Cancer Res Treat. 2009;41(3):117-121 DOI 10.4143/crt.2009.41.3.117

Review Article

Immunomodulation of Breast Cancer via Tumor Antigen Specific Th1

Mary L. Disis, M.D.[1]
Kyong Hwa Park, M.D., Ph.D.[2]

[1]Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington, Seattle, WA, USA, [2]Division of Oncology/ Hematology, Department of Internal Medicine, Korea University, Seoul, Korea

Correspondence: Mary L. Disis, M.D.
Tumor Vaccine Group, Center for Translational Medicine in Women's Health, 815 Mercer Street, 2nd Floor, Box 358050, University of Washington, Seattle, WA 98195-8050, USA
Tel: 206-616-1823
Fax: 206-685-3128
E-mail: ndisis@u.washington.edu

This work is supported for MLD by grants from Gateway Foundation, Arnold and Mabel Beckman Foundation, the Ovarian Cancer Research Fund and R01 CA101190 and CA129517. The patient care described in this review was conducted through the Clinical Research Center Facility at the University of Washington (NIH grant UL1 RR025014).

It has long been assumed that the immune system plays a role in tumor eradication, however, scant clinical evidence exists to support that hypothesis. In recent years, as the immune system and its specific effector cells are better defined, convincing data supporting immune surveillance is emerging. Several studies have shown that an "immune signature" in the tumor microenvironment is associated with a superior outcome in a variety of cancer types. Moreover, studies have suggested that T cells found in high density within the tumor parenchyma are also correlated with a survival benefit. The type of adaptive immune response implicated in improved cancer outcomes is a type 1 response. That is, adaptive immunity associated with T cells that secrete pro-inflammatory cytokines, such as IFN-γ, which can not only support a proliferative antigen specific T cell response but also enhance "cross priming" by activating antigen presenting cells local to the tumor site. There are many methods available that will allow the development of clinical reagents designed to stimulate Th1 immunity; either by *in vitro* or *in vivo* manipulation. Clinical trials of a variety of immunotherapeutic strategies indicate that the generation of tumor antigen specific Th1 may be beneficial in inhibiting the growth of common solid tumors.

Key words
CD4 T cell, T helper 1, Cancer vaccine, Adoptive T cell therapy, Breast neoplasms

Introduction

Advances in molecular immunology have greatly improved our understanding of the function of the immune system, particularly in regards to immune-cancer interactions. We now see cancer as a chronic inflammatory disease where immune cells may actually function to enhance rather than inhibit tumor growth (1). If one could reverse the chronic inflammatory state of cancer and, instead, create the type of inflammation that causes tissue rejection, perhaps tumors could be eradicated by immune effector cells. One immune system cell responsible for creating such inflammation is the $CD4^+$ T helper (Th) cell. Th have several phenotypes ranging from those subtypes which can depress the immune response, such as $FOXP3^+$ T regulatory cells (Treg) or IL-10 producing Th2 cells, to those that can create an environment which will support tissue destruction via Th1 (IFN-γ secreting) and Th-17 (IL-17 secreting) effectors. Several groups are working to exploit the potential therapeutic power of tumor antigen specific Th1 immunity. Early clinical trials demonstrate that generation of tumor antigen specific Th1 may be of benefit in cancer patients.

Therapeutic Vaccines for the Treatment of Cancer

Cancer vaccines have been evaluated as potential therapeutic agents for dozens of years. Most often vaccines are tested in the setting of advanced stage disease with the stated goal to induce a tumor regression. Unfortunately, cancer vaccines have not been effective as a treatment for established disease. In a review of over 500 patients with metastatic melanoma who were immunized with a variety of vaccines directed against putative tumor antigens, less than 10% of patients demonstrated a clinical response (2).

Cancer vaccines may be better suited for testing in the adjuvant setting, where disease is at its lowest burden. For this reason, cancer vaccines are often evaluated after patients have achieved a maximal response with standard therapy in an attempt to use an immunoconsolidation approach to prevent disease relapse. However, in 2009, the results of two Phase III studies of cancer vaccines were reported that appeared to show survival benefit in advanced stage cancer patients. The first is a prostate cancer vaccine. The vaccine, Sipuleucel-T (Dendreon Corporation) was evaluated in 341 cancer patients as compared to 171 patients given placebo. The survival in the vaccinated patients was a median of 25.8 months compared to a 21.7 month survival in controls (p=0.032) (3). A second Phase III trial evaluated an idiotype-KLH vaccine, BiovaxID (Biovest International) given to patients with follicular cell lymphoma. At a median follow-up of 56 months the vaccinated patients demonstrated a 44 month survival compared to 31 months in controls (p=0.047) (4). These data lend credence to the concept of immunizing cancer patients to modulate disease.

