

Allergens, IgE, mediators, inflammatory mechanisms

Food allergen (peanut)-specific T_{H2} clones generated from the peripheral blood of a patient with peanut allergy

Esther C. de Jong, MSc,^{a, b} Steven Spanhaak, MSc,^a
Ben P. M. Martens, MD,^c Martien L. Kapsenberg, PhD,^d
André H. Penninks, PhD,^a and Eddy A. Wierenga, PhD^d
Zeist, Utrecht, and Amsterdam, The Netherlands

Background: Increasing evidence indicates a prominent role of allergen-specific T_{H2} cells, with high IL-4 and IL-5 production and low interferon- γ production, in the regulation of IgE and eosinophil production in allergic disorders. However, most studies have concentrated on T cells reactive with inhalation allergens, whereas little is known about the properties of food allergen-reactive T cells.

Objective: In this study we therefore characterized peanut-specific T cells, cloned from a patient with severe peanut allergy.

Methods: Peripheral blood mononuclear cells from patients with peanut allergy and nonallergic individuals were stimulated with crude peanut extract (CPE) to compare the proliferative responses and to select a suitable patient for the cloning of CPE-specific T cells. The resultant panel of CPE-reactive T-lymphocyte clones was serologically phenotyped by flow cytometry and analyzed for cytokine secretion by ELISA.

Results: The patients' peripheral blood mononuclear cells showed a dose-dependent proliferative response to CPE, which was significantly higher ($p < 0.05$) than in peripheral blood mononuclear cells of nonallergic donors. The CPE-specific T-lymphocyte clones generated from the selected patient were all CD4⁺/CD8⁻ T helper cells with a T_{H2} cytokine profile, secreting high amounts of IL-4 and IL-5, but little or no interferon- γ .

Conclusions: This study demonstrates that peanut-specific T cells do occur in the peripheral blood of patients with peanut allergy and suggests an increased frequency of these T cells in patients compared with nonallergic control subjects. The CD4⁺ phenotype and the T_{H2} cytokine profile of the CPE-specific T-lymphocyte clones suggest a functional role of allergen-specific T_{H2} cells in the pathophysiology of food allergy, similar to the function of inhalation allergen-specific T_{H2} cells. (*J Allergy Clin Immunol* 1996;98:73-81.)

Key words: Food allergy, peanut, T-cell clones, cytokines, phenotype

Food allergies occur in 1% to 10% of the Western population,^{1,2} especially in young children.³ Most common food allergens are proteins in

cow's milk, hen's egg, peanuts, and soy beans,⁴ which may induce a type I food allergic reaction in sensitized individuals, characteristically associated with high serum levels of specific IgE⁵ and eosinophilia.^{6,7} So far, little is known about the mechanism underlying the development of a type I food allergy.

For allergy to inhalation allergens, such as house dust mites (HDMs), various pollens, and domestic animals, several studies revealed that allergen-specific T cells generated from peripheral blood⁸⁻¹¹ or skin^{12,13} of atopic donors are T_{H2} cells producing high levels of IL-4 and IL-5 but little or no

From ^aNetherlands Organization for Applied Scientific Research Nutrition and Food Research Institute, Zeist; ^bUtrecht Toxicology Center; ^cAllergy Center Utrecht; ^dDepartment of Cell Biology and Histology, University of Amsterdam.

Received for publication Feb. 9, 1995; revised Sept. 7, 1995, accepted for publication Sept. 8, 1995.

Reprint requests: Steven Spanhaak, MSc, TNO Nutrition and Food Research Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/69399

Abbreviations used

APCs:	Antigen-presenting cells
CLB:	Central Laboratory Blood Transfusion Service
CPE:	Crude peanut extract
EBV:	Epstein-Barr virus
FITC:	Fluorescein isothiocyanate
HDM:	House dust mite
[³ H]-Tdr:	Tritiated thymidine
IFN- γ :	Interferon- γ
IMDM:	Iscove's Modified Dulbecco's medium
PBMCs:	Peripheral blood mononuclear cells
PE:	Phytoerythrin
PNA:	Peanut agglutinin

interferon- γ (IFN- γ). IL-4 plays an important role in the regulation of production of IgE antibodies, both in vitro¹⁴⁻¹⁸ and in vivo,¹⁹ by inducing an isotype switch to IgE in B cells.^{14, 17, 18} This IL-4-induced isotype switch is inhibited by IFN- γ .²⁰⁻²² Consequently, allergen-specific T_{H2} cells from atopic donors are efficient helper cells for IgE synthesis.^{9, 23-25} IL-5 has been shown to induce eosinophil proliferation and activation.^{26, 27} Eosinophils play a role in the late-phase allergic reaction, causing tissue damage by toxic mediators released by degranulation.^{28, 29}

