

Selective expansion of T cells expressing V β 2 in peanut allergy

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Peanuts are the most common cause of fatal and near-fatal food-induced anaphylaxis. The immune basis for susceptibility to peanut allergy is poorly understood. The current study examined the possibility that patients with peanut allergy, as compared to normals, use different T cell receptor variable beta regions (V β) in the recognition of peanuts. The results demonstrate that stimulation of T cells from patients with peanut allergy results in the selective expansion of V β 2⁺ T cells.

B. J. Dorion, D. Y. M. Leung

From the Departments of Pediatrics, ¹The National Jewish Center for Immunology and Respiratory Medicine, ²University of Colorado Health Sciences Center, Denver, CO, USA

Donald Y. M. Leung, Dept of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St, Denver, CO 80206, USA

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Peanuts are the most common cause of fatal and near-fatal food-induced anaphylaxis (1). Unlike other common food allergies, e.g. to eggs or cow's milk, peanut allergy persists indefinitely. The basis for susceptibility to peanut allergy and the mechanisms by which immune responses in such peanut allergic individuals contrasts to non-peanut allergic normal individuals are poorly understood. In previous work, we showed that peripheral blood mononuclear cells (PBMC) from peanut allergic patients in culture with peanut allergen produce significantly less interferon-gamma than controls (2). In this study, we examined the possibility that patients with peanut allergy, as compared to normals, use different T cell receptor variable beta regions (V β) in the recognition of peanuts.

Material and methods

Approval for this study was obtained from the Institutional Review Board at the National Jewish Center for Immunology and Respiratory Medicine. Eight peanut allergic individuals and eight healthy controls were studied. All 8 peanut allergic subjects had a positive immediate prick skin test to peanut and a positive double blind placebo controlled food

challenge to peanuts. The control group had no history of allergy.

Lymphocyte stimulation studies and analysis of T cell repertoire

Donor PBMC were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ), washed three times in Hanks' balanced salt solution (GIBCO; Grand Island, NY) and resuspended at 1.0×10^6 cells/ml in AIM media (GIBCO) supplemented with 2 mM L-glutamine, 1.5 mM sodium pyruvate, 15 mM Hepes, 50 μ g/ml streptomycin and 100 U/ml penicillin but no serum. PBMC from both groups of subjects were cultured for 7 days with crude peanut allergen (200 μ g/ml; Hollister-Stier, Spokane) or casein (200 μ g/ml; Sigma, St. Louis), and restimulated on Day 7 with the respective antigen and autologous irradiated PBMC in the presence of IL-2 (20 U/ml) and IL-4 (20 U/ml). Freshly isolated CD3⁺ T cells and Day 14 CD3⁺ T cell blasts were analyzed for reactivity with a panel of 6 anti-V β monoclonal antibodies including V β 2, 3, (from AMAC, Westbrook, ME) 5.1, 5.2, 8.1, 12 (T Cell Sciences, Cambridge, MA) using two-color immunofluorescence as previously described (3). Lymphocyte proliferation in response to peanut and casein was measured in 7-day antigen-stimulated cultures using [³H]-thymidine incorporation as previously described (2). The results were expressed as stimulation index (SI) equal to the mean cpm in antigen

Abbreviations: FCS: fetal calf serum, IgE: immunoglobulin E, IL: interleukin, PBMC: peripheral blood mononuclear cells, SI: stimulation index, TCR: T cell receptor, V β : β chain variable region of the TCR.