Breast cancer as a good model for vaccination. First, the tumor is immunogenic and many tumor antigens have been identified that are expressed in breast cancer. Secondly, breast cancer is amenable to standard therapies such as surgery and chemotherapy. For this reason the patients can be treated to a very minimal residual disease state where the amount of tumor burden is potentially readily eradicated by antigen competent T cells. Finally, breast cancer is slow growing and often the doubling rate can be expressed in terms of years rather than weeks. The slow growth of the disease allows for the ability to expand T cells over time with repeated booster immunizations potentially achieving therapeutically effective levels before the disease begins to become bulky or metastasize.

What type of immune response is needed to potentially eradicate micrometastatic disease? Recent evidence would suggest that an adaptive T cell response with T cells capable of penetrating tumor stroma and capable of generating significant inflammation would be necessary for an anti-tumor effect.

Th1 Immunity and Cancer

There is increasing evidence that Th1 adaptive immunity is required for cancer inhibition. Several studies evaluating gene expression profiles in a variety of primary human tumors have demonstrated an "immune response" signature that is associated with improved outcome. Galon and colleagues identified a 7 gene classifier that predicted disease recurrence in colon cancer. Upregulation of genes associated with Th1 adaptive immunity predicted a better outcome (5). Data generated by the same group suggested that a high density of tumor infiltrating memory T cells was also associated with a survival benefit. Although these studies were performed evaluating primary tumors from colon cancer patients, upregulation of interferon related genes and tumor infiltrating T cells have been shown to be associated with a clinical benefit in patients with lung cancer, breast cancer, and ovarian cancer, just to name a few disease states (6-8).

$CD4^+$ Th cells can be a source of IFN-γ which results in activation of immune cells in the tumor microenvironment. Indeed, there has been increasing understanding of the role of $CD4^+$ Th cells in orchestrating the tumor specific immune response. Tumor antigen-specific $CD4^+$ Th1 cells can home to tumor and secrete inflammatory cytokines, modulating the microenvironment to enhance the function of antigen presenting cells (APC) (9). The generation of tumor-specific immunity occurs indirectly via "cross priming", thus, APC activation is critical for the generation of an effective response. Increased processing of endogenous tumor cells results in "epitope spreading:" the development of immunity to multiple immunogenic proteins expressed in the tumor which could counter the development of antigen negative variants (10). Finally, by providing a robust $CD4^+$ Th1 T cell response, tumor antigen-specific $CD8^+$ T cells will be elicited endogenously and the immune response generated will be long lived. It has been shown immunizing cancer patients with vaccine constructs designed to stimulate only $CD8^+$ T cells will generate short lived immune responses (11).

Generating Th1 Immunity

An initial step in contemplating strategies to elicit Th1 immunity is to define appropriate epitopes derived from tumor antigens to target, i.e. class II peptides. We evaluated a number of putative class II peptides derived from the HER-2/neu oncogenic protein for immunogenicity both in vitro as well as in a large Phase I clinical trial (12). These peptides were chosen, constructed, and tested based on a single peptide prediction algorithm, T-sites (13). After the peptides evaluated demonstrated both in vitro and in vivo immunogenicity, we performed in vitro HLA-DR competitive binding studies to determine the affinity of the peptides in binding

class II across multiple DR alleles (14). These studies demonstrated that those peptides that bound with high affinity across multiple class II alleles were most likely to be native epitopes of HER-2/neu, that is, those that elicited peptide specific T cells that could recognize native protein presented endogenously by autologous APC. These data suggest high affinity binding peptides, promiscuous across multiple HLA-DR types, were most likely to be the tumor antigen associated epitopes that would be clinically useful.

Over the last several years numerous epitope prediction algorithms have been developed and made widely available on the internet (15). The availability of such programs has now made it possible to assess predicted binding affinity of peptides derived from proteins against multiple class II alleles across multiple algorithms. It had been shown that peptides predicted to be high binders of class I, in more than 1 algorithm, were likely to be true class I epitopes, so we hypothesized that this observation might be the same for class II alleles as well (15,16). Using the self tumor antigen IGFBP-2 as a model and assessing predicted binding affinity, we have been able to create immunologic "heat maps" of class II binding "hot spots" based on affinity and promiscuity. Peptides derived from "hot spots" have been shown to elicit both peptide and protein specific T cells in vitro (15). In silico modeling of binding affinity has allowed rapid identification of peptides that would be suitable to use in vaccines directed against preventing cancer relapse.

