1B1), which is overexpressed on almost all human tumors.[12] About 30% of patients in that study developed immunity to CYP1B1, and half of those patients developed disease stabilization. With the exception of 1 patient who did not develop immunity to CYP1B1, all patients progressed and did not respond to salvage therapy. The patients who developed immunity to CYP1B1 required salvage therapy for progressive, metastatic disease and had a marked response to their next treatment regimen, which lasted for >1 year.

In addition, Wheeler et al[13] analyzed survival and the time to disease progression in vaccinated (tumor-pulsed DCs) and nonvaccinated patients with glioblastoma who received chemotherapy. Vaccinated patients who received subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times and had significantly longer postchemotherapy recurrence times and survival relative to patients who received vaccination or chemotherapy alone. These data suggest that vaccination against cancer-specific antigens can sensitize the tumor against subsequent chemotherapeutic treatment. Although the mechanisms that underlie such a synergistic effect have not been elucidated to date, it is speculated that the vaccination-induced increase in the frequency of primed T cells may constitute a major advantage by the time the tumor microenvironment is modified by cytotoxic drugs (eg, disruption of tumor

stroma, decreased suppressive activity, increased sensitivity of tumor cells to granzymes, and up-regulation of TAAs)[8]. Moreover, chemotherapy-induced systemic effects (eg, enhanced cross-presentation of tumor antigens, depletion of suppressor cells, activation of DCs)[3] may further promote the antitumor activity of vaccine-specific CTLs.

Polyepitope Vaccines

A significant improvement in the immunogenicity of therapeutic single-peptide vaccines was achieved using long peptides that were generated by chemical linkage of multiple immunogenic epitopes to form stable linear complexes.[14,15] In addition to those artificial polyepitope vaccines, naturally linked and usually overlapping CTL and T_h epitopes also performed well as vaccines in preclinical mouse models.[16,17] Furthermore, naturally occurring, linked CTL and T_h epitopes with improved immunogenicity are present in humans, such as human papillomavirus (HPV),[18,19] NY-ESO-1 (a cancer testis antigen),[20] and HER-2/*neu*.[21,22]

One of the major mechanisms for the enhanced vaccine potency of long peptides is most likely that, unlike 8-mer to 10-mer CTL epitopes, these are not able to bind directly to MHC class I molecules, so their presentation to CTL precursors must follow processing by DCs.[2,23] Thus, long-peptide vaccines obviate binding to MHC class I-expressing, nonspecialized APCs, which often results in transient CTL responses or tolerance.[24,25] DCs are implicated in CTL induction, probably at the injection site or in draining lymph nodes, before the peptides are degraded by proteases in serum or extracellular fluid,[2,26] so the same cell may present both CTL epitopes and T_h epitopes through class I and class II MHC, respectively. This presentation may be more efficient than others in which class I and II epitopes are presented independently by different APCs. The greater efficiency of presentation by the same APC may be because it brings the T_h cell and the CTL precursor together for more effective transmission of small quantities of labile lymphokines and/or because the APC is licensed by the T_h cell for more efficient presentation of peptide to the CTL.[27,28] In either case, the same APC would be more efficient than 2 different APCs; therefore, the linked determinants would be more effective than those that could diffuse apart once they are injected in vivo (which may occur when vaccines are delivered as single peptide mixtures).

The strong vaccine potential elicited by long peptide-based vaccines also may be the result of prolonged presentation of the epitopes in inflamed lymph nodes in close proximity to the vaccination site, resulting in the clonal expansion of interferon-γ (IFN-γ)-producing effector T cells.[23] This may be important for CTL epitopes that have low binding affinity for class I MHC, which frequently is the case for tumor peptides.[29] Tumors may escape from immunosurveillance through the mutation of a single, immunodominant T-cell epitope or through the down-regulation of a specific human leukocyte antigen (HLA) allele.[30,31] By including multiple immunogenic epitopes, long-peptide vaccines may interact with many more HLA class I and class II alleles than short peptides. If CTLs could be raised against additional epitopes, creating a broad T-cell response against many epitopes, then such tumor escape variants would have less opportunity to establish metastases. Immunodominance depends on the frequency and avidity of T cells against a given epitope that preexists in the organism (ie, before any type of vaccination). Accordingly, when vaccinating with long peptides, immunodominant peptides will be recognized by high-avidity CTLs, which will increase in number. This implies that immunodominant epitopes will be processed efficiently and will be presented by APCs to ensure a level of expression high enough to induce a T-cell response. In this context, subdominant CTL epitopes will induce only weak responses, which are unlikely to be effective in destroying tumor cells.

