

Antigen-specific recall urticaria to a peptide-based vaccine

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Recall urticaria (RU) is a localized urticarial response that occurs at a site of previous antigen injection on reexposure to that antigen at a remote site.¹ The response is rare and typically associated with allergy immune therapies. The mechanism for RU is poorly understood, but possibilities include increased accumulation and degranulation of mast cells at the site of previous allergy injections.¹ RU has not been described in the setting of peptide-based vaccines.

In recent years several immunogenic proteins associated with human malignancies have been identified, allowing the development of antigen-specific cancer vaccines.² Our group has focused on the HER-2/neu oncoprotein as a tumor antigen. HER-2/neu is a cancer-related protein that is overexpressed in 30% of breast and ovarian cancers. Studies in animal models have demonstrated that HER-2/neu peptide-based vaccines, mixed with GM-CSF as an adjuvant, effectively generate HER-2/neu-specific immunity.³

A phase-I trial of HER-2/neu peptide-based vaccines was approved by both the University of Washington Human Subjects Division and the United States Food and

Abbreviations used

DTH: Delayed-type hypersensitivity

RU: Recall urticaria

Drug Administration. Patients with stable stage III or IV breast, ovarian, or non-small cell lung cancers were vaccinated monthly for 6 months. GM-CSF was used as a vaccine adjuvant. This case report describes 1 patient in whom RU developed 30 days after the last vaccination.

CASE REPORT

A 48-year-old woman with a history of stage IV HER-2/neu-overexpressing breast cancer, which was in complete remission, received intradermal synthetic peptide immunizations (Multiple Peptide Systems, San Diego, Calif) composed of 3 peptides derived from HER-2/neu extracellular domain: 500 µg each of p42-56, p98-114, and p328-345, with 125 µg of GM-CSF. The peptides were synthesized by using t-boc chemistry, and amino acid sequences were greater than 95% correct as determined by both electrospray mass spectrometry and HPLC analysis. Vaccinations were given intradermally at the same site in the left deltoid at each visit. Lymphocytes and serum were obtained monthly for determination of HER-2/neu-specific immune response. Delayed-type hypersensitivity (DTH) skin testing to the immunizing peptides was performed before and after 6 immunizations.

In a prevaccination evaluation, neither lymphocyte proliferation nor a DTH response was detectable to HER-2/neu peptides. By the third immunization, a lymphocyte proliferative response to p98-114, as measured by a stimulation index of 3.2, was observed.⁴ This correlated to a local DTH (22 × 24-mm induration) at the immunizing site that developed 24 hours after inoculation and lasted for 2 to 3 days.

Within 5 minutes of her sixth and final vaccination, the patient experienced pruritis in her hands and feet, urticarial plaques at the

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A. DTH and Stimulation Index (S.I.)

Antigen Tested	Induration in mm at 48 hours	S.I.
P42-56	15x12	6.0
P98-114	12x12	18.2
P328-345	8x10	3.5
P1166-1180	0	0.7
Sterile Water	0	NA
GM-CSF	0	0.9
Prior vaccination site (Recall DTH)	25x10	NA