Some clinical symptoms of food allergy such as the oral allergy syndrome³⁰ and gastrointestinal problems³¹ appear to be related to allergen uptake in the digestive tract and therefore confined to this type of allergy. Other clinical manifestations, however, are very similar to those seen in inhalation allergy (e.g., atopic dermatitis, asthma, anaphylaxis, and eosinophilia). Because of the key role of allergen-specific T cells in the pathophysiology of these disorders, we attempted to determine whether food allergen-specific T cells have properties similar to those of the previously described inhalation allergen-specific T cells. To address this question, we generated and characterized peanut protein-specific T-cell clones from a patient with peanut allergy. We focused on peanut allergy because this is a common food allergy in adults which is very persistent and can cause severe symptoms, such as anaphylactic shock, followed by death.³²⁻³⁴ For this study peanut proteins were extracted from raw, unshelled peanuts. The obtained crude peanut extract (CPE) was used to compare peanut-specific T-cell reactivity in peripheral blood mononuclear cells (PBMCs) of nonal-

lergic subjects and patients with peanut allergy. The proliferative response to peanut agglutinin (PNA), the naturally occurring lectin in peanuts, was studied as a parallel control because this lectin may induce mitogenic stimulation as demonstrated in bovine PBMCs.³⁵ On the basis of this comparative study, one patient with peanut allergy was selected for the generation and characterization of CPE-specific T-cell clones.

METHODS

Subjects

The nonallergic control group consisted of 10 individuals (2 men, 8 women; mean age, 31.9 ± 10.0 years) with no history of allergy and negative RAST and skin prick test scores for peanut allergen and seven other common allergens (grass, cat, dog, *Alternaria* spp., birch, bird, and HDM). The mean total serum IgE titer was 102 ± 148 IU/ml. The peanut-allergic group consisted of seven patients (1 man, 6 women; mean age, 27.1 ± 6.7 years) with peanut allergy as indicated by clinical symptoms and positive peanut-specific RAST ($>3+$) and skin prick test scores. Approval for this study was obtained from an independent medical-ethical committee. Informed consent was obtained from all subjects. Peanut-specific T-cell clones were generated from a patient with severe peanut allergy (NvD), demonstrating strong proliferative responses to CPE in PBMCs. This patient was a 15-year-old girl with a severe peanut and coriander allergy that caused symptoms such as oral allergy syndrome, asthma, and anaphylactic shock. At the time of blood collection, 4 weeks after a severe episode of anaphylactic shock, the total serum IgE level was 708 IU/ml with high levels of peanut-specific IgE ($5+$) as determined by RAST.

Peanut protein extraction

Peanut proteins were extracted from raw, unshelled peanuts of the Runner Jumbo 38/42 variety (van Zijl, Hilversum, The Netherlands) by using a method modified from Barnett et al.³⁶ Briefly, peanuts were ground, and fat was extracted by Soxhlet (Soxtec system HT extraction unit; Tecator Hicol bv, Oud-Beijerland, The Netherlands) with petroleum ether at 40 to 60°C. The defatted flour was ground again and suspended in 0.1 mol/L ammonium bicarbonate. This suspension was stirred for 4 hours and centrifuged at 10,000 g for 30 minutes at 4°C to remove insoluble compounds. The supernatant was dialyzed overnight against distilled water with a 3.5 kd cutoff membrane (Spectrum Medical Industries, Inc., Houston, Texas). The dialyzed extract, referred to as CPE, was lyophilized and stored at -20°C. The obtained CPE consisted of approximately 90% protein as determined by the Bio-Rad protein assay (Bio-Rad, München, Germany). On sodium dodecylsulfate-polyacrylamide gel electrophoresis with silver staining, CPE consisted of several bands between 70 and 10 kd. The

PNA concentration in CPE was estimated by intensity of the bands at approximately 1%.

Culture media

The cloning procedure and proliferation assays were performed in Iscove's Modified Dulbecco's medium (IMDM) (Gibco, Paisley, U.K.) supplemented with 10% pooled complement inactivated normal human serum (Central Laboratory Blood Transfusion Service [CLB], Amsterdam, The Netherlands) and gentamicin (50 µg/ml; Flow Laboratories, Irvine, U.K.).

Epstein Barr virus (EBV)-transformed B cells were cultured in IMDM supplemented with 10% fetal calf serum (Hyclone, Logan, Utah) and gentamicin (50 µg/ml).

Proliferation assays with PBMCs

PBMCs were isolated from heparinized venous blood by using density gradient separation on Ficoll-Plaque (Pharmacia LKB, Uppsala, Sweden) essentially as described by Böyum.³⁷ Recovered cells were cultured ($2 \times 10^5/200$ µl) in triplicate at 37° C in 5% CO₂ for 7 days in IMDM supplemented with human serum in the absence or presence of increasing concentrations of CPE (1, 10, 50, 100, 150, 200, and 250 µg/ml) or PNA (lectin from *Arachis hypogaea*; 1, 10, 50 and 100 µg/ml; Sigma, St. Louis, Mo.) in 96-well flat-bottom culture plates (Nunc, Roskilde, Denmark). Proliferation was measured by using tritiated thymidine (³H]-TdR) incorporation. After 7 days, [³H]-TdR (0.4 µCi/well; Amersham, Aylesbury, U.K.) was added, and the cells were incubated for another 18 hours and then harvested (Harvester 96; Tomtec, Orange, Calif.). [³H]-TdR incorporation was measured by using a 1450 Microbetacounter (Wallac, Turku, Finland).

