

Polyclonal and clonal analysis of human CD4⁺ T-lymphocyte responses to nut extracts

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SUMMARY

The induction of IgE antibodies to aeroallergens depends upon antigen-specific CD4⁺ helper T cells of an 'interleukin-4 (IL-4)-dominant' phenotype. Nuts also drive IgE-mediated hypersensitivity and are the most dangerous of the orally encountered allergens. We have studied the polyclonal T-cell responses of atopic and non-atopic individuals to extracts of peanut, brazilnut and hazelnut. Strong proliferative responses were observed in all patients but specific IgE was only present in the nut-allergic patients suggesting a similar pathogenic mechanism to aeroallergen-mediated hypersensitivity. To investigate this hypothesis a panel of peanut-reactive T-cell clones was raised from a peanut- and brazilnut-allergic individual without hazelnut allergy. The antigen specificity, major histocompatibility complex (MHC) class II restriction and cytokine profiles of the T-cell clones were determined. With the exception of one T-cell clone, which proliferated in response to both peanut and hazelnut extract, the peanut T-cell clones were not cross-reactive with hazelnut or brazilnut. The T-cell clones recognized antigen in association with HLA-DR and HLA-DP but not HLA-DQ class II molecules. The peanut-specific clones produced high levels of IL-4 and low levels of interferon- γ (IFN- γ), exhibiting the 'Th2-like' profile which dominates the aeroallergen response. In contrast, the T-cell clone that was cross-reactive on both peanut and hazelnut allergen had a Th0-like phenotype, consistent with the lack of specific serum IgE to hazelnut. These results support the importance of functionally distinct T-cell populations that recognize oral allergens. The relative production of IL-4 and IFN- γ of the cloned T cells in the peanut-allergic patients plays a role in determining whether or not IgE antibody responses are induced with the associated potential to develop anaphylactic reactions.

INTRODUCTION

Immunoglobulin E (IgE)-dependent allergic hypersensitivity to dietary protein is clearly distinguished from food intolerance which is not immune mediated. Allergic individuals may display sensitivity to antigens in related and unrelated food substances. The clinical symptoms of food allergy range from mild irritation of the oropharynx, abdominal pain and vomiting, to life-threatening systemic anaphylaxis.¹

Peanuts, together with milk and eggs, are common sources of food allergens with respect to the total number of patients affected² and are the main cause of severe and sometimes fatal anaphylaxis owing to foods. IgE cross-reactivity between peanut allergens and proteins of other legumes has been described but it is unusual for patients who are allergic to peanut to react to other legumes.³ However, peanut allergy is often associated with cosensitivity to brazilnut and hazelnut.⁴

Patients with hazelnut allergy are frequently allergic to hazel pollen⁵ and have associated multiple cross-reactivities with pollen from the *Fagales* family.⁶ Thus food hypersensitivity may trigger or exacerbate reactions to other food substances and to non-food allergens.

The role of CD4⁺ T lymphocytes in the induction of allergic inflammatory response is well established.^{7,8} Activation of CD4⁺ T cells follows engagement of T-cell antigen receptors by peptide fragments of antigen complexed with major histocompatibility complex (MHC) class II molecules together with ligation of costimulatory receptors.^{9–11} Analysis of the functional properties of CD4⁺ T cells suggests the presence of subsets based on their cytokine profiles (reviewed in ref. 12). Th1 CD4⁺ cells secrete predominantly interferon- γ (IFN- γ), interleukin-2 (IL-2), tumour necrosis factor- β (TNF- β) and participate in delayed-type hypersensitivity reactions, whereas Th2 cells secrete predominantly IL-4, IL-5, IL-6 and IL-10 and mediate B-cell help. The characteristics of aeroallergen-reactive CD4⁺ T-cell clones from atopic individuals appear to be similar to Th2-type cells displaying an 'IL-4-dominant' profile, with variable amounts of IFN- γ .^{13–15} The balance between IL-4 and IFN- γ in the regulation of IgE synthesis is now well

Received 26 July 1994; revised 24 August 1994; accepted 5 September 1994.

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established for both human and mouse B cells. The abundance of IL-4- and IL-5-producing cells in bronchial biopsies and in the periphery of atopic individuals with specific IgE for common environmental aeroallergens illustrates the involvement of this functional subset of CD4⁺ T cells in allergic diseases.^{16,17}

Although peanut and nut anaphylaxis are well recognized clinically as serious food allergies, analysis of the T-cell responses has been only minimally investigated to date with research focusing on the isolation of the major allergens and characterization of B-cell epitopes. In this report, we have investigated the T-cell repertoires, of atopic and non-atopic individuals, reactive with extracts of the common food allergens from peanut, brazilnut and hazelnut. To further characterize the mechanisms of nut allergies, a panel of T-cell clones reactive with peanut has been raised and the antigen specificity, MHC class II restriction and cytokine phenotype of the T-cell clones have been analysed.