However, in the presence of strong adjuvants or CD4-positive T_h epitopes, such responses may be enhanced significantly.[2] It is noteworthy that immune responses toward immunodominant epitopes that exist in certain portions of long peptides may increase considerably the likelihood of generating natural responses to other epitopes of the same protein and to epitopes of other protein antigens.[32] According to the epitope-spreading hierarchy, the immune responses against the immunodominant epitopes are mediated by high-avidity T-cell clones, which serve as driver clones for spreading to other subdominant determinants with lower avidity T-cell clones.[33] Once elicited, antitumor responses mediated by low-avidity T-cell clones are efficient in protecting hosts with growing tumors.[34] Therefore, the injection of long-peptide vaccines may help ensure pluralism in the antitumor response in which several tumor-specific, CD4-positive, and CD8-positive T cells will contribute to more efficient tumor cell killing.

Clinical Trials Using Polyepitope Long-Peptide Vaccines

The necessity of CD4-positive T-cell help to generate and sustain the MHC class I-restricted, CD8-positive T-cell

responses has led to the use of MHC class II-restricted epitopes derived from the same protein[16,35] and also to universal, nonspecific MHC class II-restricted epitopes, such as pan-DR T_h epitope (PADRE), in clinical vaccination trials that included patients with melanoma who were immunized with melanoma antigen 3 (MAGE-3) CTL peptides and patients with cervical carcinoma who were vaccinated with HPV16 E7 CTL peptides.[36,37] Although those studies demonstrated the capacity of PADRE to induce potent responses to PADRE itself, this did not increase CTL activity.

Two clinical studies[21,22] tested whether HER-2/*neu*–specific CD8 T-cell immunity could be elicited using HER-2/*neu*–derived MHC class II helper peptides that encompassed HLA-A2–binding motifs. Nineteen HLA-A2 patients who had HER-2/*neu*–overexpressing cancers received a vaccine preparation consisting of putative HER-2/*neu* helper peptides p369 to p384, p688 to 703, and p971 to p984. Contained within these sequences are the HLA-A2–binding motifs p369 to p377, p689 to p697, and p971 to p979. After vaccination, the mean peptide-specific T-cell precursor frequency to the HLA-A2 peptides increased in the majority of patients. In addition, the peptide-specific T cells were able to lyse tumor cells. The responses were long-lived and were detectable for more than 1 year after patients received the final vaccination. These results demonstrated that HER-2/*neu* MHC class I epitopes encompassed within MHC class II epitopes were able to induce long-lasting, HER-2–specific, IFN-γ–producing, CD8-positive T cells. In addition, the generation of protein-specific immunity after peptide immunization was associated with epitope spreading, reflecting the initiation of a broader endogenous immune response.

A polyepitope peptide vaccine named IDM-2101 has been evaluated for safety and induction of immune responses in phase 1 trials that included patients with colon cancer and patients with metastatic nonsmall cell lung cancer (NSCLC).[21,22] It also has been evaluated for clinical efficacy in phase 2 trials in NSCLC patients.[38] The IDM-2101 vaccine was designed to induce CTLs against 5 TAAs that frequently are overexpressed in NSCLC (ie, carcinoembryonic antigen, p53, HER-2/*neu*, and the melanoma antigens MAGE-2 and MAGE-3). IDM-2101 is composed of 10 synthetic peptides from these TAAs, 9 of which represent CTL epitopes. Each CTL epitope is restricted by HLA-A2.1 and by at least 1 other member of the HLA-A2 superfamily of MHC class I molecules, providing coverage of approximately 45% of the general population.

