

Generation of T-Cell Immunity to the HER-2/neu Protein After Active Immunization With HER-2/neu Peptide-Based Vaccines

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Purpose: The HER-2/neu protein is a nonmutated tumor antigen that is overexpressed in a variety of human malignancies, including breast and ovarian cancer. Many tumor antigens, such as MAGE and gp100, are self-proteins; therefore, effective vaccine strategies must circumvent tolerance. We hypothesized that immunizing patients with subdominant peptide epitopes derived from HER-2/neu, using an adjuvant known to recruit professional antigen-presenting cells, granulocyte-macrophage colony-stimulating factor, would result in the generation of T-cell immunity specific for the HER-2/neu protein.

Patients and Methods: Sixty-four patients with HER-2/neu-overexpressing breast, ovarian, or non-small-cell lung cancers were enrolled. Vaccines were composed of peptides derived from potential T-helper epitopes of the HER-2/neu protein admixed with granulocyte-macrophage colony-stimulating factor and administered intradermally. Peripheral-blood mononuclear cells were evaluated at baseline, before vaccination, and after vaccination for antigen-specific T-cell immunity.

Immunologic response data are presented on the 38 subjects who completed six vaccinations. Toxicity data are presented on all 64 patients enrolled.

Results: Ninety-two percent of patients developed T-cell immunity to HER-2/neu peptides (stimulation index, 2.1 to 59) and 68% to a HER-2/neu protein domain (stimulation index range, 2 to 31). Epitope spreading was observed in 84% of patients and significantly correlated with the generation of a HER-2/neu protein-specific T-cell immunity ($P = .03$). At 1-year follow-up, immunity to the HER-2/neu protein persisted in 38% of patients.

Conclusion: The majority of patients with HER-2/neu-overexpressing cancers can develop immunity to both HER-2/neu peptides and protein. In addition, the generation of protein-specific immunity, after peptide immunization, was associated with epitope spreading, reflecting the initiation of an endogenous immune response. Finally, immunity can persist after active immunizations have ended.

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A VACCINE SPECIFIC for the HER-2/neu protein may have wide application and utility in the prevention of disease recurrence in many different human malignancies. HER-2/neu is a member of the epidermal growth factor receptor family.^{1,2} In humans, the HER-2/neu protein is expressed during fetal development.³ In adults, the protein is weakly detectable in the epithelial cells of many

normal tissues by immunohistochemical staining. Amplification of the gene and/or overexpression of the associated protein have been identified in many human cancers such as breast, ovarian, prostate, non-small-cell lung, and colon cancer. Overexpression of the HER-2/neu protein has been linked with a poor prognosis and high risk of cancer relapse, particularly in breast and ovarian cancer.⁴ The HER-2/neu oncogenic protein is also a tumor antigen.⁵

Cancer vaccines targeting self-tumor antigens must overcome immunologic tolerance. Tolerance may be directed toward immunodominant epitopes of self-proteins.⁶ In animal model systems, tolerance to self-proteins can be circumvented by targeting the immune response to nonimmunodominant peptide portions of the self-tumor antigen (ie, a subdominant epitope). Peptide-based vaccines using putative T-helper epitopes derived from the natural rat neu protein sequence were effective in generating rat neu-specific immunity in the rat.⁷ Furthermore, the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF) to the peptide vaccination may enhance T-cell immunity generated by stimulating the in vivo mobilization of Langerhans cells, ie, skin dendritic cells to the site of antigen deposition.^{8,9}

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The study presented here describes the clinical translation of this approach: intradermal vaccination of cancer patients, whose tumors overexpress the HER-2/neu protein, with putative CD4⁺ T-helper epitopes derived from the HER-2/neu protein admixed with GM-CSF. The objectives of the study were to (1) determine whether patients with an advanced cancer that overexpressed HER-2/neu could be immunized as assessed by the measurement of T-cell responses to HER-2/neu peptides, (2) determine whether immunization with HER-2/neu peptides would result in the development of HER-2/neu protein-specific immunity, and (3) determine the safety of the vaccine.

PATIENTS AND METHODS

Patients

The University of Washington Human Subjects Division and the United States Food and Drug Administration approved a phase I trial of HER-2/neu peptide vaccines. Patients with stage III or IV breast, ovarian, or non-small-cell lung cancer were eligible for study if the following criteria were met: (1) documentation of HER-2/neu protein overexpression in the primary tumor or metastasis, (2) patients had received prior treatment for their disease so that they had no detectable disease or were stable on hormonal or local radiation therapy, (3) patients had a WBC count \geq 3,500/mL, and (4) patients had been off and were able to remain off chemotherapy or other immune modulatory therapies for a minimum of 30 days before enrollment and for the 7-month duration of the study. All patients gave written informed consent in accordance with institutional and federal regulations. Patients were enrolled onto the study between August 1996 and August 1998. Three vaccine formulations were evaluated, each containing three different peptides derived from HER-2/neu and admixed with 100 μ g of GM-CSF (Immunex Corp, Seattle, WA). Vaccines were administered intradermally once monthly for 6 months to the same regional draining lymph node site.¹⁰ After enrollment, and 48 hours before the first HER-2/neu peptide vaccine, patients received a control immunization with keyhole-limpet hemocyanin (KLH) 100 μ g subcutaneously (Per Immune, Inc, Rockville, MD). The KLH immunization was given only one time at the start of the study. Toxicity was graded according to National Cancer Institute common toxicity criteria scoring defined before August 1998.¹¹