MATERIALS AND METHODS

Subjects

Five atopic patients (two male, three female: mean age 26.5 years) were studied. Atopic status was defined by positive skin prick tests of ≥ 3 mm diameter wheal to one or more common environmental allergens [*Dermatophagoides pteronyssinus* (house dust mite), mixed grass pollen, birch pollen, nettle, cat or dog, *Aspergillus fumigatus*, *Cladosporium herbarium* or *alternaria*] in the presence of a positive histamine control and a negative saline control. All five patients gave a history of nut anaphylaxis. Four non-atopic, age- and sex-matched individuals (mean age 30.5 years) were randomly selected and studied as control subjects. Non-atopic status was established by the absence of positive skin prick tests to the panel of common environmental allergens in the presence of a positive wheal response to histamine hydrochloride.

Venous blood (10 ml) was collected and allowed to clot at room temperature overnight. Serum was separated and total serum IgE and the amount of specific IgE against brazilnut, peanut and hazelnut were determined by radio allergosorbent test (RAST; Diagnostic Products, Cardiff, UK). RAST values were graded as 0, 1, 2, 3, 4 according to the manufacturer's recommendations with RAST 0 from 0 to 0.34 IU/ml, RAST 1 from 0.35 to 1.49 IU/ml, RAST 2 from 1.5 to 2.99 IU/ml, RAST 3 from 3 to 14.9 IU/ml and RAST 4 from 15 IU/ml.

Antigens

Extracts of peanut, hazelnut and brazilnut were generously supplied in the 'soluprick' skin test form and as an aqueous solution by ALK Laboratories (Horsholm, Denmark). Those supplied in soluprick form were dialysed against phosphate-buffered saline (PBS) and protein content determined using a BCA protein assay kit (Pierce, Chester, UK). The common recall antigen *Mycobacterium tuberculosis* soluble extract (MTSE) was the kind gift of Dr A. Rees (St Mary's Hospital, London, UK).

Antibodies

The monoclonal antibodies anti-HLA-DR (L243),¹⁸ anti-HLA-DP (B7/21)¹⁹ and anti-HLA-DQ (SPV-L3)²⁰ were

isolated from the culture supernatants of the respective hybridoma cell lines and purified using protein A Sepharose.

Proliferation assays to nut extracts

Peripheral venous blood (50 ml) was collected in preservative-free heparin (CP Pharmaceuticals Ltd, Wrexham, UK; 1 U/ml). Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Milton Keynes, UK) and resuspended in complete medium, RPMI-1640 supplemented with 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin (Gibco Life Technologies, Paisley, UK) and 5% screened, heat-inactivated A⁺ serum (Blood Transfusion Service, Edgeware, UK). PBMC (2.0×10^5 /well) were cultured in round-bottomed 96-well microtitre trays (Nunc, Gibco Life Technologies) with nut extracts over a concentration range (0–400 μ g/ml). The cultures were pulsed with tritiated methyl-thymidine (1 μ Ci/well, [³H]TdR; Amersham International, Amersham, UK) after 6 days and harvested 8–16 hr later. Proliferation was determined by [³H]TdR incorporation with liquid scintillation spectroscopy. The results are expressed as mean c.p.m. for triplicate cultures with standard errors of the mean (SEM) < 25%.

Isolation of human peanut-reactive clones

Peanut-reactive T-cell clones were isolated from an allergic individual by limiting dilution cloning as described previously.²¹ PBMC (10^6 /ml) from donor A4 (Table 1) were stimulated with an optimal concentration of peanut extract (50 μ g/ml) for 7 days in complete medium. A long-term line was established by enriching lymphoblasts on Ficoll-Paque (Pharmacia) in the presence of irradiated autologous PBMC (5×10^5 /ml; 2500 rads), peanut extract (50 μ g/ml) and IL-2 (10% v/v; Lymphocult T, Biotest Folex, Frankfurt, Germany). Cells from the line (0.3 cells/well) were cloned by limiting dilution in complete medium and plated in Microtest II plates together with irradiated autologous PBMC, peanut extract and IL-2. Seven days later, clones were transferred to flat-bottom 96-well microtitre plates and then to 24-well plates. The clones were maintained with IL-2 every 3–4 days and irradiated autologous PBMC and peanut extract every 7 days. Experiments with the clones were performed 7–8 days after the last addition of antigen and antigen-presenting cells (APC).

Proliferation assays with clones

T-cell clones (2×10^4 /well) were cultured in round-bottomed 96-well microtitre trays (Nunc, Gibco Life Technologies) with nut extracts in the presence of irradiated autologous PBMC (2×10^4 /ml; 2500 rads). The cultures were pulsed with tritiated methyl-thymidine (1 μ Ci/well, [³H]TdR; Amersham International) after 48 hr and harvested 8–16 hr later. Proliferation was determined by [³H]TdR incorporation with liquid scintillation spectroscopy. The results are expressed as mean c.p.m. for triplicate curves with the percentage SEM shown in parentheses.

