Peptide and Dendritic Cell Vaccines
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Abstract
There has been a rush to convert discovery of new melanoma antigens into cancer vaccines for the therapy of melanoma. The result has been disappointing from a clinical standpoint. The premise behind rapid pursuit of peptide vaccines for melanoma therapy was that the spontaneous tumor-associated immune response was too weak to be effective. However, it is increasingly clear that the host-tumor relationship is a complex interplay of immune response, immune escape, and immune adaptation, with multiple layers of regulatory control and modulation of responses over time. The lesion in the immune response to cancer is much more complex than simply a weak immune response to defined antigens. Current results should serve as a call to take a closer look at immune regulatory processes and principles and to develop more comprehensive and multi-agent approaches to modulate the host-tumor relationship. Development of effective immune therapy for cancer will require (a) more comprehensive and real-time immune monitoring in various tissue compartments and (b) patient-specific modulation of immune responses, informed by the real-time monitoring. Peptide antigens associated with MHC class I or class II molecules are the molecular targets for T-cell recognition of cancer. To characterize the host-tumor relationship and to optimize cancer vaccines, clinical studies using defined peptide antigens offer special opportunities to advance the field and thus have an important place in the ongoing development of effective immune therapy of melanoma.

Fifteen years ago, no melanoma antigens recognized by T cells were known. Since then, dozens of peptide antigens have been identified as targets for cytotoxic and helper T cells (1). Those that are shared among melanomas hold promise as “off-the-shelf” immunogens to be incorporated in vaccines. The melanocyte differentiation proteins and cancer-testis antigens are common source proteins for these peptides. There has been a rush to convert this discovery of new antigens into cancer vaccines for the therapy of melanoma. The result has been disappointing from a clinical standpoint. Perhaps the reason it has been disappointing is that we expected more than what we should have from a single intervention into a complex biological system.

The premise behind the rapid pursuit of peptide vaccines for melanoma immunotherapy was that the spontaneous tumor-associated immune response was too weak to be effective. Thus, it was presumed that the induction of a stronger immune response to defined antigens would create a new or improved tumor-rejection response. There was also a presumption that induction of responses to one peptide or one protein would be adequate to cause tumor rejection.

There have now been multiple clinical trials of peptide-based vaccines. Some use peptide alone; some use peptide(s) plus adjuvant; some have used gene/viral constructs encoding defined antigens; some have used peptide(s) pulsed on dendritic cells. Many of these trials show that CD8+ T-cell responses can be generated by vaccination with peptide or dendritic cell vaccines. Objective clinical responses have been observed in a few cases (3-5% of patients with advanced melanoma; ref. 2). Some studies suggest a correlation between immune response and clinical response; many others do not. However, clinical responses have been rare, and thus meaningful assessments of correlations are difficult to make.

These observations have led some to conclude that peptide vaccines do not work, that cancer vaccines in general do not work, and/or that the immune response to vaccines is irrelevant to the clinical benefit of patients with melanoma. It is useful at this point to take a critical look at what we really know and what we do not know about modulation of host immunity to melanoma with cancer vaccines. Several clinical melanoma cases, with which we have had experience, may be illustrative of the complex interplay of host immunity, immune escape, and immune adaptation.

Case Reports

Case 1. Patient VMM39 presented with a large palpable mass in the right axilla, which was found to be metastatic melanoma. His history included excision 2 years previously of a skin lesion on the right arm that had become black, then faded in color. A biopsy specimen revealed the lesion to be benign. In retrospect, the original slides, when re-reviewed, revealed that the skin lesion had been a completely regressed melanocytic lesion, presumably primary melanoma. This patient...
underwent surgery and was rendered clinically free of disease. He was interested in an experimental adjuvant clinical trial of vaccination with an HLA-A2–restricted synthetic gp100 peptide (gp100\textsubscript{280–288}) in adjuvant. He was HLA-A2 positive, and by immunohistochemistry, all of his metastatic melanoma cells expressed high levels of gp100. He was thus considered an excellent candidate and was enrolled in the study. However, he progressed within a few months, exhibiting tumor in the spleen and in distant lymph nodes, and he died soon thereafter.

Laboratory evaluations revealed that the patient had a pre-existing (tumor-induced) immune response to the gp100\textsubscript{280–288} peptide that was included in the vaccine, that his metastatic tumor cells had a mutation in its antigen-processing machinery, and that his tumor cells were not recognized by gp100\textsubscript{280–288}–reactive T cells. On the other hand, T cells could be generated in vitro from his lymphocytes by stimulation with autologous tumor and they recognized autologous tumor. A possible explanation is that this patient experienced regression of his primary melanoma, which was mediated by tumor-induced immune responses against one or more melanoma antigens, one of which was to the gp100\textsubscript{280–288} Peptide. Furthermore, it is presumed that his metastasis escaped immune-mediated destruction by alteration in antigen presentation pathways. The tumor progressed in “immune system strongholds,” consistent with an immune escape phenotype. Nonetheless, the tumor expressed antigens that could be recognized by autologous lymphocytes. This illustrates the coexistence of partial immune protection and immune escape and that antigen expression needs to be confirmed at several levels.