The 10th synthetic peptide is the pan-DR epitope PADRE. In phase 1 studies, no significant adverse effects were reported, and a strong CTL response was observed in the majority of patients.[39] In a phase 2 study, patients initially achieved stable disease, but overall survival was comparable to that of historic patients who received conventional treatment.[38]

A novel melanoma vaccine comprised of 6 melanoma-associated peptides as antigenic targets for melanoma-reactive T_h cells was evaluated for safety and immunogenicity in a phase 1/2 trial.[40] Vaccination with the helper peptide vaccine was well tolerated. Proliferation assays revealed induction of T-cell responses to the melanoma helper peptides in 81% of patients. Among the patients who had measurable disease, objective clinical responses were observed in 2 patients (12%) with response durations of 1 year and 3.9 years. Durable stable disease was observed in 2 additional patients for 1.8 years and 4.6 years.

Welters et al,[18] initiated a phase 1/2 clinical vaccination study in patients who had end-stage cervical cancer with a vaccine that consisted of a set of 13 overlapping peptides, 27 to 35 amino acids in length, of the complete sequence of the HPV16 E6 and E7 oncogenic proteins. The vaccine, in Montanide adjuvant, was tolerated well, and 1 of 35 patients had complete and lasting disease regression. Five additional patients achieved stable disease with salvage chemotherapy after vaccination that lasted up to 2 years; however, on average, all other patients died within 5 months. This indicated that therapeutic vaccination in this category of patients has only limited therapeutic action by itself but may exert much better therapeutic activity in conjunction with conventional chemotherapy or radiation therapy. At the same time, 6 patients who had undergone surgery for HPV16-positive cervical cancer were vaccinated subcutaneously with the same set of overlapping long peptides. Vaccine-induced, HPV16 E6-specific T-cell responses were detected in 6 of 6 patients; and HPV16 E7-specific T-cell responses were detected in 5 of 6 patients. The responses were broad, consisted of both HPV16-specific CD4-positive and CD8-positive T cells, and could be detected for up to 12 months after the last vaccination. The vaccine-induced responses were dominated by effector-type CD4-positive/CD25-positive/forkhead box P3 (FOXP3)-negative, type 1 cytokine, IFN-γ–producing T cells (T_h cells) but also included the expansion of T cells with a CD4-positive/CD25-positive/FOXP3-positive phenotype (regulatory T cells [Tregs]). The presence of vaccine-induced increases in HPV16-

specific Tregs indicates that strategies to eliminate or disarm these cells should be considered for immunotherapeutic strategies against HPV-induced cancers.

The same group conducted a phase 2 vaccination study[19] in patients with HPV16-positive vulvar intraepithelial neoplasia grade 3: a premalignant condition. Patients were vaccinated 4 times with the same vaccine, which consisted of 13 overlapping long peptides of the E6 and E7 oncoproteins of HPV16. Of the patients who were analyzed, 25% achieved complete regression of all lesions, and several patients had partial regression of their lesions (>50% reduction in size) with marked relief of symptoms. These results are encouraging and suggest that further improvement may be expected from the coformulation of this vaccine with a potent, toll-like receptor (TLR) ligand rather than just Montanide.

*Immunogenic HER-2/*neu *Peptides and Ii-Key/HER-2/*neu *Hybrid Peptides as Enhancers of Antitumor Responses*

Given the accumulating body of evidence that T_h-cell stimulation is essential for generating memory antitumor CTLs, our recent efforts focused on the identification and presentation of HER-2/*neu* class II-restricted peptides. We have identified 2 helper peptides from the HER-2/*neu* sequence (HER-2/*neu*[776-788]) and HER-2/*neu*[884-899]) that induced potent CD4-positive T-cell responses as measured by proliferation and IFN-γ production.[41,42] More noteworthy, peptide-specific T-cell lines and clones were capable of recognizing primary HER2/*neu*-expressing tumor cells, demonstrating that both peptides were processed naturally. Those reports were the first to suggest that HER-2/*neu* possesses lysosomal targeting sequences that enable it to present its peptides in the context of MHC class II molecules.[41,42] We also observed strong immunogenicity with HER-2/*neu*(776-790), which is an extended analog of the p776 to p788 peptide.[43-45] CD4-positive T cells primed with the p776 to p790 peptide exhibited an extensive capacity to synergize with syngeneic CTLs for the rejection of HER-2/*neu*-positive tumors.[43-45] Both the p776 to p790 peptide and the p776 to p788 peptide encompass in their sequences the nonamers GVGSPYVSRL (p776-p784), VGSPYVSRL (p777-p785), and PYVSRLLGI (p780-788), which bind with intermediate to high affinity to the HLA-A2.1/A68, HLA-A11, and HLA-A3 alleles, respectively. Thus, the p776 to p790 peptide may function as a multipeptide vaccine that contains overlapping T_h and CTL epitope motifs, thereby acting as a powerful inducer of antitumor immune responses.