Fractionation of CD19⁺ from PBMC

PBMC were fractionated into B-cell (CD19⁺) and non-B-cell (CD19⁻) populations using a magnetic bead system (Dynal, Wirral, UK) as previously described.²² Briefly, PBMC were suspended in complete medium with beads at a ratio of 5:1 (beads CD19⁺ cells) and rotated at 4° for 30 min. The CD19⁺

Table 1. Total and specific IgE levels (IU/ml), RAST grades (0–4) and polyclonal responses ($\times 10^{-3}$ c.p.m.) to nut extracts. Proliferation of PBMC was determined over a concentration range of 0–400 μ g/ml with the maximum response for each individual patient and antigen shown. The results are expressed as mean c.p.m. for triplicate cultures with SEM < 25%

		RAST value						Proliferation (c.p.m. × 10 ⁻³)			
		Brazilnut		Peanut		Hazelnut					
		Total IgE (IU/ml)	RAST	IU/ml	RAST	IU/ml	RAST	IU/ml	Medium	Brazilnut	Peanut
<i>Atopic</i>											
A1	505	3	3.50	1	1.10	3	14.50	3.5	6.1	20.1	51.1
A2	87	0	0.10	4	31.20	0	<0.35	13.1	18.5	75.2	62.5
A3	527	1	0.45	4	28.60	1	0.57	2.3	26.1	55.1	72.9
A4	242	3	4.00	4	33.60	0	<0.35	0.7	43.4	54.5	33.8
A5	146	3	9.54	0	0.23	3	6.20	0.5	55.1	83.3	58.2
<i>Non-atopic</i>											
NA1	7	0	0.00	0	0.00	0	<0.35	1.4	23.5	22.5	32.5
NA2	12	0	0.10	0	0.20	0	<0.35	3.7	23.5	49.6	10.5
NA3	9	0	0.00	0	0.00	0	<0.35	4.6	28.3	24.1	19.5
NA4	13	1	1.02	0	0.38	0	<0.35	0.6	32.8	58.2	21.1

cells bound to beads were separated from the CD19⁺ cells using a Magnetic Particle Concentrator (MPC; Dynal) according to the general instructions supplied by the manufacturer. Proliferation assays were performed over 7 days as described earlier. PBMC were resuspended in complete medium at 2×10^5 /well, CD19⁺ cells at 2×10^4 /well, CD19⁺ cells and irradiated (2500 rads) CD19⁺ cells at 1.8×10^5 /well with peanut (200 μ g/ml), brazilnut (250 μ g/ml), hazelnut (225 μ g/ml) and pokeweed mitogen (1/100; Gibco Life Sciences).

Cytokine analysis

Supernatants were collected from the cells following 24 hr of activation. Cloned T cells (1×10^6 /ml) were incubated with autologous PBMC as APC (irradiated with 3000 rads; 1×10^6 /ml) and optimum antigen. Control cultures of APC with and without antigen were prepared in parallel. As a positive control, cytokine production following a non-specific stimulus was also measured. Cloned T cells were incubated in tubes precoated with 10 μ g/ml anti-CD3 (a gift from DNAX, Palo Alto, CA) and 1 ng/ml PMA phorbol 12-myristate 13-acetate (Sigma). IFN- γ and IL-4 were measured in enzyme-linked immunosorbent assays (ELISA) using the manufacturer's instructions [IFN- γ (Endogen) and IL-4 (Mabtech) (both supplied by Eurogenetics, Teddington, UK)].

RESULTS

Polyclonal responses to nut extracts

T cells from both atopic and non-atopic individuals responded to all three nut extracts in a dose-dependent manner with maximal responses tabled, ranging from 6100 to 83 300 c.p.m. (Table 1). There was a limited proliferative response to brazilnut by the T cells of patients A1 and A2 with proliferation less than twice the background levels of medium alone. The background proliferation was in the range of 500–13 100 c.p.m. T cells from the atopic subjects responded more

vigorously to peanut and hazelnut extracts than the control T cells. The peanut extract stimulated the highest level of proliferation in both groups. Additionally, maximal responses of T cells from atopic individuals were achieved at lower concentrations of the nut extracts compared to the non-atopic subjects (Fig. 1). However, in atopics, T-cell responses did not correlate with levels of specific serum IgE levels. The proliferative results are expressed as mean c.p.m. for triplicate cultures with SEM < 25%.

Serum IgE responses to nut extracts

Total IgE levels in the atopic individuals were considerably higher (87–527 IU/ml) than those measured in the non-atopic subjects (7–13 IU/ml; Table 1). Only two of the five atopic subjects, A1 and A3, had measurable specific IgE for all three nuts (Table 1). A1 had a RAST grade 3 to brazilnut and hazelnut, but only a RAST grade 1 to peanut, with a corresponding specific IgE of 1.1 IU/ml. A3 had a high RAST value of 4 for peanut accompanied by high levels of specific IgE (28.6 IU/ml) but RAST values and specific IgE responses to brazilnut and hazelnut were very low (1 and <0.6 IU/ml, respectively). With the remaining patients, A2 exhibited specific IgE only for peanut (RAST 4, 31.2 IU/ml specific IgE); A5 had RAST values of 3 to brazilnut and hazelnut with specific IgE levels of 9.54 IU/ml and 6.2 IU/ml, respectively, but did not respond to peanut. None of the non-atopic subjects had a positive RAST value to any of the nuts tested with the exception of NA4 who had a RAST grade 1 to brazilnut. However, all specific IgE levels in these subjects were below 1.02 IU/ml.