HER-2/neu Peptide-Based Vaccines

All peptides constructed were potential helper epitopes of the HER-2/neu protein predicted by computer modeling and empiric testing to be immunogenic.¹² Peptides derived from the extracellular domain (ECD) and the intracellular domain (ICD) were evaluated separately. The aim of the study was to determine whether patients could be immunized against a self-protein, HER-2/neu, and therefore whether tolerance to self would potentially be a major factor in limiting the generation of immunity.⁶ The ECD of HER-2/neu is known to circulate in a patient's sera, and thus is more likely to be tolerogenic.¹² The final vaccine formulation consisted of helper epitopes that encompassed in their natural sequence HLA-A2-binding motifs. This vaccine was designed to specifically elicit cytotoxic T-lymphocyte responses directed against HER-2/neu. The CD8⁺ responses specific for this vaccine have been previously reported.¹³

The ECD vaccine contained peptides p42 to 56 (p42), p98 to 114 (p98), and p328 to 345 (p328) that are derived from the ECD of the HER-2/neu protein. The ICD vaccine included peptides p776 to 790 (p776), p927 to 941 (p927), and p1166 to 1180 (p1166) derived from the ICD of HER-2/neu. The final vaccine formulation, HLA-A2 vaccine, consisted of peptides p369 to 384 (p369), p688 to 703 (p688), and p971 to 984 (p971). Each of these peptides is contained within the natural amino acid sequence of an HLA-A2-binding motif of HER-2/neu.¹³ The peptide dose in each vaccination was 500 μ g per peptide. Patients were tested for the presence of an HLA-A2 phenotype at the start of the study and received the HLA-A2 vaccine if positive. Non-HLA-A2 patients alternated between receiving the ECD or ICD vaccine in the order in which they were enrolled.

Detection of Peripheral-Blood T-Cell Responses

HER-2/neu-specific T-cell responses were measured 30 days after each vaccination, before the next immunization. T-cell proliferation was assessed using a modified limiting dilution assay designed for detecting low-frequency lymphocyte precursors on the basis of Poisson distribution¹⁴ and as previously described.¹⁰ Results are reported as a standard stimulation index (SI), defined as the mean of all 24 experimental wells divided by the mean of the control wells (no antigen). Phytohemagglutinin incubated with patient T cells at a concentration of 5 μ g/mL was used as a positive control for the ability of T cells to respond to antigen and resulted in an SI more than 2.0 in all assays reported (data not shown). Peripheral-blood mononuclear cells (PBMCs) from 30 female volunteer donors without cancer, age range 32 to 58 years, were evaluated in similar assays to establish baseline values. The mean and three SDs of the T-cell response in the reference population to any of the HER-2/neu antigens tested was a maximum SI of 1.98; therefore an SI more than 2 was considered evidence of an immunized response. If subjects had an SI more than 2 at baseline (ie, pre-existent immunity to HER-2/neu),¹⁵ a postvaccination response was defined as positive if it was a minimum of two times baseline.

Peptide-Specific Delayed-Type Hypersensitivity Response After Immunization

Patients were skin tested against their immunizing peptides (100 μ g) 1 month after their last vaccination.¹⁶ Biopsy specimens were analyzed by immunohistochemical staining to characterize infiltrating cell populations (Alan Gown, MD, Phenopath, Seattle, WA).

Generation of Antigen-Specific T-Cell Lines

Antigen-specific T-cell lines were generated as previously described with the following modifications.¹³ For the expansion of CD4⁺ T-cell lines from patient 0756, peptides p369 to 384, p776 to 790, and p971 to 984 were added to PBMCs in individual flasks at a concentration of 1 μ mol/L. The flasks were incubated at 37°C and 5% CO₂. On day 3 and every other subsequent day, interleukin-2 was added to the media to a final concentration of 5 U/mL. On day 10, in vitro stimulation was performed with peptide-pulsed, irradiated autologous PBMCs. The cultures were further incubated for an additional 10 days with periodic interleukin-2 administration.¹³ After the second in vitro stimulation, the antigen-specific T-cell lines were examined for proliferative activity using tritiated thymidine incorporation as previously described.¹⁷ Lines were tested against peptide antigens (10 μ g/mL; p369, p776, and p1166), and protein antigens (1 μ g/mL; HER-2/neu recombinant domain proteins and ovalbumin as an irrelevant control). Results are expressed as the mean \pm SD of six well replicates.