T-cell cloning procedure

CPE-specific T-cell clones were generated from heparinized venous blood as described previously.²⁵ In parallel to the previously described proliferation experiments, identical triplicate cultures were started in separate culture plates. If cultures in the first plate indicated PNA- or CPE-induced T-cell proliferation on day 7, cultures in the parallel plate were used for the generation of specific T-cell clones. To further promote the expansion of CPE- or PNA-reactive T cells, recombinant IL-2 (Eurocetus, Amsterdam, The Netherlands) was added to each well on day 7 at a final concentration of 20 IU/ml. After another 7 days of culture, cells were cloned from the thus formed short-term CPE-specific T-cell lines by limiting dilution at 1 or 0.3 cells/100 µl/well in 96-well flat-bottom culture plates and mitogenic stimulation with phytohemagglutinin (final concentration, 5 µg/ml; Difco, Detroit, Mich.). To all wells, 100 µl of a feeder mix was added; feeder mix consisted of irradiated (3000 rad) PBMCs of two unrelated donors (each 10^6 cells/ml), irradiated (3000 rad) cells of the EBV-transformed B-cell line JY (2×10^5 cells/ml), and

rIL-2 (20 IU/ml) as growth factor. Approximately 3 weeks later, expanded clones were tested for CPE reactivity in an antigen-specific proliferation assay performed in IMDM supplemented with human serum in duplicate in 96-well flat-bottom culture plates. To 100 µl of T-cell suspension (10^5 cells), 50 µl of irradiated (3000 rad) freshly isolated autologous PBMCs (2×10^5), as a source of antigen-presenting cells (APCs), and 50 µl of CPE (200 µg/ml) were added. After 48 hours, proliferation was measured by [³H]-TdR incorporation as described previously. Criteria for antigen-specific proliferation were a minimum of 1000 cpm and a stimulation index of 5 or more. The stimulation index was the measured counts per minute in the presence of antigen divided by the measured counts per minute in the absence of antigen. CPE-specific T-cell clones were maintained in culture by biweekly restimulation with phytohemagglutinin in the presence of the feeder mix, as described previously. All experiments were performed on day 10 after restimulation.

Surface marker analysis of T-cell clones

For analysis of surface marker expression by the CPE-specific T-cell clones, the following fluorescein isothiocyanate (FITC-) or phycoerythrin (PE)-conjugated monoclonal antibodies were used: CD3-PE (T3), CD4-FITC (T4), CD8-PE (T8), CD45RA-PE (2H4), CD29-PE (4B4), and HLA-DR-FITC (I3), all purchased from Coulter (Hialeah, Fla.). Before labeling, the T-cell clones were separated from the remaining feeder mix cells by using Ficoll-Paque as described for the isolation of PBMCs. Per experiment, 10^5 cells were suspended in 100 µl of phosphate-buffered saline (Coulter) containing 1% bovine serum albumin (Organon Teknika, Boxtel, The Netherlands) and labeled with 10 µl of undiluted solution of the appropriate antibody. After 30 minutes of incubation, the cells were washed twice in 2 ml of phosphate-buffered saline containing 1% bovine serum albumin by centrifugation for 5 minutes at 250 g at 4° C. The final pellet was resuspended in 300 µl of phosphate-buffered saline containing 1% bovine serum albumin. Analysis was performed with an Epics Elite flow cytometer (Coulter). Results were expressed as percentage of positive cells.

EBV transformation of autologous B cells

Antigen-specific proliferation assays with T-cell clones to obtain culture supernatant for the determination of cytokine production were performed with EBV-transformed autologous B cells (EBV-B) as APCs. For EBV transformation, 10^6 PBMCs in 500 µl of IMDM supplemented with 10% fetal calf serum and 4 µg/ml cyclosporine were added to 500 µl of supernatant of a 10-day culture of the marmoset monkey B95-8 cell line³⁸ containing EBV and cultured overnight at 37° C in a 24-well culture plate (Nunc). Cells were washed three times and resuspended in 200 µl of IMDM with 10% fetal calf

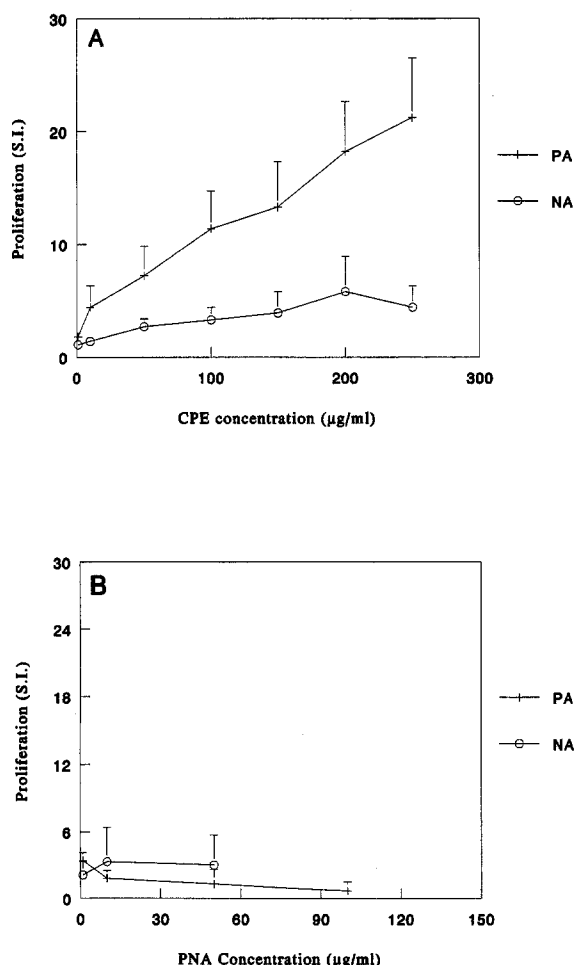


FIG. 1. Proliferative response of PBMCs from patients with peanut allergy (PA, $n = 7$) and nonallergic donors (NA, $n = 10$) to CPE (A) and to PNA (B). Proliferation of PBMCs (2×10^5 cells/well) was determined by measuring [^{3} H]-TdR incorporation after 7 days of incubation in the absence or presence of increasing amounts of CPE or PNA. Results are expressed as mean stimulation index (SI) $\pm$ SEM.