Polyclonal responses of donor A4

Polyclonal T cells from nut-allergic donor A4 responded to all three nut extracts in a dose-dependent manner (Fig. 1). Maximal proliferation was induced by a peanut extract which

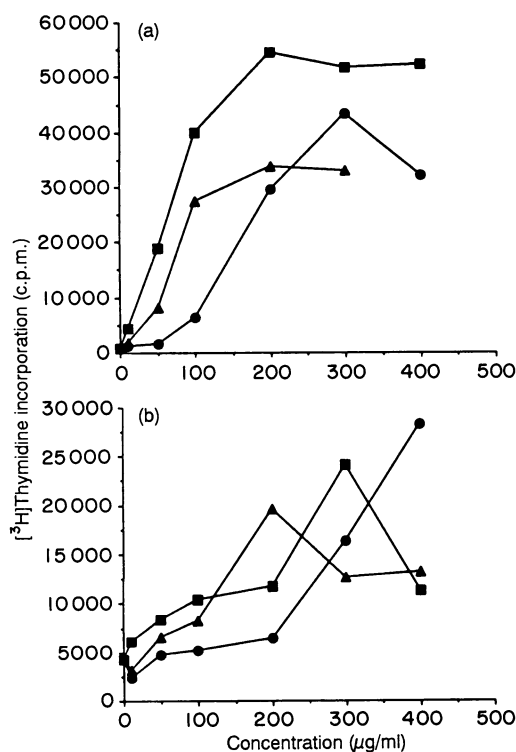


Figure 1. Polyclonal responses of atopic donor A4 (a) and non-atopic donor NA3 (b) to nut extracts were determined in 7-day proliferation assays as correlated with $[^3\text{H}]\text{TdR}$ incorporation. PBMC ($2 \times 10^5/\text{well}$) were incubated with brazilnut extract (●), peanut extract (■) and hazelnut extract (▲) over a concentration range. Dose-dependent stimulation was observed with all nut extracts and the SEM of triplicate assays $< 25\%$.

corresponded to high levels of specific IgE (33.6 IU/ml; RAST grade 4) for peanut (Table 1). A4 had considerably lower levels of specific IgE to brazilnut (4.0 IU/ml; RAST grade 3) and in addition a lower T-cell response to this extract was observed. Although the patient had no specific IgE (< 0.35 IU/ml; RAST grade 0) a T-cell proliferative response was observed to hazelnut.

Proliferation of PBMC to nut extracts is antigen specific

The possibility that the responses were non-specific and mediated by mitogens such as agglutinins in the extracts was investigated. The capacity of the nut extracts to non-specifically stimulate T cells was tested by culturing peanut extract with cloned house dust mite-reactive CD4^+ T cells in the presence of irradiated APC (data not shown). Although phytohaemagglutinin (PHA) stimulated the cloned T cells, the peanut extract failed to elicit proliferation. Potential B-cell mitogenicity was also assessed by culturing PBMC fractionated into B-cell (CD19^+) and non-B-cell (CD19^-) populations. All three nut extracts and pokeweed mitogen induced proliferation in unfractionated PBMC and the CD19^- population (Fig. 2). As expected, the CD19^- cells alone failed to respond to any of the nut preparations or to pokeweed mitogen. Addition of irradiated CD19^- cells to the CD19^+ cells to reconstitute T-cell-dependent B-cell function resulted in a proliferative

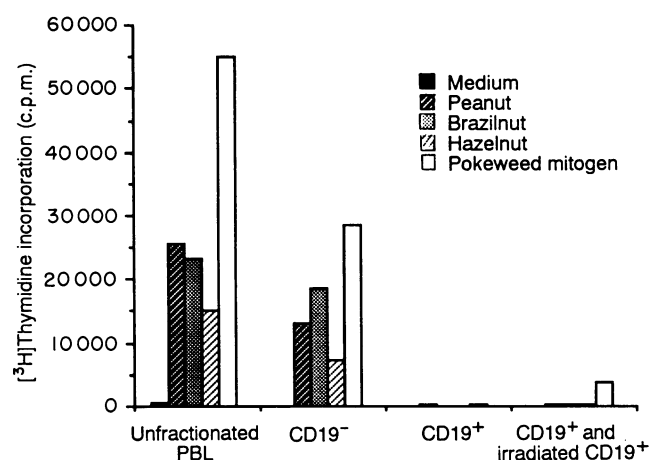


Figure 2. Exclusion of mitogenicity as the trigger for T-cell proliferative responses to peanut extracts. The ability of the nut extracts to induce proliferation in a B-cell population was determined in 7-day proliferation assays with fractionated populations of PBMC separated using CD19^+ magnetic beads. Medium (■), peanut extract (200 $\mu\text{g/ml}$), brazilnut extract (250 $\mu\text{g/ml}$), hazelnut extract (225 $\mu\text{g/ml}$) and pokeweed mitogen (1/100) were incubated with PBMC ($2 \times 10^5/\text{well}$), CD19^- cells ($1.8 \times 10^5/\text{well}$), CD19^+ cells ($2 \times 10^4/\text{well}$) and CD19^+ cells ($2 \times 10^4/\text{well}$) reconstituted with irradiated (2500 rads) CD19^- cells ($1.8 \times 10^5/\text{well}$). The nut extracts did not induce proliferation in the CD19^+ cells nor, in contrast to pokeweed mitogen, did they induce T-cell-dependent B-cell proliferation in the reconstituted cell fractions.

response to pokeweed, but there was no response to any of the nut extracts. Collectively, these results suggest that the responses of PBMC to the nut extracts are owing to specific T-cell proliferation and that the nut extracts contain neither T- nor B-cell mitogens.