Statistical Considerations

To assess the correlation of the development of protein-specific immunity and response (taken as the maximum of ECD or ICD), linear regression models were fit, one regression model for each of the nine peptides analyzed. Generalized estimating equations (GEEs) were used to account for the multiple samples per patient. The GEE model appropriately uses the multiple samples per patient to adjust the variance of the estimate of the slope parameter in the regression line. The association of epitope spreading with development of an immune response was assessed with logistic regression, where a GEE model was used to account for the multiple samples per patient. All *P* values from regression models are derived from the Wald test, and no adjustments were made for multiple comparisons. SAS (Version 8; SAS Institute, Inc, Cary, NC) was used for all statistical analyses.

RESULTS

Immunization With HER-2/neu Peptide-Based Vaccines Was Well Tolerated and Did Not Result in the Development of Autoimmunity

Sixty-four patients met eligibility criteria and were enrolled onto the study, and 38 of them completed all six vaccines (ECD, *n* = 13; ICD, *n* = 11; HLA-A2, *n* = 14). Among these 38 patients, the median age was 50 years (range, 33 to 85 years); 31 had breast cancer, five had ovarian cancer, and two had non-small-cell lung cancer. The median time from last chemotherapy in this group was 5 months (range, 1 to 75 months). Twenty-six patients did not complete the study, 23 because of progressive disease requiring treatment, one because of travel inconvenience, one because of skin rash secondary to the vaccine, and one because of treatment dose corticosteroids required for control of chronic asthma. Immune response data presented here detail the 38 patients who completed all six scheduled immunizations. Toxicity data are reported on all 64 patients.

There were no grade 3 or 4 toxicities observed on the study. One subject developed grade 1 and six subjects developed grade 2 skin toxicity characterized by a scattered erythematous and/or pruritic rash. One patient's grade 2 rash occurred at the time of her last immunization and she was treated with a short course of oral prednisone, and one patient withdrew from the study after four vaccinations because the reoccurrence of the rash was bothersome, although it did not require treatment. One subject developed urticaria at the time of skin testing 30 days after her last immunization, recall urticaria at her vaccine site, and subsequent recall delayed-type hypersensitivity (DTH).¹⁸ Two patients described grade 1 myalgias. No patient developed any detectable evidence of autoimmune toxicity, particularly in organs known to express basal levels of HER-2/neu protein such as the liver, digestive tract, and skin.³

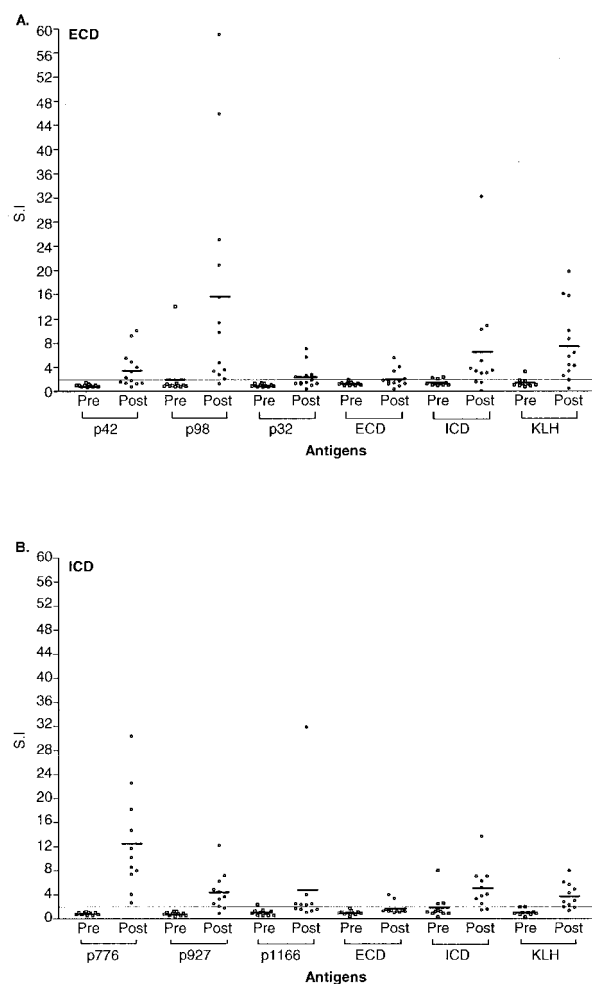


Fig 1. Peptide and protein T-cell immunity elicited after active immunization is similar in magnitude to KLH response. (A) ECD peptide responses. (B) ICD peptide responses. Responses to ECD, ICD, and KLH proteins are shown. Each symbol represents an individual patient. Bold bars indicate the mean.