serum and cultured in a 96-well flat-bottom culture plate at 37°C in 5% CO₂ until proliferating cells were observed by light microscopy. EBV-B cells were maintained at a concentration of approximately 10^6 cells/ml in IMDM with 10% fetal calf serum.

Cytokine assays

Cytokine secretion profiles of the CPE-specific T-cell clones were determined after both polyclonal stimulation with monoclonal antibodies against CD3 and CD28 and specific stimulation with CPE in the presence of APCs. For polyclonal stimulation, triplicate cultures of 10^5 cells/well were stimulated in a 96-well flat-bottom culture plate with anti-CD3 monoclonal antibody (CLB-1xE; CLB) and anti-CD28 monoclonal antibody (CLB-28/1; CLB) both at a final dilution of 1:1000 in a volume of 200 μl/well in IMDM

with 10% human serum. For antigen-specific stimulation, 10^5 cells/well in triplicate cultures were stimulated in 200 μl of IMDM/10% human serum with CPE (final concentration, 50 μg/ml) in the presence of 2×10^4 EBV-B/well as APCs. After 36 hours, the culture plates were centrifuged at 300 g for 10 minutes at room temperature. Supernatants from the triplicate cultures were collected, pooled, and stored at -20°C until determination of the cytokine concentration. Both IFN-γ and IL-4 secretion were measured with ELISA kits from Gibco and CLB, respectively, and IL-5 with an ELISA as described before.³⁹

Statistical analysis

Statistical analysis was performed by creating an orthogonal polynomial with linear, square, and cubic components. These polynomials were created for the proliferative response to increasing amounts of CPE of every subject. The mean polynomials of the peanut-allergic and nonallergic groups were tested for statistically significant differences by using a Student's *t* test with a *p* value of less than 0.05 as level of significance.

RESULTS

Peanut-specific proliferation in PBMCs of patients with peanut allergy and nonallergic donors

A clear dose-dependent proliferative response was measured on exposure of PBMCs of patients with peanut allergy ($n = 7$) to CPE of 9918 ± 6834 cpm in the presence of 250 μg/ml CPE (915 ± 816 without CPE), whereas PBMCs of nonallergic control subjects ($n = 10$) showed only very weak responses to CPE of 393 ± 329 cpm in the presence of 250 μg/ml CPE (163 ± 183 without CPE). In Fig. 1 these data are expressed as stimulation index, which represents the measured counts per minute in the presence of CPE divided by the measured counts per minute in the absence of CPE.

Responses in PBMCs of the peanut-allergic group were significantly higher ($p < 0.05$) than those in PBMCs of the nonallergic group from CPE concentration of 50 μg/ml. PNA, the naturally occurring lectin in peanuts, induced only very weak responses in PBMCs of both groups. The responses were not significantly different between the two groups (Fig. 1).

After the demonstration of CPE-specific T-cell reactivity in PBMCs of the patients with peanut allergy, CPE-specific T-cell clones were generated from one of the patients with peanut allergy (NvD), selected for showing a relatively strong PBMC response, which reached a plateau level of 5317 cpm in the presence of 150 μg/ml CPE (444