Clonal responses to peanut

A T-cell line reactive to peanut extract was generated from donor A4 and T-cell clones isolated by limiting dilution. The antigen specificity of five T-cell clones was determined in proliferation assays. All five clones recognized peanut extract but with the exception of AM9 did not recognize the other nut extracts (Table 2). In contrast, T cells of clone AM9 responded to both peanut and hazelnut extracts.

HLA-class II restriction specificity of the T-cell clones

Antigen-dependent proliferation of the T-cell line AM and clones AM9, AM10 and AM15 were inhibited with the anti-HLA-DR antibody (Fig. 3). In contrast proliferation of the clones AM3 and AM11 was inhibited by anti-HLA-DP antibodies indicating that these clones were restricted by HLA-DP. This also further confirms that responses to peanut were antigen specific and not related to a mitogenic response. Neither the line nor the T-cell clones were inhibited by the monoclonal antibody directed at HLA-DQ.

Cytokine production of T-cell clones

The peanut-specific clones AM3 and AM10 produced both

Table 2. Antigen specificity of five T-cell clones isolated from a peanut-induced T-cell line. The antigen specificity of T-cell clones isolated from donor A4 was determined in proliferation assays. Cloned T cells were stimulated with extracts of peanut (100 µg/ml), brazilnut (250 µg/ml) and hazelnut (225 µg/ml) in the presence of APC. Clones were also tested with APC alone and 10% IL-2. All five clones responded to peanut extract. With the exception of AM9 which was cross-reactive with hazelnut the clones did not recognize the other nut extracts. Results shown are mean c.p.m. of triplicate assays with the % SEM shown in parentheses

	APC	Hazelnut	Peanut	Brazilnut
Clone				
AM3	100 (27)	126 (19)	30 264 (8)	95 (50)
AM9	183 (63)	43 949 (5)	40 936 (4)	215 (53)
AM10	67 (40)	92 (35)	3876 (7)	65 (17)
AM11	123 (26)	107 (25)	797 (41)	76 (24)
AM15	86 (41)	110 (32)	19 042 (10)	364 (90)

IFN- γ (1500 and 460 pg/ml respectively) and IL-4 (8500 and 3500 pg/ml respectively) when stimulated with peanut in the presence of APC (Table 3). Both clones secreted six- to sevenfold more IL-4 than IFN- γ , indicating that these clones were 'IL-4 dominant' in character. All the clones demonstrated enhanced cytokine production after stimulation with anti-CD3 and PMA. Cytokine production by the peanut and hazelnut cross-reactive T-cell clone AM9 was measured following restimulation with both nut extracts. Cytokine production following stimulation with peanut was lower than that induced by hazelnut. However, following stimulation with peanut allergen the T-cell clone produced 170 pg/ml and 176 pg/ml of IFN- γ and IL-4 respectively. In contrast the levels of IFN- γ and IL-4 secreted in response to hazelnut were 577 pg/ml and 780 pg/ml. Following a non-specific stimulus AM9 produced approximately fourfold more IFN- γ than IL-4 (3550 pg/ml and 820 pg/ml respectively). Therefore this clone does not have the 'IL-4 dominant' profile exhibited by clones AM3 and AM10 but is more characteristic of the Th1/Th0 phenotype. Less than 150 pg/ml of IFN- γ and IL-4 were secreted by APC in the absence or presence of antigen.