Case 2. Patient VMM5 developed metastatic melanoma to 12 lymph nodes in his neck. With no treatment other than surgery, he remained clinically free of disease for 5 years, then disease recurred as a large node in the neck in 1997. Again, with no additional therapy other than surgery, he lived free of disease for 6 more years until his death at the age of 91 years due to cardiovascular disease. His original tumor expressed HLA-A2 and his circulating lymphocytes and tumor-associated lymphocytes exhibited prominent immunologic reactivity to multiple HLA-A2–associated peptides from melanocytic differentiation proteins. The dominant reactivity was to the MART-1/MelanA peptide AAGIGILTV. However, in his second lymph node metastasis, there was a marked down-regulation of HLA-A2 expression mediated by a new acquired mutation in β2m, and tumor cells thereby escaped recognition by MART-1/MelanA–reactive T cells. However, lymphocytes infiltrating the second metastasis had a dominant reactivity to the tyrosinase peptide YMDGTMSQV, which had not been targeted by T cells in the original metastasis. This is an example of a patient with multiple mechanisms of immune escape in the tumor where an adaptive change in the immune response occurred and may have contributed to long-term survival (3).

Case 3. Patient VMM22 developed extensive metastatic melanoma from a left leg primary tumor, which ultimately involved >90% of the skin of her left lower extremity, with extensive intravascular melanoma deposits in the femoral artery and vein. However, no distant metastases were found. A high amputation (hip disarticulation) was done for palliation. A lymph node that contained tumor was found (and removed) at the margin of resection. Surprisingly, the patient remains clinically well and free of disease more than 8 years later. This is a dramatic case of regional progression of melanoma without systemic evidence of disease. Because tumor was present in the arterial and venous systems of the lower extremity, there must have been melanoma cells in the systemic circulation. This suggests that there may have been systemic immune protection simultaneous with regional tumor progression.

Future Investigations

These cases illustrate the complexity of the natural tumor-associated immune response to melanoma and suggest several premises that deserve further characterization:

- Clinical progression of melanoma in an immunocompetent patient is de facto evidence of immune escape by the melanoma.
- Multiple mechanisms of immune escape can occur and may reflect immune editing; they define a complex molecular phenotype of each melanoma.
- The host-tumor relationship is a complex process that occurs over time and involves immune responses, immune escape, immune editing, and immune adaptation.
- Antitumor immunity (or failure of antitumor immunity) may be regional rather than systemic.
- The lesion in the immune response to cancer is much more complex than simply a weak immune response to defined antigens.

Given the complexity of the host-tumor relationship and the layers of regulatory control and avenues of immune escape shown by melanoma cells, it is remarkable that single interventions ever lead to objective clinical responses in any patients. Thus, the single digit response rates with melanoma vaccines, although disappointing results for melanoma therapy, are an encouraging proof of principle for T-cell–directed cancer vaccines. These results should serve as a call to take a closer look at immune regulatory processes and principles and to develop more comprehensive and multitarget approaches to modulate the host-tumor relationship.

Cancer vaccines using defined peptide antigens may well have a place in the future of such therapies. However, the greatest value of peptide vaccines in the present era is their value in dissecting the host-tumor relationship. Vaccinating with defined peptide antigens enables studies of immune responses to the minimal epitope(s) in isolation (4, 5). In addition, at present, there is no consensus about how best to vaccinate with defined antigens. Thus, current studies with peptide antigens permit measures of immunogenicity to guide improved vaccine strategies.

Questions of the current era have dwelled too much on comparing the efficacy of various strategies; e.g., are dendritic cell vaccines, whole melanoma cell vaccines, peptide vaccines, or viral vaccines most effective? The answer thus far is that all have some promise but all fall far short of our goals. Thus, the goals of the next generation of cancer vaccines should be to enable new technology and new understanding of the host-tumor relationship.

We have developed approaches for multipeptide vaccines using 12 MHC class I–associated peptides (6), and new and planned strategies for vaccine trials include addition of
melanoma-associated helper peptides (ECOG 1602 and UVMel44), combination of vaccines with chemotherapy (Mel44), and combination of vaccines with targeted molecular therapies (CTEP LOI 7190). Immune monitoring has included evaluation of peptide-reactive responses in a vaccine-draining lymph node, as well as in peripheral blood. Evaluation of responses in peripheral blood has revealed an interesting dichotomous response pattern, with persistent responses to some peptides and transient responses to others, when responses are evaluated frequently over time during the vaccine regimen (5). The responding T cells also evolve phenotypically over time and seem to include both memory and effector phenotypes (7). In contrast, preexisting tumor-associated immune responses to defined antigens may be more stable over time.6

Development of effective immune therapy for cancer will require more complete immune monitoring in various tissue compartments. Technological developments that are needed include the following:

- Rapid high-throughput characterization of cellular immune responses to a large array of defined antigens in real-time.
- Monitoring of cellular immune responses in blood, lymph nodes, and tumor compartments.
- Patient-specific modulation of immune responses, informed by the real-time monitoring (compare use of real-time blood pressure monitoring to direct and to inform therapy for hypotension or use of blood cultures to modify antibiotic therapy for sepsis).
- Economical methods for screening patients’ tumor deposits for immune escape mechanisms with evaluation of their response to immune modulation.
- Selection of immune therapy based on pretreatment assessments of the patient-specific host response and the tumor escape mechanisms.
- Determination of the effector or memory T-cell phenotypes (cytotoxic and helper) that are optimal for tumor control.
- Reliable methods for inducing antigen-reactive T cells of a desired functional phenotype in high numbers.