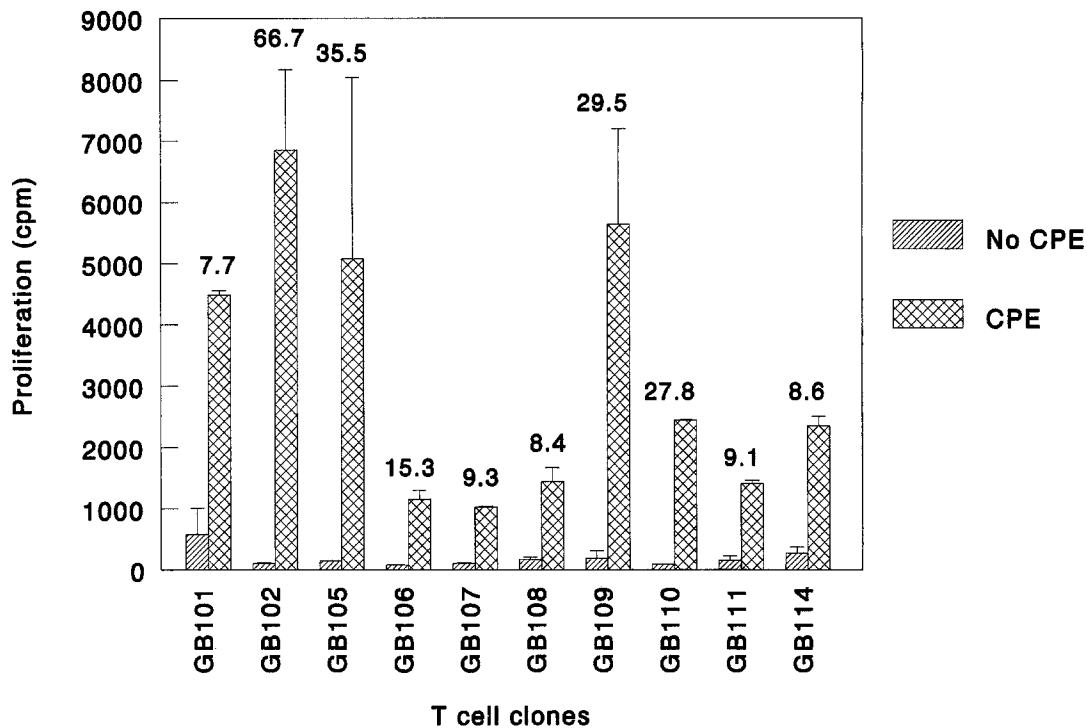


FIG. 2. CPE-specific proliferative responses of T-cell clones from peanut-allergic patient NvD. Cloned cells (10^5 cells/well) were cultured with 10^5 3000 rad irradiated autologous PBMCs as APCs in the absence or presence of 50 μ g/ml CPE. Proliferation was measured after 48 hours of incubation with [^{3} H]-TdR incorporation. Bars express mean values $\pm$ SEM of triplicate cultures, and stimulation index is given on top of the bars.

cpm without CPE). Also, in PBMCs of this patient stimulation with purified PNA induced only a weak proliferative response.

CPE-specific T-cell clones

From a CPE-reactive T-cell line obtained by stimulation of PBMCs of this patient with 150 μ g/ml CPE for 14 days, 49 T-cell clones were generated. When this panel of T-cell clones was tested for CPE specificity, 10 clones proved to be reactive, which were designated GB101, GB102, GB105, GB106, GB107, GB108, GB109, GB110, GB111, and GB114 (Fig. 2). These 10 clones were further studied for their expression of certain surface markers by flow cytometry and for their cytokine secretion profile by ELISA.

Surface marker expression by CPE-specific T-cell clones

The surface marker phenotype of the 10 CPE-specific T-cell clones was determined by flow cytometric analysis 10 days after restimulation with feeder mix. All T-cell clones expressed a typical phenotype of activated memory T_H cells: $CD3^+$,

$CD4^+$, $CD8^-$, $CD45RA^-$, $CD29^+$, and $HLA-DR^+$. The percentage of activated cells expressing HLA-DR varied greatly (10% to 100%) between the different clones.

T_H2 cytokine secretion profile of CPE-specific T-cell clones

Cytokine secretion of the CPE-specific T-cell clones was measured in culture supernatants after 36 hours of stimulation of 10^5 cells with either a combination of anti-CD3 and anti-CD28 or CPE in the presence of APCs. Reactivity of the clones to these different modes of stimulation was confirmed by [^{3} H]-TdR incorporation in identical parallel cultures. As shown in Fig. 3, the CPE-reactive T-cell clones secreted high levels of IL-4 and IL-5 and little or no IFN- γ after strong stimulation, indicating a T_H2 profile. Stimulation with CPE showed a similar profile, except that lower concentrations of the secreted cytokines were measured. IL-4 was produced at a concentration of 200 to 500 pg/ml, IL-5 concentrations ranged from undetectable levels (≤ 200 pg/ml) to 600 pg/ml, and IFN- γ concentrations ranged from undetectable levels