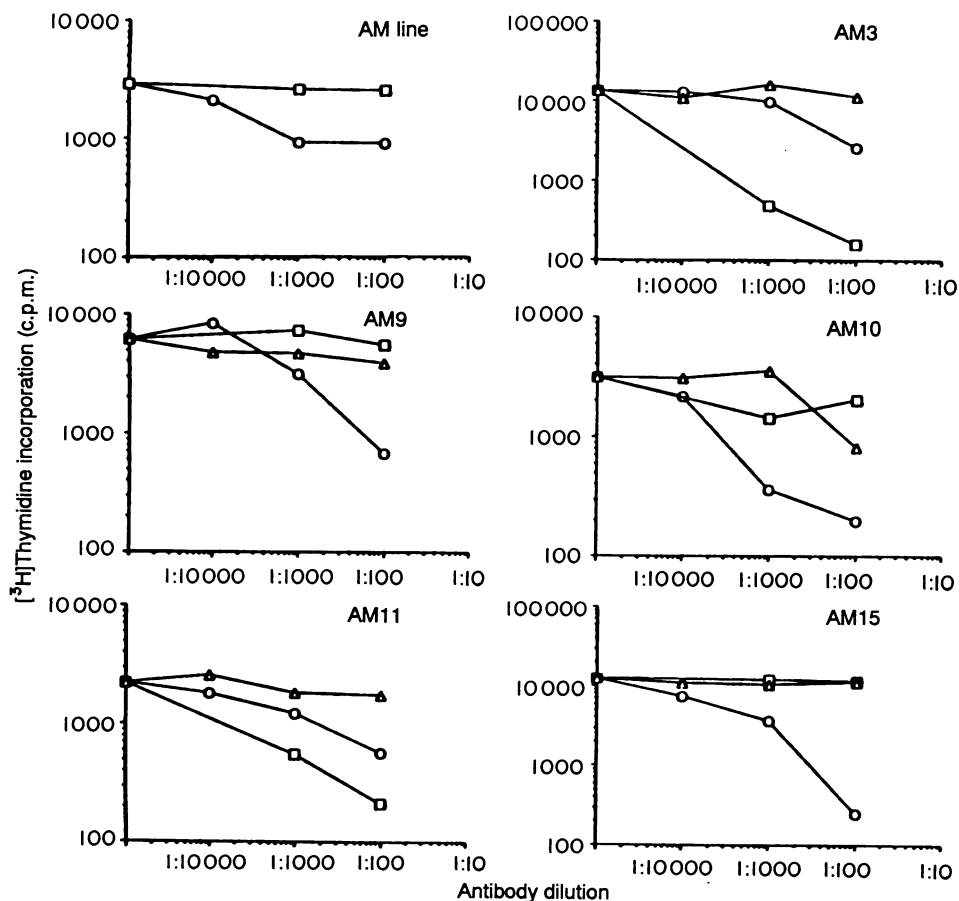


Figure 3. MHC class II restriction specificity of T-cell recognition of peanut allergens. The genetic restriction of the T-cell line and the T-cell clones were determined by the addition of anti-HLA-DQ (SPV-L3) (Δ), anti-HLA-DP (B7/21) ($\square$) and anti-HLA-DR (L243) antibodies ($\circ$) to proliferation assays with APC and peanut extract (50 µg/ml). The T-cell line and three of the T-cell clones (AM9, AM10 and AM15) were inhibited by the anti-HLA-DR antibody indicating that the peanut-reactive T cells from this individual are predominantly restricted by HLA-DR class II molecules. Antigen-specific proliferation of the clones AM3 and AM11 were inhibited by anti-HLA-DP antibodies indicating that these clones were restricted by HLA-DP. Neither the lines nor the clones were inhibited by anti-HLA-DQ antibody.

Table 3. Cytokine production (pg/ml $\pm$ SD) of peanut-reactive T-cell clones at 24 hr following stimulation with antigen in the presence of autologous PBMC as APC or stimulation with anti-CD3 antibody and PMA. IFN- γ and IL-4 secretion were measured by ELISA using a commercially available kit. The sensitivity of the assays were 10 pg/ml for the IFN- γ assay and 20 pg/ml for the IL-4 assay

Treatment	IFN- γ (pg/ml)	IL-4 (pg/ml)
APC	133 $\pm$ 12	100 $\pm$ 6
APC and peanut	111 $\pm$ 14	95 $\pm$ 3
APC and hazelnut	156 $\pm$ 9	104 $\pm$ 4
AM3, APC and peanut	1500 $\pm$ 13	8500 $\pm$ 32
AM9, APC and peanut	170 $\pm$ 12	176 $\pm$ 9
AM9, APC and hazelnut	577 $\pm$ 35	780 $\pm$ 0
AM10, APC and peanut	460 $\pm$ 13	3500 $\pm$ 263
AM3 anti-CD3/PMA	5644 $\pm$ 482	12 000 $\pm$ 348
AM9 anti-CD3/PMA	3552 $\pm$ 127	820 $\pm$ 50
AM10 anti-CD3/PMA	1340 $\pm$ 168	19 000 $\pm$ 6595

DISCUSSION

The clinical importance of IgE-mediated food allergies, such as reactions to peanut, hazelnut and brazilnut, is clearly demonstrated by the severity of symptoms displayed; death from anaphylactic shock is not uncommon with seven deaths reported in the UK recently. Clinical management is based on dietary avoidance and symptomatic relief using adrenaline-based medications. However, even with specific allergen avoidance, allergic manifestations may continue in some individuals owing to cross-reactivity with other common environmental allergens and the wide use of nuts as ingredients in other foods.

In this study we have analysed the T-cell responses of atopic and non-atopic individuals to extracts of peanut, hazelnut and brazilnut. Peripheral blood T cells in both groups of subjects responded to the nut extracts, whether or not the individuals had detectable levels of specific IgE. However, the T-cell proliferative responses of the atopic patients were in general greater and induced with lower concentrations of extract. The possibility that mitogenic components of the peanut extract were responsible for the strong proliferation observed was excluded by demonstration of the inability of the extracts to stimulate cloned T cells and also by the demonstration of MHC class II restriction. A superantigenic component of the extract was judged unlikely because a high antigen concentration was required to induce maximal proliferation. Furthermore, although superantigen-induced CD4⁺ T-cell activation requires the presence of MHC class II molecules, it is not restricted unlike responses to nominal antigens²³ and the proliferation induced by superantigens is not easily inhibited by anti-MHC class II antibodies as was demonstrated here with the T-cell line and clones.