Once class II epitopes have been identified which are suitable for vaccine formulations, the next step is to present the peptides in such a way that Th1 are preferentially elicited. One method would be to elicit antigen presentation via dendritic cells (DC) that were of a DC1 phenotype. Although DC are only rarely found circulating in the peripheral blood, the cells are found in large numbers in the skin as Langerhans cells or dermal dendritic cells. Several adjuvants have been shown to induce type 1 responses when given intradermally or transcutaneously including GM-CSF and toll like receptor agonists (17,18). The type of vaccine adjuvant used to initiate the immune response can skew a T cell phenotype to Th1 or Th2 (19).

Antigen specific $CD4^+$ Th1 are ideally suited for initiating and sustaining a tumor antigen specific immune response. However, their ability to locally secrete high levels of type I cytokines in the tumor bed, thus, potentially reversing local immune suppression, also results in generating a broader immune response. Activation of APC in the tumor bed by Th1 enhances cross priming and epitope spreading. Epitope spreading has been well defined in autoimmune disease and is a mechanism which may enhance tissue destruction. The development of epitope spreading after active immunization with a self antigen cancer vaccine has been associated with improved survival (20). We evaluated 52 patients immunized on our initial HER-2/neu Th peptide based vaccine studies for disease outcome (12). The patients were advanced stage with HER-2/neu overexpressing cancers (breast, ovarian, and lung cancer). The median follow-up time was 112 months for surviving patients. In multivariate analysis, the number of chemotherapy regimens prior to vaccination (HR=5.7 (CI 95%, 1.5～23; $p \leq 0.001$)), and the development of epitope spreading after HER-2/neu vaccination (HR=0.34 (CI 95%, 0.12～1.0; p=0.05)) were independent predictors of overall survival. Median overall survival for subjects (n=33) who developed epitope spreading was 84 months vs. 25 months for 16 subjects who did not develop epitope spreading (21). Thus, epitope spreading which may be uniquely associated with the generation of a pro-inflammatory Th response may be a candidate for an immunologic surrogate of clinical response and should be further explored as a desired outcome of active immunization.

Th1-based Immunotherapeutics in Breast Cancer

It is possible to drive Th1 polarization of the tumor specific T cell response for potential clinical benefit. Differentiation into a Th1 phenotype from an undifferentiated state can be achieved by manipulating the cytokine environment at the initiation of the immune response via vaccine adjuvants. Alternatively, one can isolate tumor antigen specific T cells and impact their phenotype by varying the in vitro conditions used for T cell propagation and culture (19). Our group has exploited both these methods, in vivo and in vitro manipulation of Th1 tumor specific T cells, for the immunomodulation of breast cancer.

We have recently completed a clinical trial of immunizing advanced stage breast cancer patients with Th peptides derived from the HER-2/neu oncogenic protein. Twenty-two patients with HER-2/neu overexpressing Stage IV breast cancer were vaccinated with 3 Th peptides admixed in GM-CSF and administered intradermally (22). Previous studies from our group have demonstrated that GM-CSF, as a vaccine adjuvant, activates local skin derived APC and results in elevated levels of circulating IFN-γ secreting antigen specific T cells (17,23). Patients enrolled on this study were vaccinated while concurrently receiving trastuzumab. Approximately half the patients began vaccination in a complete remission of their disease while the other half were immunized in a stable disease state. Data from our group has suggested that pre-existent immunity to HER-2/neu is only found in the minority of HER-2/neu positive patients (～10%), however, these studies were performed prior to the widespread use of trastuzumab (24). In this current trial we found over 50% of patients had evidence of a pre-existent immune response to HER-2/neu prior to starting vaccination. This observation suggests that trastuzumab may, in itself, stimulate a HER-2/neu specific immune response via antibody dependent cell mediated cytotoxicity (25). Despite the presence of pre-existing HER-2/neu specific T cell immunity, over 70% of patients augmented immunity to the HER-2/neu protein achieving levels of antigen specific IFN-γ secreting cells as high as 1 in 250 peripheral blood mononuclear cells (PBMC).