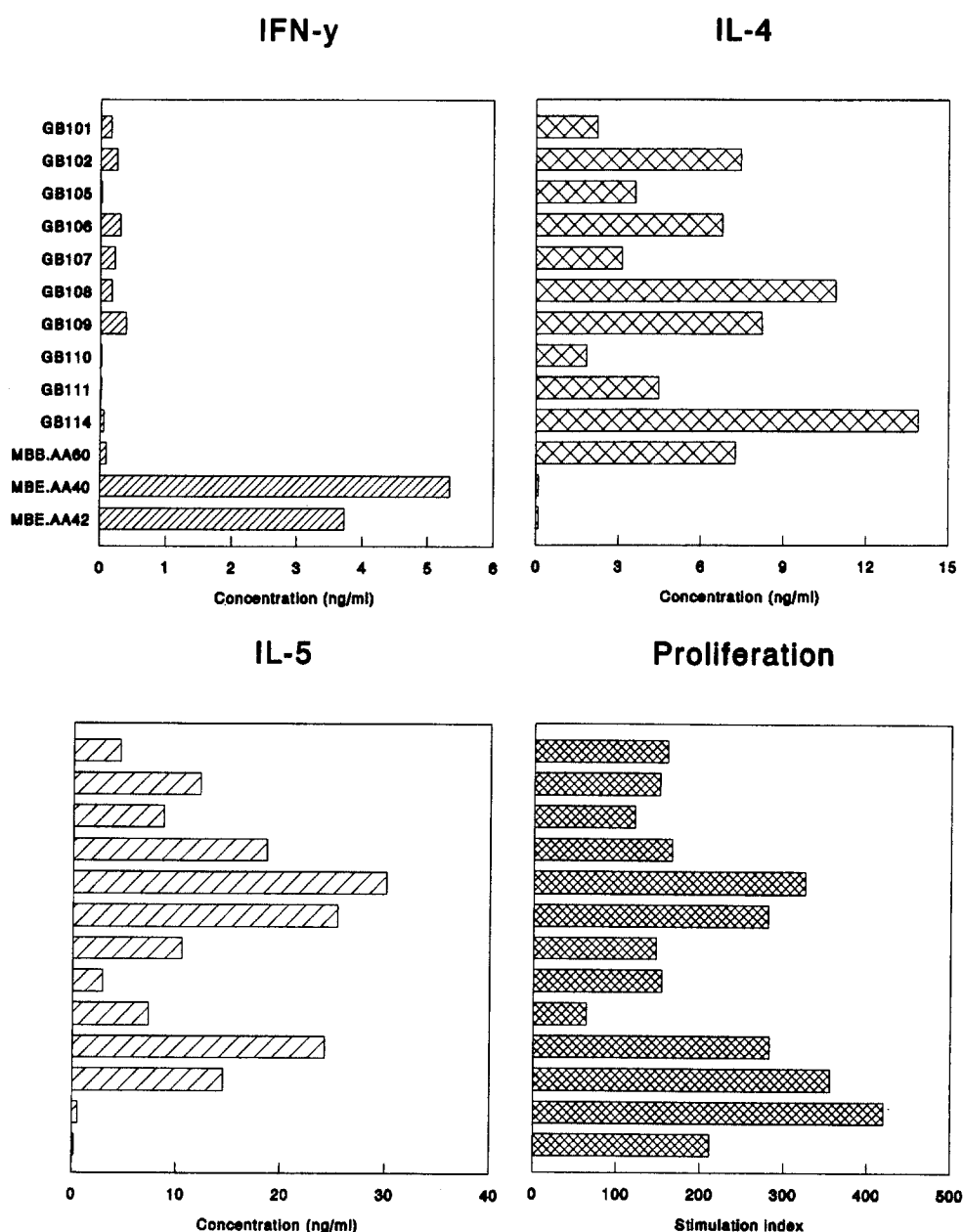


FIG. 3. Cytokine secretion by CPE-specific T-cell clones. Cloned cells were stimulated (10^5 cells/well) for 36 hours with anti-CD3 plus anti-CD28 (both, 1:1000 final dilution), after which the cytokine concentrations in the culture supernatants were measured by ELISA. Secretion levels were compared with those of well-characterized aeroallergen-specific T_{H1} (MBA.AA40 and MBE.AA42) and T_{H2} (MBB.AA60) clones tested identically. Detection levels: IFN- γ , 20 pg/ml; IL-4, 2.4 pg/ml; IL-5, 0.2 ng/ml.

(≤ 20 pg/ml) to 450 pg/ml. The cytokine production was compared with the previously described, well-characterized HDM-specific T-cell clones with a T_{H1} (MBE.AA40 and MBE.AA42) or T_{H2} (MBB.AA60) phenotype, measured under identical experimental conditions.⁴⁰

DISCUSSION

This study shows that PBMCs of patients with peanut allergy harbor peanut protein-responsive T cells, which on cloning appeared to be T_{H2} cells producing high levels of IL-4 and IL-5 but little or no IFN- γ .

The proliferative responses of PBMCs of patients with peanut allergy to CPE were significantly higher than those of nonallergic individuals. This phenomenon has been described previously for inhalation^{25, 41, 42} and food-induced intolerances,⁴³⁻⁴⁵ and this could indicate a higher frequency of responsive T cells. This has been contradicted by others.^{46, 47} Recently, Dorion et al.⁴⁷ showed no difference between patients with peanut allergy, patients with asthma, and nonallergic subjects in response to Ara h 2, a major peanut allergen that has been identified by immunoblotting techniques with patients' sera.⁴⁸ This difference in response could be due to the amount of consumption of peanuts, which is much higher in the American population compared with the Dutch population. Another explanation could be that the lymphocyte stimulation studies of Dorion et al.⁴⁷ were performed in medium without serum, whereas we used medium supplemented with 10% pooled human serum. This serum could contain certain factors that could inhibit or favor the outgrowth of certain cells. Also, Higgins et al.⁴⁹ studied the proliferative response to peanut proteins of patients with peanut allergy and nonallergic individuals. They found a good response in nonallergic individuals as well, although this response tended to be lower than that observed in patients with peanut allergy and higher concentrations of peanut extract (up to 400 µg/ml) were needed. It could very well be that if we had used higher concentrations of our extract, we would have found a response.

It should be mentioned, however, that some patients with peanut allergy whose PBMCs showed no significant proliferative response to CPE were excluded from this comparative study. The nonresponsiveness of this small subgroup of patients could be a result of their peanut-free diet, resulting in a very low frequency of reactive T cells in peripheral blood.⁵⁰ Another possible explanation is that these patients selectively respond to non-soluble peanut compounds or to allergens smaller than 3.5 kd, which are not present in our CPE preparation.