HER-2/neu Peptide and Protein T-Cell Immunity, Elicited After Active Immunization, Is Similar in Magnitude to the KLH Response After Active Immunization

Figure 1 shows the preimmunization and postimmunization T-cell responses of the 38 patients who completed six vaccines. The range of responses was heterogeneous. Figure 1A depicts results from the 13 patients who received the ECD vaccine. Seven of 13 patients developed T-cell immunity to p42 (mean SI, 3.6; range, 0.7 to 10), 10 of 13 patients developed T-cell immunity to p98 (mean SI, 15.7; range, 1.2 to 59), and five of 13 patients developed an immune response to p328 (mean SI, 2.3; range, 0.3 to 7). Eight (62%) of 13 patients developed T-cell immunity to one of

the HER-2/neu protein domains, three to the ECD (mean SI, 2; range, 0.3 to 5.5) and eight to the ICD (mean SI, 6.4; range, 0 to 32.1). Nearly all patients, 11 of 13, responded to KLH after active immunization (mean SI, 7.6; range, 0.4 to 19.7). Figure 1B shows preimmunization and postimmunization results from the 11 patients who received the ICD vaccine. Eleven of 11 patients developed T-cell immunity to p776 (mean SI, 12.5; range, 2.6 to 30.3), eight of 11 patients developed T-cell immunity to p927 (mean SI, 4.4; range, 0.8 to 12.1), and six of 11 patients developed an immune response to p1166 (mean SI, 4.7; range, 1 to 31.8). Nine (82%) of 11 patients developed T-cell immunity to one of the HER-2/neu protein domains, two to the ECD (mean SI, 1.6; range, 0.9 to 3.9), and seven to the ICD (mean SI, 5.0; range, 1.4 to 13.6). Eight of 11 patients in this group responded to KLH after active immunization (mean SI, 3.8; range, 1.3 to 7.9). Results for patients receiving the HLA-A2 vaccine have been previously reported in detail.¹³ Thirteen of 14 patients developed T-cell immunity to at least one of the immunizing peptides (range SI, 1.2 to 35.6). Nine (64%) of 14 patients developed T-cell immunity to one of the HER-2/neu protein domains (range SI, 1.0 to 18.2). Nine of 14 patients in this group responded to KLH after active immunization (mean SI, 4.1; range, 1.2 to 13.0).

The Majority of Patients Immunized With HER-2/neu Peptide-Based Vaccines Develop Both HER-2/neu Peptide- and Protein-Specific T-Cell Immune Responses

The majority of patients could be immunized to at least one of the HER-2/neu peptides (Fig 2). Overall, 92% of those who completed all six vaccines developed HER-2/neu peptide-specific T-cell immunity to at least one peptide in their immunizing mix. Only a few patients had pre-existent immune responses to some of the predicted T-cell epitopes, two patients to p369 (SI, 2.0 and 2.4), and two patients to p688 (SI, 2.1 and 2.5). The overall immune competence of patients was corroborated by their ability to be immunized to the foreign antigen KLH. The majority of patients, 28 (74%) of 38, developed T-cell immunity to KLH during the 6-month period of the study.

The majority of patients developed HER-2/neu protein-specific immunity after peptide immunization. Twenty-six percent of patients (n = 10) who completed all six vaccines developed T-cell immunity to the HER-2/neu ECD protein, and 63% (n = 24) developed T-cell immunity to the ICD protein. Twenty-one percent (n = 8) developed detectable immune responses to both proteins. None of the patients had significant pre-existent immune responses to the ECD (range SI, 0.3 to 1.6); however, five responded to the ICD before immunization (range SI, 2.0 to 7.9).

There was no vaccine formulation that was more associated with the development of HER-2/neu protein-specific T-cell immunity than another. In addition, there was no individual peptide, in any of the three formulations, where the development of a T-cell response to that peptide was associated with the development of a significant HER-2/neu protein-specific T-cell immune response.

Epitope Spreading Occurs With Active Immunizations and Correlates to the Development of an HER-2/neu Protein-Specific Immune Response