The presence of aeroallergen-reactive T cells in the peripheral blood of atopic and non-atopic subjects has been previously described,^{24,25} but it is the qualitative nature of the T-cell help that determines the induction of specific IgE antibody. This observation has been made in many studies on

aeroallergen-reactive T-cell lines and clones^{13,14} but the clinical response of aeroallergen-reactive patients differs significantly from patients with nut hypersensitivity and, therefore, it could not be assumed that the same Th2 pattern of response was the sole underlying mechanism. T-cell lines and clones reactive with allergens from peanut and other nuts have not previously been reported or characterized with respect to T-cell recognition and subsequent antibody synthesis.

In order to begin to dissect the mechanisms acting to induce nut anaphylaxis, polyclonal responses were analysed in nine subjects together with the isolation of a panel of T-cell clones that were reactive with peanut extract from one of the allergic patients. All of the T-cell clones tested responded to peanut extract with the exception of one clone (AM9), which also proliferated to extracts of hazelnut. Analysis of the polyclonal T-cell responses of this individual demonstrated the presence of T cells reactive with peanut and hazelnut, whereas serologically the individual demonstrated specific IgE for peanut but no IgE directed at hazelnut could be detected. The failure of this patient to produce IgE to hazelnut implies that T-cell recognition of this food may be predominantly by 'Th1/Th0-type' cells. Although both peanut and hazelnut allergen induced proliferation of AM9 it was only in response to hazelnut that either IL-4 or IFN- γ was secreted. The ability to dissociate T-cell effector functions such as proliferation from cytokine release has been reported for murine T-cell clones in response to stimulation with peptide in which there are substitutions of the residues that contact the T-cell receptor (TCR).²⁶ The possibility exists that amino acid differences in the sequences of the cross-reactive epitope in peanut and hazelnut recognized by AM9 may be T-cell contact residues and so function as altered T-cell ligands capable of dissociating cytokine release and proliferation. The presence of specific IgE to peanut in contrast suggests a 'Th2-type' response to this antigen. This hypothesis was supported by the pattern of cytokine secretion from the T-cell clones that were specific for peanut. They exhibited an 'IL-4-dominant' phenotype suggesting that they were capable of supporting IgE synthesis to this allergen. Taken together, these observations suggest the notion that the mechanism underlying clinical hypersensitivity to peanut and other nut allergens is driven by 'Th2-like' T-cell responses.

This study supports and extends a previous study²⁷ which demonstrated increased IFN- γ production by PBMC from control patients compared to nut-allergic patients. Although increased mRNA for IL-4 was detected by polymerase chain reaction (PCR) in all PBMC it was comparable in both control and nut-allergic patients, and failed to correlate with serum IgE levels and the presence of clinical allergy in the patient group. Moreover, as found by many others, the secretion of the protein, IL-4, could not be detected in polyclonal cultures perhaps reflecting the low frequency of IL-4-secreting T cells in the peripheral blood. Investigations using cloned T lymphocytes as reported here allow enhancement of protein secretion to detectable levels in supernatants, thereby confirming that there is indeed differential cytokine production with the secretion of IL-4 in patients matching the tendency to specific allergen IgE synthesis, as has been clearly established for aeroallergen responses.

Extensive cross-reactivity has been described in patients with food hypersensitivities. In addition, food sensitivity may

be exacerbated by immunocompetent cells primed to other environmental allergens, for example, patients allergic to hazelnut are sometimes only reactive in the hazel pollen season.¹ Recognition of antigen components of several related allergens by cross-reactive IgE will lead to multiple allergies. Similarly, T cells that recognize cross-reactive epitopes may provide bystander help and promote the production of specific antibodies to another allergen and so contribute to the extensive multiple allergies usually described in these patients.

The peanut-reactive T-cell clone AM9 was cross-reactive with hazelnut. Peanut contains an 18 000 MW allergen, *Ara h* II,²⁸ and an 18 000 MW allergen has been defined in hazelnut.²⁹ Although IgE cross-reactivity between hazelnut, peanut and brazilnut is not evident, many patients are sensitive to more than one of these nuts.³ Identification of defined components of these nut preparations will allow identification of both T and B epitopes, facilitating the development of new therapeutic approaches.

ACKNOWLEDGMENTS

This work was supported by grants from the Wellcome Trust and the Medical Research Council, UK. R.E.O. is the recipient of a Wellcome Senior Research Fellowship in Clinical Sciences.