Although PNA, the naturally occurring lectin in peanuts, can induce strong proliferative responses in bovine PBMCs by means of agglutination,³⁵ it induced a slight increase in [³H]-TdR incorporation in human PBMCs of both patients with peanut allergy and nonallergic control subjects. This weak PNA-induced proliferative response cannot account for the specific CPE-induced response in the patients' PBMCs, because CPE used in this study

contained only about 1% PNA (data not shown). Indeed, compared with the proliferation induced by 1 µg/ml or even 10 µg/ml PNA, 150 µg/ml CPE induced a significantly higher proliferation ($p < 0.05$). Moreover, if the proliferative response induced by CPE was caused by the mitogenic activity of PNA, a similarly high proliferative response should also have been observed in PBMCs of nonallergic individuals. However, it cannot be excluded that agglutination of the PBMCs by PNA facilitated the CPE-specific T-cell response to some extent.

The 10 CPE-specific T-cell clones generated from patient NvD, with a strong PBMC response to CPE, all expressed the characteristic phenotype of activated T helper cells (i.e., CD3⁺, CD4⁺, CD8⁻, CD29⁺, and CD45RA⁻). No CPE-specific CD8⁺ T cells were cloned, although approximately 10% of the obtained non-CPE-specific T-cell clones were CD8⁺. The strong differences in HLA-DR expression tested for all clones in parallel, at the same time point in the restimulation cycle, suggest that the panel comprises different CPE-specific T-cell clones, although future experiments on their protein specificity and HLA restriction of antigen recognition will provide more definite data on this matter.

On strong stimulation with anti-CD3 plus anti-28 monoclonal antibodies, all CPE-specific T-cell clones produced considerable to high levels of IL-4 and IL-5 but little or no IFN-γ, indicating a T_{H2} cytokine profile²⁴ as observed by Higgins et al.⁴⁹ Antigen-specific stimulations with 50 µg/ml CPE and autologous EBV-transformed B cells as APCs, resulted in the same cytokine profile but in lower absolute concentrations. Some of the CPE-specific T-cell clones produced detectable amounts of IFN-γ (20 to 500 pg/ml), but compared with IFN-γ production by the well-characterized HDM-reactive T_{H1} clones (MBE.AA40 and MBE.AA42), the concentrations are very low. Compared with an HDM-specific T_{H2} clone, MBB.AA60, tested under identical experimental conditions and with observations of others with several inhalation allergen-specific T-cell clones,^{8-13, 25} the CPE-specific T-cell clones produced IL-4 within a similar concentration range. IL-5 was not detectable in the supernatants of two clones after stimulation with CPE, although these clones are capable of producing high concentrations of IL-5 after strong mitogenic stimulation (Fig. 3). This could be due to the high detection level of the method used or to the small amount of cells used to obtain the supernatant.

So far, the precise protein specificity of these clones has not been determined. Little is known about peanut protein constitution and the allergens it may contain, but recently Dorion et al.⁴⁷ showed that PBMCs of patients with peanut allergy proliferate in response to Ara h 2. Current isolation of proteins from CPE in our laboratory may enable us to determine the specificity and diversity of proteins that are recognized by our panel of CPE-specific T-cell clones.

The observation that all T-cell clones reactive with the total peanut protein extract are IL-4- and IL-5-producing T_{H2} cells and the commonly increased production of IgE and eosinophilia in patients allergic to food suggest an important role of food allergen-specific T_{H2} cells in the pathophysiology of food allergy, similar to the role of T_{H2} cells in inhalation allergy.

We thank Dr. Catharien Hilken (Department of Cell Biology and Histology, University of Amsterdam) for the measurements of IL-5, Hillie Pellegrum (TNO Nutrition and Food Research) for technical assistance, and Dr. Geert Houben (TNO Nutrition and Food Research) for critical reading of the manuscript.