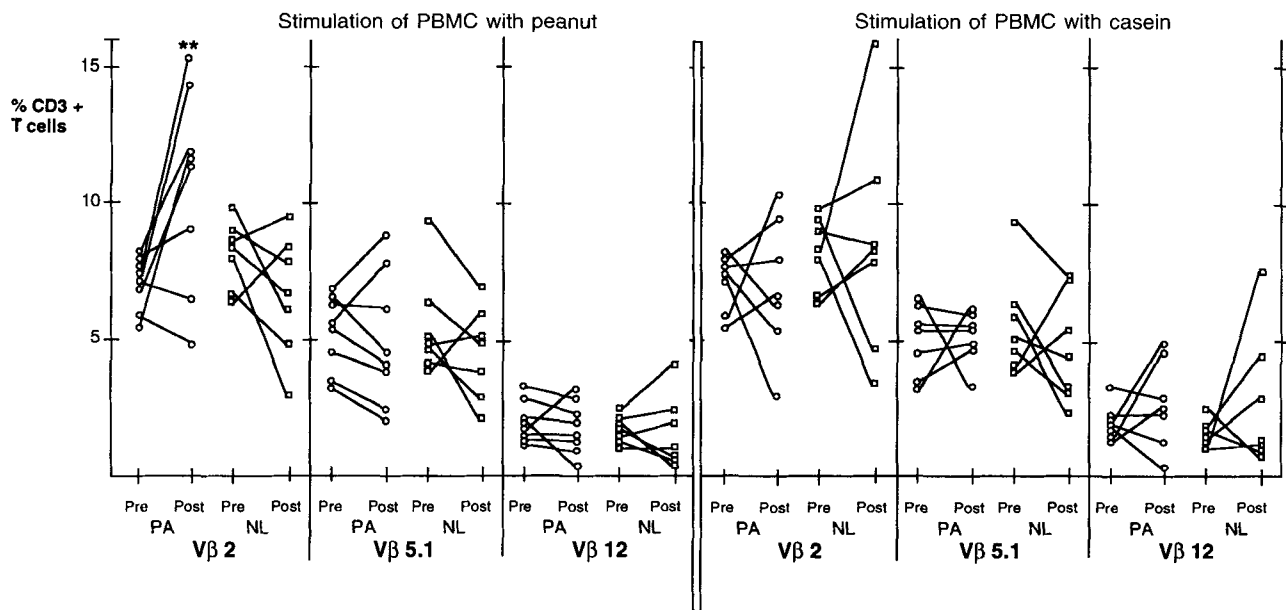


Fig. 1. T cell receptor expression after stimulation with peanut allergen vs casein. PBMC from different patients with peanut allergy (PA) or normal donors (NL) were studied prior to (Pre) and after (Post) a 14-day *in vitro* stimulation with the respective allergens as described in the methods section. The cells used for TCR Vβ analysis on Day 0 were freshly isolated CD3⁺ T cells, and on Day 14 CD3⁺ T cell blasts. Peanut stimulated the selective expansion of Vβ2⁺ T cells (** $P=0.04$; by two-tailed paired t-test) in PBMC from patients with peanut allergy, but not in PBMC from normal donors. Casein did not stimulate selective expansion of T cell Vβ's in peanut allergic subjects, nor in normal donors.

stimulated cultures divided by mean cpm in unstimulated cultures.

Results and discussion

Thymidine incorporation studies revealed that the proliferative response to crude peanut and casein of PBMC from peanut allergic patients (mean $\pm$ SI = 85 ± 18 to peanut and 64 ± 23 to casein) was not significantly different ($p=0.2$ for peanut and 0.6 for casein) from normal donors (mean SI = 46 ± 16 to peanut and 43 ± 11 to casein). However, as shown in Fig. 1, following stimulation with peanut, PBMC from peanut allergic patients, as a group, demonstrated a significant increase ($P=0.04$) in the expression of Vβ2⁺ T cells. The expansion of Vβ2⁺ T cells in response to peanut was observed in 6 of the 8 peanut allergic patients. None of the other 5 TCR Vβ's studied were significantly increased in patients with peanut allergy. In contrast, stimulation of normal donors with crude peanut did not induce any significant expansion of T cells expressing particular Vβ variable regions.

The peanut-induced Vβ2-specific response by PBMC from patients with peanut allergy appeared to be antigen specific because casein did not induce selective expansion of TCR Vβ regions in these same individuals. Furthermore, casein did not induce a selective expansion of the other 6 TCR Vβ's studied in normal PBMC.

Numerous studies, have demonstrated the association of IgE-dependent hypersensitivity reactions with food allergy. To date, however, studies characterizing various food allergens have not demonstrated what makes peanuts such a potent allergen as compared with other foods, e.g., egg, milk or soy products. Indeed, a recent study failed to demonstrate the presence of elevated tryptase levels in sera from patients with fatal or near fatal peanut-induced anaphylaxis suggesting the possibility of non-mast cell mediated mechanisms in this condition (1). Therefore, other potential factors need to be considered. In this regard, the host immunologic reactivity, e.g. the nature of the T lymphocytes stimulated in response to a particular antigen, may also play an important role. Indeed, our current observation that mononuclear cells from patients with peanut allergy selectively expand their Vβ2⁺ T cells in response to incubation with peanut, but not with casein indicate that, in addition to producing IgE responses to peanut, such patients have an abnormal T cell reactivity to peanut.

Of interest, selective expansion of Vβ2⁺ T cells is seen in toxic shock syndrome. Furthermore, in experimental animals it has been demonstrated that lethal shock triggered by superantigens are mediated by T cells secreting tumor necrosis factor- α (4). Our current observations do not support the concept that peanuts induce hypotension via a superantigenic mechanism because superantigens would have been expected to stimulate the expansion of Vβ2⁺ T cells

from both atopic and non-atopic donors in a MHC non-restricted manner. However, it remains possible that V β 2⁺ T cells in a majority of patients with peanut allergy play a role in anaphylaxis, possibly via the secretion of cytokines such as TNF- α which are known to participate in hypotensive events triggered in other illnesses. Furthermore, our data do not support a role for lectins in the peanut as the mitogen for V β 2⁺ T cell expansion because lectins, e.g. PHA, are not known to induce V β -specific T cell expansion.

Our observation that two of the eight patients with peanut allergy did not have increases in V β 2 following *in vitro* stimulation with peanut allergen is not surprising. The frequency of antigen-specific T cells in a mixed T cell population is generally very low, i.e. less than one in a hundred T cells. Thus, the lack of detectable V β 2⁺ T cell expansion in these two patients may reflect a low frequency of circulating peanut-reactive T cells. Alternatively, T cells expressing other β chain variable regions, not measured in this study, may be involved.

Previous studies have demonstrated that crude peanut contains a multiplicity of potential allergens (5). Our current study demonstrating that the majority of patients with peanut allergy selectively expand their V β 2⁺ T cells in response to crude peanut is therefore somewhat surprising. Since the expansion of T cells bearing specific TCR V β 's probably reflects the response to a particular peptide antigen, our present findings suggest that crude peanut is likely to contain an immunodominant antigen which is primarily responsible for T cell responses to peanut. Indeed recent studies by Burks and colleagues (6) have demonstrated that crude peanut does contain major peanut allergens reactive with IgE in sera from patients with peanut allergy. In the future, it will be of interest to determine whether these major peanut allergens are responsible for the V β 2⁺ T cell expansion induced by crude peanut in PBMC from peanut allergic individuals.

Taken together, our study suggest that despite the complexity of crude peanut, there is restricted T cell receptor V β usage to this common food allergen. To date, much of the research in food allergy has focused on IgE responses to specific allergens. However other arms of the immune response, perhaps T cell mediated, may also be important in regulating the magnitude of allergic reactions. The present findings provide an important new direction in which potential mechanisms for allergy to peanuts and other foods can be studied.

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