The majority of patients who completed all six immunizations (84%) developed epitope spreading or determinant spreading.^{10,19} As an example, Fig 3A demonstrates HER-2/neu peptide-specific immune responses before and after completion of all six immunizations in one patient (patient no. 0756) with stage IV breast cancer and a supraclavicular lymph node as the sole metastatic site. The patient received a vaccine consisting of peptides p369, p688, and p971 and developed a T-cell response to p369 but not the other peptides in her immunizing mix (Fig 3A). However, she also developed immunity to a peptide with which she was not immunized, p776. Moreover, she developed immunity to both the HER-2/neu ECD and ICD proteins. Short-term CD4⁺ peptide-specific T-cell lines generated on this patient demonstrated that both the immunizing peptide, p369, and the nonimmunizing peptide, p776, could elicit T cells that responded in vitro to the appropriate HER-2/neu recombinant domain protein, ECD and ICD, respectively (Fig 3B). No antigen-specific T cells could be generated to p971, an immunizing peptide to which she did not respond. Figure 4 demonstrates the percentage of patients who finished all six immunizations and who developed T-cell immunity against a peptide in their immunizing mix as well as the percentage of patients who developed epitope spreading to a particular peptide. Twenty percent of patients who received the ICD and HLA-A2 vaccines developed immunity to the ECD peptide p42 (range SI, 0.2 to 7.5), 60% to p98 (range SI, 0.2 to 18.8), and 36% to p328 (range SI, 0 to 8.7). Fifty-six percent of patients who received the ECD and HLA-A2 vaccines developed immunity to the ICD peptide p776 (range SI, 0.2 to 29.6), 73% to p927 (range SI, 0.3 to 24.3), and 33% to p1166 (range SI, 0.2 to 6.9). Finally, 54% of patients who received the ECD or ICD vaccine developed immunity to the helper peptides, which encompassed HLA-A2 epitopes, p369 (range SI, 0.2 to 6.7), 13% to p688 (range SI, 0 to 3.6), and 29% to p971 (range SI, 0 to 10.3).

Epitope spreading was significantly associated with the development of an HER-2/neu protein-specific immune response in patients who finished all six immunizations (n

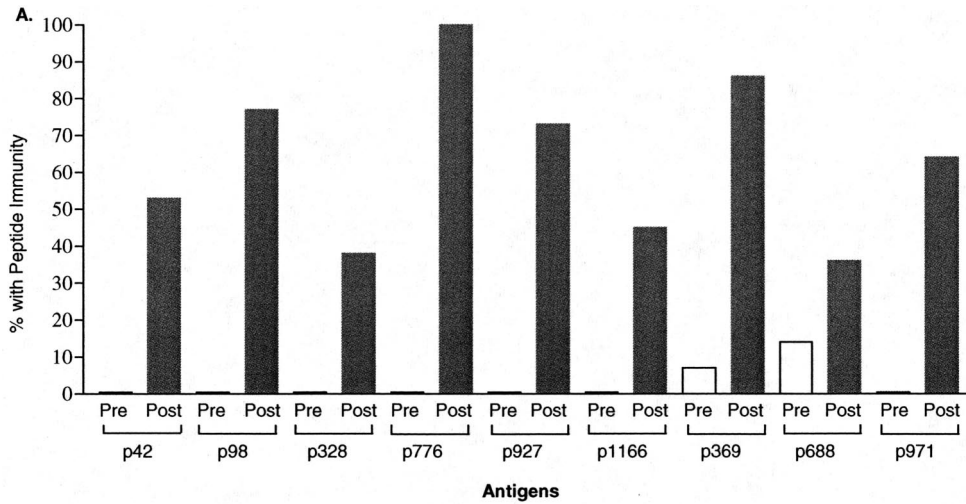
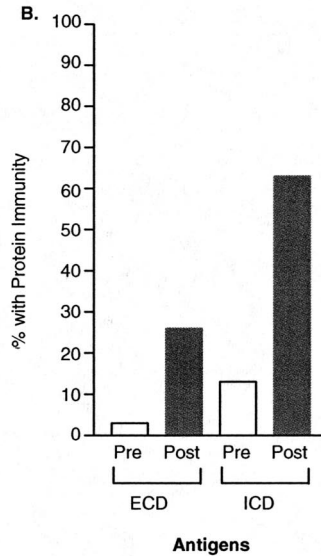


Fig 2. The majority of patients immunized develop HER-2/neu peptide- and protein-specific T-cell responses. Bars show the percentage of subjects who demonstrated responses before (□) and after (■) immunization. Responses to the ECD and ICD domains of HER-2/neu protein are also shown.



= 38) ($P = .03$) as well as in all patients enrolled ($n = 64$) ($P = .004$).

T Cells Could Traffic to the Site of Antigen

Twenty-four of 27 patients who underwent skin testing before and after six vaccinations had new DTH responses more than 5 mm at 48 hours to peptide skin tests placed on a site distant from the vaccine site. Biopsy specimens of positive DTH sites revealed a predominant $CD3^+$, $CD4^+$ lymphocytic infiltrate with significant influx of $CD1a^+$ cells above what is seen in normal skin. One example is shown in Fig 5.

Elicited T-Cell Immunity Persisted in Some Patients After Active Immunization Ended

Thirteen subjects were followed a median of 12 months after their last vaccination. At 1 year, immunity was detected to peptides and to HER-2/neu domain proteins in 38% (Fig 6A) and 38% (Fig 6B) of patients, respectively.