REFERENCES

- Belanti JA. Developmental aspects of food allergy in infancy and childhood. *Immunol Allergy Clin* 1991;11:885-91.
- Schreiber RA, Walker WA. Food allergy: facts and fiction. *Mayo Clin Proc* 1989;64:1381-91.
- Kajosaari M. Food allergy in Finnish children aged 1 to 6 years. *Acta Paediatr Scand* 1982;71:815-9.
- Burks AW, Williams LW, Mallory SB, Shirrell MA, Williams C. Peanut protein as a major cause of adverse reactions in patients with atopic dermatitis. *Allergy Proc* 1989;10:265-9.
- Sampson HA, Metcalf DD. Food allergies. *J Am Med Assoc* 1992;20:2840-4.
- Businco L, Meglio P, Ferrara M. The role of food allergy and eosinophils in atopic dermatitis. *Pediatr Allergy Immunol* 1993;4(suppl 4):33-7.
- Jaffe JS, Metcalf DD. Cytokines and their role in the pathogenesis of severe food hypersensitivity reactions. *Ann Allergy* 1993;71:362-4.
- Wierenga EA, Snoek M, de Groot C, et al. Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-6.
- Del Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. *Allergy* 1992;47:450-5.
- Ebner E, Széphalusi Z, Ferreira F, et al. Identification of multiple T cell epitopes on *Bet v* I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993;150:1047-54.
- van Neerven RJ, van de Pol MM, van Milligen FJ, Jansen HM, Aalberse RC, Kapsenberg ML. Characterization of cat dander-specific T lymphocytes from atopic patients. *J Immunol* 1994;152:4203-10.
- van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML. High frequency of IL-4 producing CD4⁺ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991;97:389-94.
- Ramb-Lindhauer Ch, Feldmann A, Rotte M, Neumann Ch. Characterization of grass pollen reactive T cell lines derived from lesional atopic skin. *Arch Dermatol Res* 1991;183:71-6.
- Snapper CM, Finkelman FD, Paul WE. Differential regulation of IgG1 and IgE synthesis by interleukin-4. *J Exp Med* 1988;167:183-96.
- Romagnani S, Del Prete G, Maggi E, et al. Role of interleukins in induction and regulation of human IgE synthesis. *Clin Immunol Immunopathol* 1989;50:S13-S23.
- Schultz CL, Coffman RL. Control of isotype switching by T cells and cytokines. *Curr Opin Immunol* 1991;3:350-4.
- Vercelli D, Geha RS. Regulation of IgE synthesis in humans: a tale of two signals. *J Allergy Clin Immunol* 1991;88:285-95.
- Heusser ChH, Bews J, Brinkmann V, et al. New concepts in IgE regulation. *Int Arch Allergy Appl Immunol* 1991;94:87-90.
- Lebedin YS, Raudla LA, Chuchalin AG. Serum levels of interleukin 4, interleukin 6 and interferon-gamma following in vivo isotype-specific activation of IgE synthesis in humans. *Int Arch Allergy Appl Immunol* 1991;96:92-4.
- Coffman RL, Carty J. T cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J Immunol* 1986;136:949-54.
- Snapper CM, Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulated Ig isotype production. *Science* 1987;236:944-7.
- Pène J, Rousset F, Brière F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferon γ and α and prostaglandin E₂. *Proc Natl Acad Sci USA* 1988;85:6880-4.
- Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for IgE synthesis induced *in vitro* by human T cell clones. *J Immunol* 1988;140:4193-8.
- Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-73.
- Wierenga EA, Snoek M, Bos JD, Jansen HM, Kapsenberg ML. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *Eur J Immunol* 1990;20:1519-26.
- Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-23.
- Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 1989;73:1504-12.
- Gleich GJ. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 1990;85:422-36.
- Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991;324:1110-8.
- Enberg RN. Food induced oropharyngeal symptoms: the oral allergy syndrome. *Immunol Allergy Clin* 1991;11:767-72.
- Metcalf DD. Food allergy. *Curr Opin Immunol* 1991;3:881-6.
- Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989;83:900-4.

33. Bush RK, Taylor SL, Nordlee JA. Peanut sensitivity. *Allergy Proc* 1989;10:261-4.
34. Yunginger JW, Squillace DL, Jones RT, Helm RM. Fatal anaphylactic reactions induced by peanuts. *Allergy Proc* 1989;10:249-53.
35. Nagi AM, Babiuk LA. Peanut agglutinin (PNA): binding and stimulation of bovine intestinal and peripheral blood leukocytes. *Vet Immunol Immunopathol* 1989;22:67-79.
36. Barnett D, Baldo BA, Howden MEH. Multiplicity of allergens in peanuts. *J Allergy Clin Immunol* 1983;72:61-8.
37. Bøyum A. Isolation of leucocytes from human blood. *Scand J Clin Lab Invest* 1968;21(suppl 97):9-14.
38. Miller G, Lipman M. Release of infectious Epstein-Barr virus by transformed marmoset leukocytes. *Proc Nat Acad Sci USA* 1973;70:190-4.
39. McNamee LA, Fattah DI, Baker TJ, Bains SK, Hissey PH. Production, characterisation and use of monoclonal antibodies to human interleukin-5 in an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991;141:81-8.
40. Wierenga EA, Snoek M, Jansen HM, Bos JD, van Lier RAW, Kapsenberg ML. Human atopen-specific type 1 and 2 T helper cell clones. *J Immunol* 1991;147:2942-9.
41. Rawle FC, Mitchell EB, Platts-Mill TAE. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1984;133:195-201.
42. Burastero SE, Fenoglio D, Crimi E, Brusasco V, Rossi GA. Frequency of allergen-specific T lymphocytes in blood and bronchial response to allergen in asthma. *J Allergy Clin Immunol* 1993;91:1075-81.
43. Scheinmann P, Gendrel D, Charlas J, Paupe J. Value of lymphoblast transformation test in cow's milk protein intestinal intolerance. *Clin Allergy* 1976;6:515-21.
44. Van Sickle GJ, Keating Powell G, McDonald PJ, Goldblum RM. Milk- and soy protein-induced enterocolitis: evidence for lymphocyte sensitization to specific food proteins. *Gastroenterology* 1985;88:1915-21.
45. Kondo N, Agata H, Fukutomi O, et al. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J Allergy Clin Immunol* 1990;86:253-60.
46. May CD, Alberto R. *In vitro* responses of leukocytes to food proteins in allergic and normal children: lymphocyte stimulation histamine release. *Clin Allergy* 1972;2:335-40.
47. Dorion BD, Burks AW, Harbeck R, et al. 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* 1994;93:93-9.
48. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification and characterization of a second major peanut allergen, *Ara h* II, with use of sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962-9.
49. Higgins JA, Lamb JR, Lake RA, O'Hehir RE. Polyclonal and clonal analysis of human CD4⁺ T-lymphocyte responses to nut extracts. *Immunology* 1995;84:91-7.
50. Shinoda S, Kondo N, Fukutomi O, et al. Suppressive effects of elimination diets on T cell responses to ovalbumin in hen's egg-sensitive atopic dermatitis patients. *Clin Exp Allergy* 1993;23:689-95.