A novel method for amplifying the activity of MHC class II epitopes entails linking a 4-amino-acid moiety (LRMK; Ii-Key) to the N-terminal end of the epitope peptide either directly or using a simple polymethylene spacer (-ava-). Ii-Key is derived from the MHC class II-associated invariant chain (Ii protein) and catalyzes binding of the linked epitope to the MHC class II molecule, thereby enhancing the overall potency of presentation.[45-47] More recently, studies have indicated that, in addition to increasing binding to the MHC class II molecule, the presence of the Ii-Key moiety also may trigger the release of activating cytokines and chemokines from DCs after class II binding (unpublished data). In several studies, Ii-Key hybrid peptides demonstrated significantly increased potency over the unmodified epitope peptide in both in vitro and in vivo vaccine studies as determined by the antigen-specific stimulation of T cells.[43] By using the Ii-Key/HER-2/*neu*(776-790) peptide, a series of Ii-Key/epitope hybrids were designed and screened in vitro using peripheral blood mononuclear cells from cancer patients who had various types of HER-2-positive tumors to identify a candidate hybrid (AE37) for clinical trials.[43,45] Furthermore, CD4-positive T cells from AE37-immunized mice potentiated CD8-positive T cells to recognize and lyse autologous tumor cells more efficiently in vitro[43,45] and in vivo.[45-47] All of the aforementioned studies paved the way for clinical trials of AE37 in patients with breast cancer.[48,49]

Large Tumors Induce Strong Immunosuppression: The Concept of Vaccinating During the Early Stages of Tumor Formation

It has been demonstrated that bulky tumors generate strong tolerizing conditions within their microenvironment that limit effective immunotherapy. Such conditions can lead to escape from immunosurveillance by allowing the cancer to become immunoresistant. Immunosuppressive tumor microenvironments inhibit the local antitumor immune response through distinct mechanisms, including the impairment of antigen presentation, the activation of negative costimulatory signals, active biosynthesis of immunosuppressive molecules, the recruitment of naturally occurring Tregs, and/or the transformation of effector T cells locally into Tregs.[50] In addition, suppressor cells produced within the tumor microenvironment can migrate to lymphoid organs,

where they can exert systemic immunosuppression. Thus, bulky tumors that emerge during the advanced stages of cancer are the potential source of suppressor cells that inhibit antitumor immunity, which may persist even after the tumor has been removed by standard therapies. This may account in part for the failure of therapeutic vaccines, when used as monotherapy, to induce objective responses in the majority of cancer patients with advanced disease.[5,51] This also holds true for clinical trials of therapeutic vaccination in the adjuvant setting (ie, after conventional chemotherapy, radiotherapy, or surgical therapy) in which no complete responses could be demonstrated; although, in most trials, improvements in recurrence-free survival were observed.[5,51] These positive but limited results from clinical studies with therapeutic vaccines in the adjuvant setting led to the consideration that vaccinating at the early stages of tumor formation, when tumor burden is too low to facilitate the establishment of strong local and systemic immunosuppressive responses, may induce more efficient antitumor immune responses with enhanced clinical benefit.