NA: not applicable

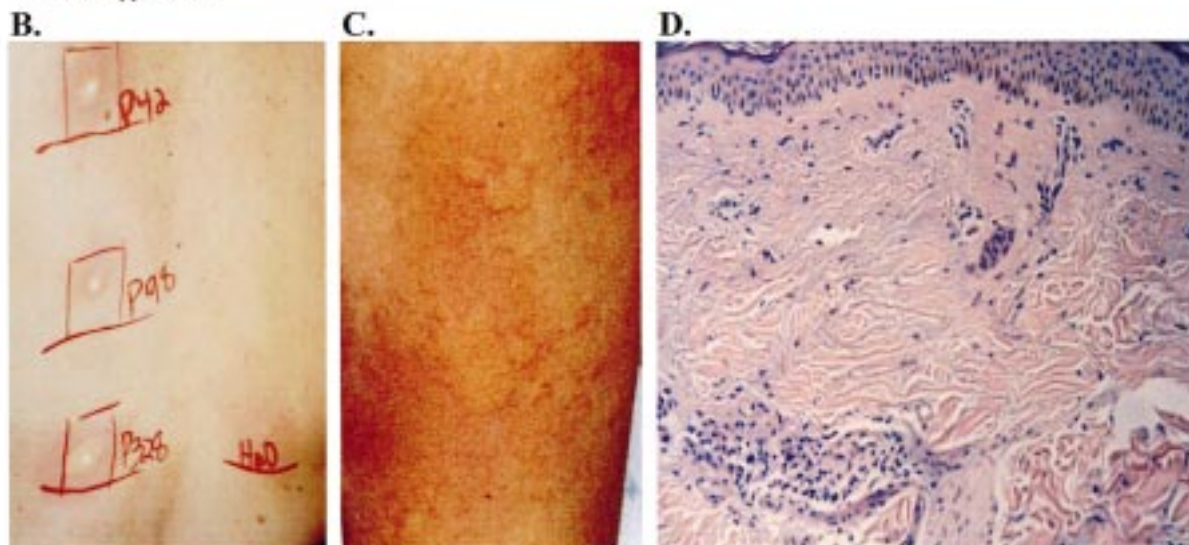


FIG 1. RU and recall DTH. **A**, DTH to peptides and controls were measured as induration in millimeters at 48 hours. HER-2/neu peptide-specific T-cell proliferation was measured by tritiated thymidine incorporation in 24-well replicates. PBMCs (2×10^5 /well) were incubated with 25 μ g/mL of the various HER-2/neu peptides. After 5 days, wells were pulsed with 1 μ Ci of tritiated thymidine for 8 to 10 hours and counted. Responses were quantified as a standard stimulation index (S.I.), which is defined as the mean of all 24 experimental wells divided by the mean of the control wells (no antigen). **B**, Urticarial reactions to each peptide 5 minutes after placement. No urticarial response appeared with sterile water, GM-CSF (not shown), or an irrelevant peptide (not shown). **C**, RU developing at the vaccine site on the left deltoid immediately after peptide skin tests were performed on the back. **D**, Hematoxylin and eosin staining of the dermal cellular infiltrate, which occurred at the site of recall DTH.

vaccination site, lip swelling consistent with angioedema, and a patchy macular rash on her trunk. All symptoms resolved after administration of oral diphenhydramine. Within 48 hours, the patient had a local DTH reaction at the injection site, measuring 60 $\times$ 75 mm, that lasted for 2 weeks. At the time of this final vaccination, lymphocyte proliferative responses were detectable to all of the immunizing peptides (Fig 1, A).

During DTH testing 1 month later, 100 μ g of each immunizing peptide was placed intradermally on the patient's back, a site distant from the original vaccination site. Sterile water (0.1 mL) was placed as a control. Initially, GM-CSF was not tested separately because it was thought to possibly have caused the previous month's allergic reaction. Within 5 minutes of peptide skin test application, the patient experienced local erythema and urticaria at all of the peptide injection sites (Fig 1, B); response to sterile water was not observed.

The patient also had pruritis and RU in her left deltoid at the site of her previous vaccinations (Fig 1, C). Within 15 minutes, she again experienced lip swelling and a macular truncal rash, which resolved with oral diphenhydramine. The patient returned 48 hours later for evaluation. DTH responses were present at the sites of pep-

tide inoculation (Fig 1, A). In addition, she had a recall DTH at the site of RU (Fig 1, A). Biopsy specimens of the positive DTH sites on her back showed a CD3⁺, T-cell infiltrate with CD4⁺ predominance, as determined by immunocytochemistry. Biopsy of the area of recall DTH showed a similar appearing lymphocytic infiltrate (Fig 1, D). At this time, GM-CSF (167 μ g in sterile saline) and a control peptide (p1166-1180, 100 μ g in sterile saline) were tested and caused neither urticaria nor a DTH response.

DISCUSSION

RU has not been reported in the setting of peptide-based vaccine administration. This report describes a patient who was challenged with HER-2/neu peptide antigens to evaluate a DTH response. She experienced immediate urticaria and angioedema, most likely as a result of antigen-specific IgE-mediated histamine release. She also had RU at the location of her past vaccinations.

The etiology of RU in this patient is unknown but may possibly be due to chronic antigen-presenting cell infiltration at the vaccine site, with antigen retention. Repeated GM-CSF administration may have played a significant role by stimulating Langerhans' cells, specialized antigen-presenting dendritic cells, in the dermis. GM-CSF is known to promote maturation and enhance effector function of antigen-presenting cells.

Furthermore, GM-CSF has been observed to act as a chemoattractant for dendritic cells when injected intradermally.⁵ Repeated local injections with GM-CSF may have allowed the chronic accumulation of Langerhans' cells and mast cells. Langerhans' cells and dermal mast cells bind IgE with high affinity and have the potential to elicit a peptide-specific, IgE-mediated cutaneous phenomenon such as urticaria.

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REFERENCES

1. Karaayvaz M, Ozanguc N. Recall urticaria: a case report. *J Allergy Clin Immunol* 1996;97:1419-20.
2. Disis ML, Cheever MA. Oncogenic proteins as tumor antigens. In: Alt F, Marrack P, editors. *Current opinion in immunology*. London: Current Biology Ltd; 1996. p. 637-42.
3. 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.
4. Disis M, Grabstein K, Sleath P, Cheever M. 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. In press.
5. Kaplan G, Walsh G, Guido LS, Meyn P, Burkhardt RA, Abalos RM, et al. Novel responses of human skin to intradermal recombinant granulocyte/macrophage-colony-stimulating factor: Langerhans' cell recruitment, keratinocyte growth, and enhanced wound healing. *J Exp Med* 1992;175:1717-28.