DISCUSSION

The current investigation focused on immunizing cancer patients against putative $CD4^+$ T-helper epitopes derived from the HER-2/neu oncogenic protein,¹² a well-defined tumor antigen in many adenocarcinomas. Data presented

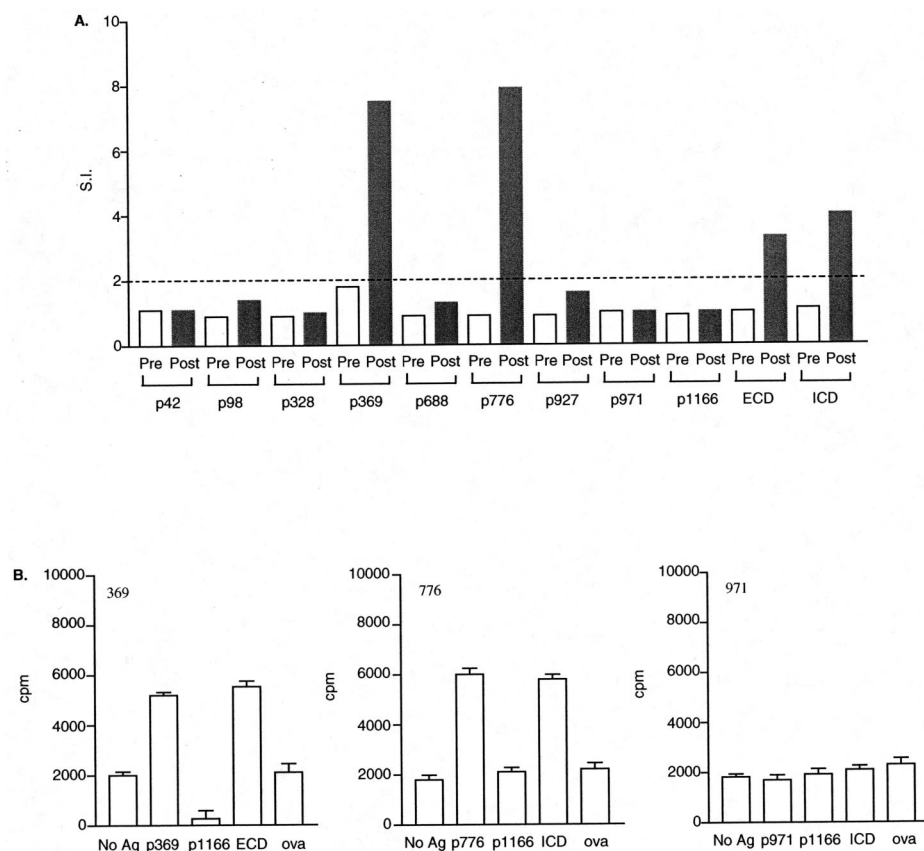


Fig 3. Immunity can develop to epitopes not in the patient's vaccine: (A) before (□) and after (■) immunization; (B) T-cell lines generated to peptides p369, p776, and p971 and tested against no antigen, cultured peptide, irrelevant peptide, HER-2/neu, and ovalbumin (mean \pm SD for six replicates).

demonstrate that (1) the majority of cancer patients who completed all scheduled vaccines developed both HER-2/neu peptide- and protein-specific T-cell responses, (2) epitope spreading was associated with the generation of an HER-2/neu protein-specific immune response after peptide immunization, and (3) both peptide- and protein-specific T-cell immunity persisted after active immunization had ended.

The majority of patients immunized with HER-2/neu peptide-based vaccines developed both HER-2/neu peptide- and protein-specific T-cell immunity. Generation of peptide-specific immunity after peptide immunization is a reflection of a patient's immune competence. Indeed, the patients eligible for this study were those whose disease was treated to a maximal response and who were stable on adjunct therapies, such as tamoxifen, or patients without evidence of disease. The high rate of successful peptide immunization may directly reflect the low tumor burden and excellent functional status of these patients. The choice of this population was intentional and determined on the basis of the premise that, much like an infectious disease vaccine, cancer vaccines will have their greatest utility in preventing disease or its recurrence. Moreover, we presume that the in

vitro surrogate of a potential in vivo response to tumor would be the generation of T cells that could recognize the HER-2/neu protein domains. Measurable protein-specific T-cell responses in vitro require antigen processing and presentation by antigen-presenting cells (APCs) present within the patient's PBMCs. The T-cell responses directed against the HER-2/neu protein domains were of a magnitude similar to immunity elicited to the positive control antigen KLH. In addition, although HER-2/neu is a self-protein and weakly detectable on some normal tissues, no patient developed evidence of autoimmune disease. This finding mirrors studies performed in rodent models⁷ and, theoretically, may reflect a difference in the peptide repertoire presented when malignant cells overexpress the protein as compared with epitopes presented in the major histocompatibility complex (MHC) when the protein is present at reduced levels.^{6,20,21}

Epitope spreading occurred with active immunization in the majority of patients. In addition, the identification of epitope spreading itself correlated to the development of HER-2/neu protein-specific T-cell immunity. Epitope, or determinant spreading, is a phenomenon first described in