The development of spontaneous mammary tumors or prostate adenocarcinoma in transgenic animals[52,53] can be prevented efficiently through vaccination when preneoplastic lesions are present. An example of a vaccine currently in clinical trials that may benefit from early administration is a breast cancer vaccine that consists of an MHC class I HER-2/*neu* epitope peptide (E75) mixed with granulocyte-macrophage–colony-stimulating factor; phase 2 studies have indicated that it is successful in reducing disease recurrence in lymph node-positive and high-risk/lymph node-negative patients with minimal disease burden.[54] The modified MHC class II Ii-Key/HER-2/*neu*(776-790) epitope hybrid peptide also has been applied as a preventive vaccine in a phase 1 clinical trial in disease-free patients with lymph node-negative breast cancer.[48] Surprisingly, the Ii-Key–modified vaccine produced enhanced immunologic responses even in the absence of an immunoadjuvant.[48]

Currently, we are using the Ii-Key/HER-2/*neu*(776-790) hybrid peptide in a phase 1 trial of patients with HER-2/*neu*-positive prostate cancer. Only grade 1 toxicity has been observed in 10% of patients, whereas the majority of patients have achieved increased immunologic responses to the vaccine in vivo, measured as delayed-type hypersensitivity, and in vitro (as determined with the IFNγ-based enzyme-linked immunospot assay).[55] We are observing similar results using this Ii-Key–conjugated HER-2/*neu* peptide in an ongoing phase 2 study of patients with HER-2/*neu*-positive/lymph node-positive and high-risk/lymph node-negative breast cancer for the prevention of recurrences (unpublished observations). In a randomized, double-blind study, patients with stage II breast cancer but no evidence of disease received injections with oxidized mannan-mucin 1 (MUC1) to immunize against MUC1 and to prevent cancer recurrence/metastases.[56] Immunized patients had no recurrences (0 of 16 patients) for >5 years after treatment, whereas the recurrence rate among patients who received placebo was 27% (4 of 15 patients). In the majority of vaccinated patients, both humoral and cellular immunity to MUC1 was evident.

Challenges for Peptide Vaccination Strategies

To increase the chances for clinical success and to apply peptide-based vaccination to patients with different types of cancer, it is necessary to be very precise in selecting the treatment modalities and the time period during which these should be applied. However, this may be complicated by the dictates of chemotherapeutic protocols and, to a certain extent, by the type of cancer to be treated. Therefore, it is uncertain whether the theoretically optimal treatment strategies for both standard chemotherapy and peptide vaccination will be possible. Another problem may arise from the finding that, in many cases, patients already will have received treatments that may exert long-lasting negative effects on their immune systems, thus weakening the effect of the vaccine. All of these factors may impede the accurate assessment of clinical trial results and hinder the standardization of clinical protocols.

A prerequisite for successful anticancer vaccination in the therapeutic setting is the induction of a cell-mediated immune response that is robust enough to efficiently eliminate malignant cells. To some extent, as outlined above, the use of polyepitope vaccines, including CTL and T_h epitopes (either in the form of chemically linked epitopes, or as long peptides, or linked to the Ii-Key moiety) (Fig. 1), should meet this requirement, because such molecules will be presented efficiently by DCs. However, because established bulky disease clearly subverts the immune system, it is unlikely that single-component therapies, such as vaccine-only strategies, will be effective. Therefore, polyepitope-based therapeutic vaccination should be combined with other modalities, such as chemotherapy and radiotherapy; or, alternatively, they should be applied at the earliest possible stages of diagnosed malignancy (Fig. 1). Even so, antigenic competition and the induction of T_h peptide-specific Tregs constitute 2 important obstacles for the efficient generation of