**Summary**

Peptide antigens associated with MHC class I or class II molecules are the molecular targets for T-cell recognition of cancer. To characterize the host-tumor relationship and to optimize cancer vaccines, clinical studies using defined peptide antigens offer special opportunities to advance the field and thus have an important place in the ongoing development of effective immune therapy of melanoma.

**Open Discussion**

Dr. Hwu: It’s encouraging that a better immune response looks like its correlating with increased survival in your patients. The question is whether that’s just because the patients are healthier and they would respond to anything better or if it is actually the antigen-specific cells mediating that immune response. Do you have a control antigen?

Dr. Slingluff: We don’t. We initially didn’t because we didn’t want to have something that would compete with the immune response. The difference in outcome with peptides and interleukin 2 obviously is not necessarily just a test of vaccines, although this was a randomized group. Therefore, the group that had a better immune response is the group that has better outcome.

Dr. Kirkwood: Have you looked at the patients’ time to progression or issues such as epitope spreading or responses to individually defined antigens?

Dr. Slingluff: We have not seen epitope spreading in the adjuvant setting. We’re set up to evaluate that, at least for some antigens, in a fairly systematic way because we’re looking at responses. The patients who got the four-peptide mix were evaluated for responses to 12. We saw one patient or two patients with small responses to one antigen, although it was really not meaningful. That may be different in the advanced stage patients and we’ll be able to look more at this concept in more detail in the E1602 trial.

Dr. Kirkwood: A sequel to that is compartmental analysis by tetramer flow cytometry with CD27, CD28, CCR-7, and CD45. What is the current best approach to doing that?

Dr. Slingluff: We have eight tetramers that are working well for us for the 12 antigens. There are some that don’t work so well. Our plan is to identify at least initially those patients who have transient and persistent responses. We’re particularly interested when it’s antigen-specific differences rather than patient specific. Then we will try to characterize whether there are changes that explain that phenomena that we may be able to target. As our initial screen, we are looking at CD27, CD28, CD45 RA, and CCR-7 because these are classic markers, but we’ll also be looking more at granzyme B.

Dr. Essner: We also see with our vaccine that you get peak responses at ~8 to 12 weeks and then the immune response plateaus. Does this relate to the presence or regrowth of tumor or does it seem to be unrelated?

Dr. Slingluff: It’s not clear that it’s related to tumor regrowth. Sometimes we see in the same patients a peak in response to one antigen and a plateau of others, but I don’t think it’s that simple. We’ve done some booster vaccines in subsequent patients, such as once every 3 months, which tends to maintain some of the responses better, which is encouraging.

Dr. Essner: In these patients, is immune response maintained years later?

Dr. Slingluff: We have blood samples from most of those patients out to 2 years. In general, what we’ve seen is that often patients who didn’t get booster vaccines don’t have much immunity at later time points. In Mel-39, 50% of patients maintain response to ~6 to 9 months, which is as far as we’ve looked. With the booster vaccines, we’re starting to see responses that persist at a higher level even further out.

Dr. Gajewski: In terms of markers to assess circulating tumor antigen-specific T cells, I wonder if we should give more attention to molecules that reflect what we expect good activated T cells to be able to do, such as chemokine receptors that support trafficking into the peripheral tissues rather than the lymph node, and expression of granule-related molecules that might predict cytolytic potential?

Dr. Slingluff: David Mullins has done some chemokine receptor work with tetramer-positive cells and other antigen reactive cells, which suggests that this may actually predict

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survival in patients. The question is whether or not some of this is vaccine induced or whether it actually defines a polymorphism that defines outcome. There is also interesting work being done by Dr. Mullins and Dr. Engelhard on the regionality of the immune response. If you vaccinate with dendritic cells in the skin in the murine models, you get a nice response in the node and protection against tumor in the skin. However, you don’t get protection against tumor in the lung. If you vaccinate with dendritic cells i.v., then you get a nice response in the spleen, not much in the node, and good protection against lung metastases but not skin metastases. The concept of regional immunity goes along with the compartmentalization of response and being able to monitor differential effects in different compartments.

Dr. Atkins: What would you like to see in a phase 2 trial that would encourage you to take this to a phase 3 trial?

Dr. Slingluff: I would like to see phase 2 trials that are designed to answer biological questions and also, usually at the same time, to identify whether one vaccine gives better immune responses than others so that we can make progress stepwise.

Dr. Atkins: So, we’re talking about trying to induce immune potency rather than to necessarily induce a clinical effect. Hopefully, immune potency will be a surrogate for clinical effect.

References
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