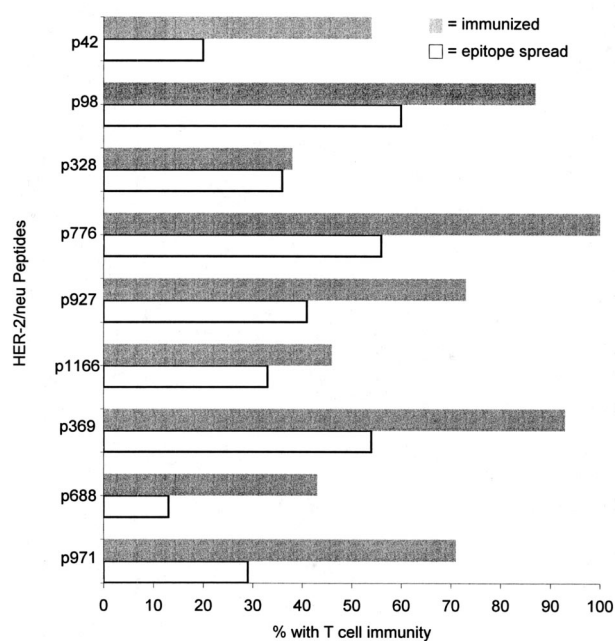


Fig 4. Epitope spreading occurs with active immunizations. Data are shown as the percentage of patients with T-cell immunity against peptides in their immunizing mix (□) or peptides that were indicative of epitope spreading (ie, not in their immunizing mix) (■).

autoimmune disease¹⁹ and has been associated with both MHC class I- and MHC class II-restricted responses.^{22,23} The phenomenon represents the generation of an immune response to a particular portion of an immunogenic protein and then the natural spread of that immunity to other areas of the protein. In terms of our study, epitope spreading reflected the extension of a significant T-cell immune response to portions of the HER-2/neu protein that were not contained in the patient's vaccine. How does epitope spreading develop? Theoretically, a broadening of the immune response may represent endogenous processing of antigen at sites of inflammation initiated by a specific T-cell

response or "driver clone."^{23,24} That is, the initial immune response can create a microenvironment at the site of the tumor that enhances endogenous immune effector cells present locally. These immune cells (eg, APCs and T cells) may begin to respond more effectively to tumor antigen that is present in the body.

The observation that the T cells from patient no. 0756 (Fig 3) (specific for a peptide [p776] not in her immunization mix and elicited as a consequence of vaccination) respond to recombinant HER-2/neu protein suggests that peptide p776 represents a native epitope of HER-2/neu. DTH histology from sites where peptides were administered as recall skin tests demonstrate that HER-2/neu antigen-specific T-cells elicited after active immunization can traffic to the site of antigen deposition and set up an inflammatory response. Presumably, if this inflammation occurred at sites of tumor, APCs would take up tissue debris and present a variety of tumor-associated antigens to infiltrating T cells.²³ The development of epitope spreading may be due to a more effective presentation of subdominant epitopes facilitated by the use of GM-CSF as a vaccine adjuvant. Local GM-CSF deposition results in the preferential recruitment of skin Langerhans cells as APCs (Fig 5).^{8,9} Investigations in murine models have suggested that dendritic cells may be more effective in presenting epitopes from antigens not recognized because of poor display by other nonprofessional APCs.^{25,26} We demonstrate intramolecular epitope spreading within the HER-2/neu protein; a critical determination would be to discern whether intermolecular epitope spreading occurs between other tumor antigens expressed in the patient's cancer. As additional antigens implicated in breast, ovarian, and non-small-cell lung cancer become defined, this issue can be effectively addressed. Epitope spreading diversifies the immune response beyond what was expected and represents the development of immunity to naturally processed antigen. Perhaps the more appropriate biologic marker for monitoring a potentially therapeutic anticancer response will be the demonstration of epitope

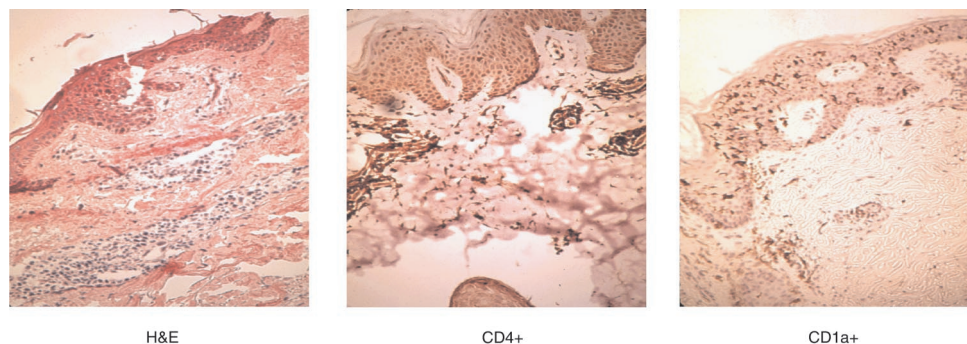


Fig 5. T cells could traffic to the site of antigen. Patient no. 7062 received the ICD vaccine and developed DTH responses of 11 mm² to peptide p776. Illustrated are hematoxylin and eosin staining ($\times 10$), CD4⁺ staining ($\times 10$), and CD1a⁺ staining ($\times 10$).