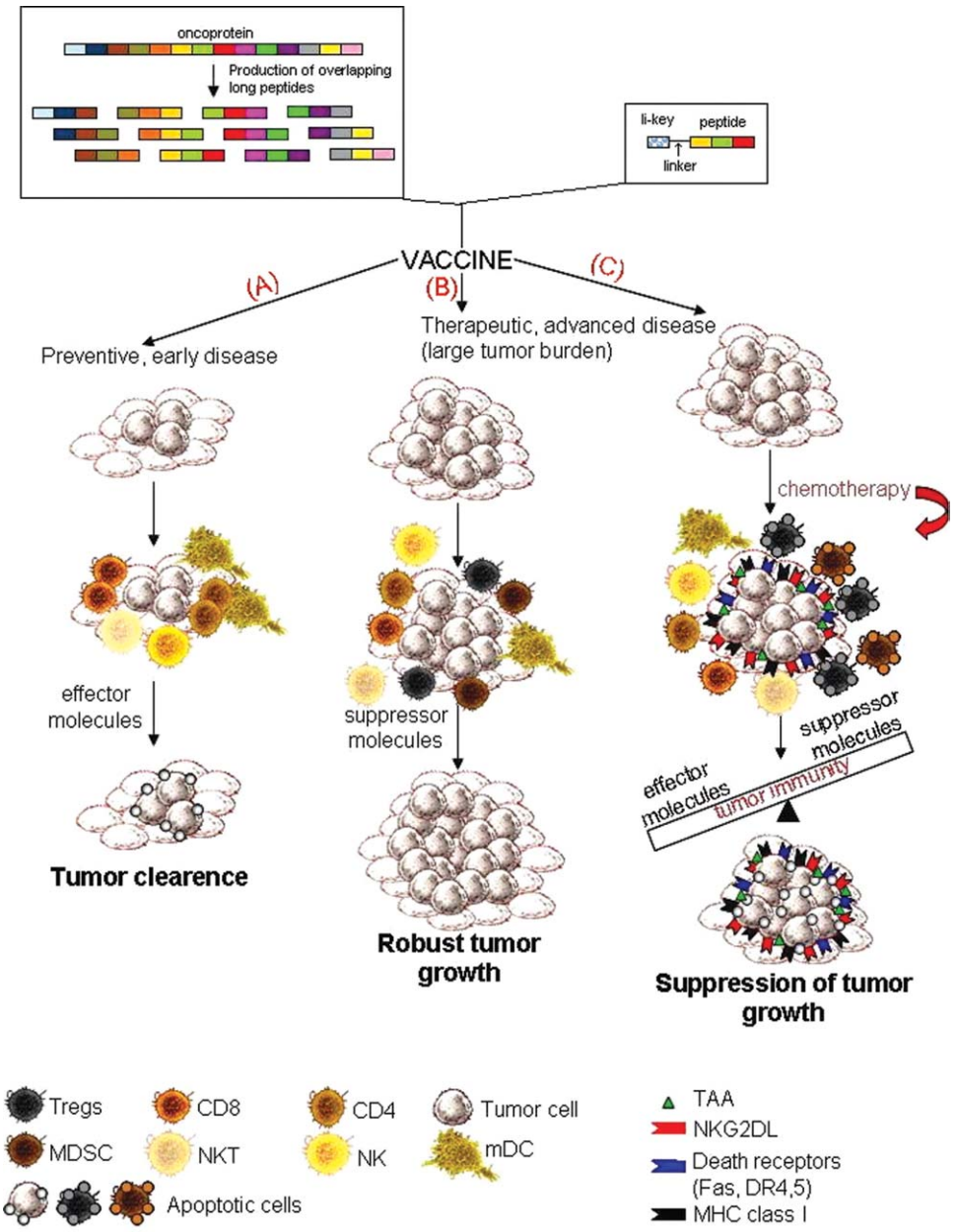

Figure 1. This schematic drawing illustrates the manipulation of antitumor immunity by vaccination with multiepitope vaccines (eg, long peptides, Ii-Key/polypeptide hybrids). (A) Vaccines are administered either at early stages of tumor development or after conventional treatment in the clinical setting of minimal residual disease (preventive vaccination). An inflammatory milieu consisting of vaccine-activated (CD4-positive, CD8-positive) T cells, mature dendritic cells (DCs), cytokines, and coactivated natural killer (NK) cells and NK T (NKT) cells (eg, through T-cell–derived interferon γ and/or DC-derived interleukin 12 [IL-12]/IL-18) will induce efficient tumor killing through various mechanisms (eg, tumor necrosis factor-related apoptosis-inducing ligand [TRAIL] and perforin). (B) Induced by a therapeutic vaccine, bulky tumors establish a suppressive milieu consisting of suppressor cells and molecules that dominate antitumor T cells, rendering them functionally inactive and allowing tumor growth. (C) Substantial clinical benefits for patients with advanced disease can be achieved when therapeutic vaccination is combined with chemotherapy. This synergistic modality may include multiple mechanisms like the elimination of immunosuppressive cells and the up-regulation of recognition molecules on tumor cells, including tumor-associated antigens (TAAs), major histocompatibility complex (MHC) class I molecules, ligands for NK-activating receptors (NKG2D ligand [NKG2DL]), and death receptors (Fas, DR4,5). These multimodal effects of chemotherapeutic agents will enable efficient tumor killing by the vaccine-induced T cells. Tregs indicates regulatory T cells; MDSC, myeloid-derived suppressor cells.