REFERENCES

- METCALFE D.D. (1985) Food Allergens. *Clin Rev Allergy* **3**, 331.
- SPEER F. (1976) Food allergy: the ten common offenders. *Am Fam Physician* **13**, 106.
- BERNHISEL-BROADBENT J. & SAMPSON H.A. (1989) Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J Allergy Clin Immunol* **83**, 435.
- ARSHAD S.H., MALMBERG E., KRAFT K. & HIDE D.W. (1991) Clinical and immunological characteristics of Brazilnut allergy. *Clin Exp Allergy* **21**, 373.
- ANDERSEN K.E. & LOWENSTEIN H. (1978) An investigation of the possible immunological relationship between allergen extracts from birch pollen, hazelnut, potato and apple. *Contact Dermatitis* **4**, 73.
- IPSEN H., BOEWADT H. & JANNICHE H. (1985) Immunological characterization of reference alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) pollen extracts and partial immunochemical identity between the major allergens of alder, birch and hazel pollens. *Allergy* **40**, 510.
- ISHIZAKA K. (1984) Regulation of IgE synthesis. *Annu Rev Immunol* **2**, 159.
- LETT BROWN M.A., ALAM R. & GRANT J.A. (1989) Regulation of human basophils and mast cells. Activation by cytokines. *Year Immunol* **5**, 195.
- SCHWARTZ R.H. (1985) T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Annu Rev Immunol* **3**, 237.
- MOLLER G. (1987) Antigen requirements for activation of MHC restricted responses. *Immunol Rev* **98**, 1.
- DAVIS M.M. & BJORKMAN P. (1988) T cell antigen receptor genes and T cell recognition. *Nature* **334**, 395.
- COFFMAN R.L., SEYMOUR B.W., LEBMAN, D.A. *et al.* (1988) The role of helper T lymphocyte products in mouse B cell differentiation and isotype regulation. *Immunol Rev* **102**, 5.
- O'HEHIR R.E., BAL V., QUINT D. *et al.* (1989) An *in vitro* model of allergen-dependent IgE synthesis by human B lymphocytes: comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite). *Immunology* **66**, 499.
- WIERENGA E.A., SNOEK, M., DE GROOT C. *et al.* (1990) Evidence for the compartmentalisation of functional subsets of CD4⁺ T lymphocytes from atopic patients. *J Immunol* **144**, 4651.
- PARRONCHI P., MACCHIA D., PICCINNI M.-P. *et al.* (1991) Allergen- and bacterial antigen-specific T cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci USA* **88**, 4538.
- HAMID Q., AZZAWI M., YING S. *et al.* (1991) Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* **87**, 1451.
- FIELD E.H., NOELLE R.J., ROUSE T., GOEKEN J. & WALDSCHMIDT T. (1993) Evidence for excessive Th2 CD4⁺ subset activity *in vivo*. *J Immunol* **141**, 48.
- LAMPSON L.A. & LEVY R. (1980) Two populations of Ia-like molecules on a human B cell line. *J Immunol* **125**, 293.
- WATSON A.J., DE MARS R., TROWBRIDGE I.S. & BACH F.H. (1983) Detection of a novel human class II antigen. *Nature* **304**, 358.
- SPITS H., KEIZER G., BORST J., TERHORST C., HEKMAN A. & DE VRIES J.E. (1983) Characterisation of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma* **2**, 423.
- O'HEHIR R.E., YOUNG D.B., KAY A.B. & LAMB J.R. (1987) T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite): a comparison of T- and B cell antigen recognition. *Immunology* **62**, 635.
- FUNDERUD S., ERIKSTEIN B., ASHEIM H.C. *et al.* (1990) Functional properties of CD19⁺ve B lymphocytes positively selected from buffy coats by immunologic separation. *Eur J Immunol* **20**, 201.
- VERHOEF A., HIGGINS J.A., THORPE C.J. *et al.* (1993) Clonal analysis of the atopic immune response to the group 2 allergen of *Dermatophagoides* spp: identification of HLA-DR and DQ restricted T cell epitopes. *Int Immunol* **5**, 1589.
- O'HEHIR R.E., VERHOEF A., PANAGIOTOPOULOU E. *et al.* (1993) Analysis of human T cell responses to the group II allergen of *Dermatophagoides* spp. (house dust mite): localisation of major antigenic sites. *J Allergy Clin Immunol* **92**, 105.
- HALVORSEN R., BOSNES V. & THORSBY E. (1986) T cell responses to a *Dermatophagoides farinae* allergen preparation in allergics and healthy controls. *Int Arch Allergy Appl Immunol* **80**, 62.
- EVAGOLD B.D. & ALLEN P.M. (1991) Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science* **242**, 1308.
- DORION B.J., BURKS A.W., HARBECK R. *et al.* (1994) The production of interferon- γ in response to a major peanut allergy, *Ara h* II, correlates with serum levels of IgE anti-*Ara h* II. *J Allergy Clin Immunol* **93**, 93.
- BURKS A.W., WILLIAMS L.W., CONNAUGHTON C., COCKRELL G., O'BRIEN T. & HELM R.M. (1992) Identification and characterisation of a major peanut allergen, *Ara h* II, with use of the sera of patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* **90**, 692.
- HIRSCHWEHR R., VALENTA R., EBNER C. *et al.* (1992) Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* **90**, 927.