The vaccinated patients' PBMC were also evaluated for intramolecular epitope spreading by assessing responses directed

against HER-2/neu peptides not included in the vaccine formula yet shown to be native epitopes of the protein (14). The majority of patients demonstrated evidence of new intramolecular epitope spreading during active immunization. Moreover, peripheral blood from these patients was interrogated against other common breast cancer antigens and evidence of induced immunity directed against these proteins was also identified. Thus, immunization elicited intermolecular epitope spreading; a broadening of the tumor specific immune response beyond just the HER-2/neu protein (22). Concurrent with generating multiple tumor specific T cell populations secreting type I cytokines, we assessed the impact of immunity on serum TGF-β. Patients with advanced stage breast cancer have been shown to have elevated levels of serum TGF-β which is known to be an immune suppressive agent. The greater the magnitude of IFN-γ secreting T cells associated with epitope spreading generated with vaccination, the greater the decrease in the patient's serum TGF-β level. Although some cancer vaccines have been associated with the induction of $CD4^+CD25^+FOXP3^+$ Treg, the median peripheral blood Treg level decreased during the course of the study (26).

Patients were followed for clinical outcome a median of 36 months after the start of immunization. The median overall survival has not been reached in the study population and a third of patients have not yet experienced disease progression. Several immune parameters were assessed to ascertain whether a survival benefit could be predicted by a vaccine elicited immune response. Only the magnitude of the induced IFN-γ HER-2/neu specific immune response and the magnitude of IFN-γ secreting cells associated with intramolecular epitope spreading were correlated with survival. This study would suggest that the generation of robust levels of Th1 tumor antigen specific T cells would have a beneficial impact on the tumor microenvironment, stimulating endogenous immunity, and potentially resulting in improved clinical outcome.

A more direct evaluation of the clinical efficacy of tumor antigen specific Th1 cells is the direct infusion of these cells into tumor bearing patients and the observation of tumor regression, i.e. adoptive T cell therapy. We have completed the first arm of a study of adoptive T cell therapy of HER-2/neu specific Th1 in patients with refractory metastatic HER-2/neu positive cancers (27). Previous work by our group has shown that tumor antigen specific T cells are much more readily expanded from patients who have been primed with vaccination than from naïve donors (28). Indeed, the maximum level of immunity achieved with active immunization predicted the ability to propagate tumor specific T cells ex vivo. Moreover, we identified that expanding HER-2/neu specific T cells ex vivo with the cytokine combination of IL-2 and IL-12 resulted in a greater magnitude of IFN-γ secreting antigen specific T cells of higher avidity than those expanded in IL-2 alone (29). Our phase I dose escalation study involves collecting and expanding HER-2/neu specific T cells from patients after vaccination, pre-treating patients with Cytoxan 48 hours prior to T cell infusion, and administering increasing doses of HER-2/neu specific T cells weekly for three doses (27). T cells were readily expanded on the first 5 patients enrolled on study. The mean fold expansion from baseline was 7.1 (range 0.2～20 fold). In addition, the number of HER-2/neu specific precursors infused was markedly increased from, in some cases non-detectable, to levels as high as 1 in 130 $CD3^+$ cells. The tumor specific T cells continued to augment *in vivo* in 3 of the 5 patients and could be detected at high levels *in vivo* as long as 220 days after infusion. There was a 60% response rate (2 partial responses and one stabilization of disease) similar to the response rate reported after adoptive T cell therapy in patients with metastatic melanoma (30). Of note, T cell receptor (TCR) gene usage analysis demonstrated the development of multiple clonal populations evolving over time in some of the patients treated.

We questioned what immunologic parameters predicted those patients who achieved a clinical response after vaccination compared with those patients whose disease did not respond. The magnitude of persistent HER-2/neu specific Th1 cells and the number of clonal TCR populations developing in patients after infusion were associated with tumor regression or disease stabilization. These data suggest that tumor specific Th1 cells may be a clinically beneficial method of immunomodulation in patients with HER-2/neu overexpressing breast cancer.

Conclusions

Breast cancer is an immunogenic tumor and multiple immunotherapeutic strategies are being tested as new clinical modalities which may improve disease outcome. It has been shown that eliciting an immune response similar to what is observed in tissue rejection may indicate a beneficial outcome in cancer patients (5). $CD4^+$ Th1 cells are uniquely capable of creating such a rejection signal. In secreting high levels of type 1 cytokines these cells not only support the expansion of antigen specific cytotoxic T cells but also have the capability of directly modulating the tumor microenvironment to generate epitope spreading which would lead to tissue destruction. Clinical strategies focused on eliciting tumor antigen specific Th1 are showing promise in terms of benefiting clinical outcomes in patients with cancer.

Acknowledgement

We would like to thank Ms. Molly Boettcher for assistance in manuscript preparation.