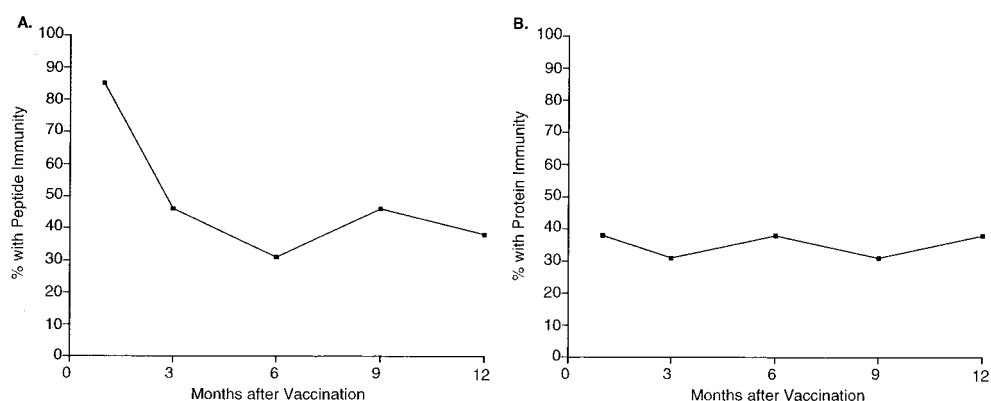


Fig 6. Elicited T-cell immunity persisted in some patients after active immunization had ended. Percentage of 13 patients followed long-term for persistent HER-2/neu peptide-specific T-cell immunity (A) and HER-2/neu protein-specific T-cell immunity (B) after active immunizations (median of 12 months after the last vaccination).

spreading rather than solely measuring the magnitude of the T-cell response that occurs with active immunization.

A major goal of vaccination is the development of effective immunologic memory. Few clinical trials have monitored a substantial number of immunized patients for persistent immunity. A recent study of 105AD7, a human anti-idiotype antibody that mimics the CD55 antigen expressed in colorectal cancer, demonstrated only one of 14 patients followed after active immunization showed a sustained antigen-specific T-cell response.²⁷ In the present study, HER-2/neu-specific T-cell responses often persisted after active immunization had ended. In addition, those patients immunized with the vaccine encoding HLA-A2 epitopes within the natural longer HER-2/neu sequence developed and sustained CD8⁺ peptide-specific precursor frequencies at the level of those measured to viral antigens such as cytomegalovirus and influenza.¹³ Of note, of the 64 patients enrolled onto this study, only 38 were able to complete all six immunizations. It is unknown how many vaccinations are needed to result in the development of immunologic memory against a self-antigen such as HER-2/neu. Our hypothesis was that multiple immunizations would be needed to stimulate a T-cell immune response against a weak tumor antigen as compared with a foreign infectious disease antigen such as hepatitis B, where three vaccinations result in protective immunity in the majority of subjects.²⁸ Our strategy was to double the number of vaccinations to HER-2/neu as compared with a hepatitis B vaccine. Current studies are ongoing with other HER-2/neu-specific vaccine formulations to determine whether effective immunity can be elicited with fewer immunizations. The immunogenicity of the antigen and the method of

vaccine delivery can impact greatly on the number of vaccines that must be given to achieve a measurable and sustained immune response.^{29,30}

Data presented here demonstrate patients with advanced-stage cancer, but minimal disease can be immunized against a self-tumor antigen, HER-2/neu. Whether the development of an HER-2/neu-specific T-cell response results in clinical benefit is currently unknown. A phase II study designed to assess clinical outcomes such as an assessment of time to progression after vaccination and the development of HER-2/neu-specific immunity is under consideration. We hypothesize that the greatest utility of cancer vaccines will lie in their ability to elicit a long-lived protective immune response to prevent cancer relapse or even the initiation of cancer. The first step in vaccine development is to identify a strategy that will result in successful antigen-specific immunity in the majority of vaccinated patients and the generation of an immune response that will persist after active immunizations have ended. The second step is to determine whether a measurable immune response directed against a target antigen will correlate with protection against cancer relapse. Finally, the broad application of a particular cancer vaccine to a specific patient population will depend on the prevalence of the expression of a particular tumor antigen or group of antigens and the immune competence of a patient population (ie, the ability to be immunized). In this study, the finding of epitope spreading occurring in the majority of patients and correlating with the generation of an HER-2/neu protein domain response suggests immunization was effective in stimulating natural processing and presentation of antigen *in vivo*.

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