antitumor responses. The former usually occurs when CTL peptides are injected into a single site, suggesting antigenic competition at the level of local draining lymph nodes, or when vaccines are applied in the context of bacterial/viral vectors, in which antivector responses dominate over vaccine-specific responses. Thus, the proper way to avoid antigenic competition is the administration of polyepitope peptides at different sites in the absence of vectors and with the inclusion of adjuvants. In addition, strategies to eliminate or disarm Tregs should be considered.

Another important issue is immunologic targeting of cancer stem cells (CSCs) for more effective therapies. Although some targeting of stem cell molecular pathways, such as Hedgehog, Notch, and β-catenin, have been initiated in clinical trials,[57] immunotherapy largely has ignored the CSC phenomenon. Moreover, the evaluation of CSCs in the context of host-immune responses largely has been disregarded, and most studies on CSCs have been done using immunocompromised rodent models.[58,59] It must be noted that, as normal stem cells, CSCs are characterized by a very low constitutive MHC class I antigen expression as well as by a lack of MHC class II molecules.[60,61] This would allow CSCs to escape from T-cell immunosurveillance; however, at the same time, they would become more sensitive to natural killer cell recognition and killing.

It is noteworthy that recent reports have revived interest in the involvement of adaptive immune responses toward CSCs. Pellegatta et al[62] produced a vaccine that consisted of DCs pulsed with lysates from glioma stem cells and observed that vaccine-specific, CD8-positive T cells were highly effective in protecting vaccinated mice from the outgrowth of syngeneic gliomas or glioma stem cells. Furthermore, Gedye et al[63] generated clonogenic, CD133-positive melanoma cells with self-renewal capacity that expressed NY-ESO-1 (the cancer testis antigen) and HLA-A2 and were amenable to killing by NY-ESO-1-specific, CD8-positive T cells. Most important, it has been demonstrated that CTLs specific for transduction proteins involved in the downstream signaling of CSCs efficiently eradicated CSCs.[64] These data provide initial evidence for the possible targeting of CSCs by tumor peptide-specific CTLs and suggest that the successful elimination of CSCs in vivo may require targeted inhibition of a suitable signaling pathway combined with immune targeting of the antigens expressed by these cells.

Future Directions

Although both the clinical setting in which vaccines are used as well as their design and mode of application (eg, long peptides, Ii-Key/hybrid peptides) clearly are important parameters, it is evident from the discussions above that this still may be an oversimplification. The complexity of interactions between developing tumors and immune cells may require combined therapeutic treatments for generating the most effective protective antitumor immunity. The subversion of key tolerizing conditions, the potentiation of immunostimulatory pathways, and the blockade of tumor growth and angiogenesis may improve cancer immunotherapy substantially. Such a combined therapeutic strategy will be feasible for application in disease-free patients to prevent or delay recurrences and in patients with advanced disease who are receiving therapeutic vaccinations combined with conventional chemotherapy.

Although we have begun to see the potential clinical benefit in cancer patients using combined treatment strategies, the mechanisms underlying such modalities are complex and need to be defined precisely. To this end, it will be essential to characterize more specifically the interaction between tumor cells and the immune system at a molecular and cellular level. Appropriate dynamic and quantitative models are required to gain insight into the development of immunoregulatory pathways during tumor progression. An improved understanding of these aspects will help us to develop and refine novel immunopotentiating treatments and to estimate the optimal timing of their application.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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