References

1. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
2. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005;5:296-306.
3. AUA. American Urologic Association Annual Meeting April, 2009.
4. Schuster S, Neelapu S, Gause B. Idiotype vaccine therapy (BiovaxID) in follicular lymphoma in first complete remission: phase III clinical trial results. J Clin Oncol. 2009;27(Suppl 18):abstr 2.
5. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960-4.
6. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, et al. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res. 2007;9:R65.
7. Roepman P, Jassem J, Smit EF, Muley T, Niklinski J, van de Velde T, et al. An immune response enriched 72-gene prognostic profile for early-stage non-small-cell lung cancer. Clin Cancer Res. 2009;15:284-90.
8. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203-13.
9. Cohen PA, Peng L, Plautz GE, Kim JA, Weng DE, Shu S. $CD4^+$ T cells in adoptive immunotherapy and the indirect mechanism of tumor rejection. Crit Rev Immunol. 2000;20:17-56.
10. Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol. 2002;2:85-95.
11. Knutson KL, Schiffman K, Cheever MA, Disis ML. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short-lived peptide-specific immunity. Clin Cancer Res. 2002;8:1014-8.
12. Disis ML, Gooley TA, Rinn K, Davis D, Piepkorn M, Cheever MA, et al. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. J Clin Oncol. 2002;20:2624-32.
13. Disis ML, Grabstein KH, Sleath PR, Cheever MA. Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. Clin Cancer Res. 1999;5:1289-97.
14. Salazar LG, Fikes J, Southwood S, Ishioka G, Knutson KL, Gooley TA, et al. Immunization of cancer patients with HER-2/neu-derived peptides demonstrating high-affinity binding to multiple class II alleles. Clin Cancer Res. 2003;9:5559-65.
15. Park KH, Gad E, Goodell V, Dang Y, Wild T, Higgins D, et al. Insulin-like growth factor-binding protein-2 is a target for the immunomodulation of breast cancer. Cancer Res. 2008;68:8400-9.
16. Lu J, Celis E. Use of two predictive algorithms of the world wide web for the identification of tumor-reactive T-cell epitopes. Cancer Res. 2000;60:5223-7.
17. Disis ML, Bernhard H, Shiota FM, Hand SL, Gralow JR, Huseby ES, et al. Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines. Blood. 1996;88:202-10.
18. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol. 2005;6:769-76.
19. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology. 2008;123:326-38.
20. Butterfield LH, Ribas A, Dissette VB, Amarnani SN, Vu HT, Oseguera D, et al. Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. Clin Cancer Res. 2003;9:998-1008.
21. Salazar LG, Goodell V, O'Meara M. Persistent immunity and survival after immunization with a HER2/neu (HER2) vaccine. J Clin Oncol. 2009;27(Suppl 15):abstr 3010.
22. Disis ML, Wallace D, Gooley TA. Concurrent trastuzumab and HER-2/neu specific vaccination in patients with metastatic breast cancer. J Clin Oncol. 2009;in press.
23. Knutson KL, Schiffman K, Disis ML. Immunization with a HER-2/neu helper peptide vaccine generates HER-2/neu CD8 T-cell immunity in cancer patients. J Clin Invest. 2001;107:477-84.
24. Disis ML, Knutson KL, Schiffman K, Rinn K, McNeel DG. Pre-existent immunity to the HER-2/neu oncogenic protein in patients with HER-2/neu overexpressing breast and ovarian cancer. Breast Cancer Res Treat. 2000;62:245-52.
25. Arnould L, Gelly M, Penault-Llorca F, Benoit L, Bonnetain F, Migeon C, et al. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer. 2006;94:259-67.
26. Nakai N, Katoh N, Kitagawa T, Ueda E, Takenaka H, Kishimoto S. Immunoregulatory T cells in the peripheral blood of melanoma patients treated with melanoma antigen-pulsed mature monocyte-derived dendritic cell vaccination. J Dermatol Sci. 2009;54:31-7.
27. Disis ML, Salazar LG, Coveler A. Phase I study of infusion of HER2/neu (HER2) specific T cells in patients with advanced-stage HER2 overexpressing cancers who have received a HER2 vaccine. J Clin Oncol 2009;27(Suppl 15):abstr 3000.
28. Dang Y, Knutson KL, Goodell V, dela Rosa C, Salazar LG, Higgins D, et al. Tumor antigen-specific T-cell expansion is greatly facilitated by *in vivo* priming. Clin Cancer Res. 2007;13:1883-91.
29. Knutson KL, Disis ML. IL-12 enhances the generation of tumour antigen-specific Th1 CD4 T cells during *ex vivo* expansion. Clin Exp Immunol. 2004;135:322-9.
30. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol. 2005;23:2346-57.