

Specific T cell lines for ovalbumin, ovomucoid, lysozyme and two OA synthetic epitopes, generated from egg allergic patients' PBMC

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Summary

Background Proteins of hen egg white are common ingredients of food and difficult to eliminate. Allergens of egg white induce allergic symptoms among relatively high numbers of patients suffering from food allergy. B cell epitopes to hen egg white major allergens have been reported. Considering that IgE antibody formation is mostly T cell dependent, the study of T cell epitopes is essential for both T cell dependent and independent IgE response.

Objectives Little information on T cell epitopes recognizing food allergens has been reported. T cell responses to hen egg white allergens and two synthetic OA peptides located at amino acid residues No. 105–122 and 323–339 were investigated.

Methods Peripheral blood mononuclear cells from hen egg allergic patients were investigated. Various allergens of hen egg white were used for stimulation. Primary proliferation responses were detected followed by the generation of long-term cultures which were examined for their specificity, phenotype, cytokine profile and IgE production. The allergen specific T cell lines were mapped using a panel of 13 synthetic peptides of ovalbumin.

Results Human T cells recognizing ovomucoid, lysozyme and ovalbumin epitope 105–122 are reported for the first time. The cell lines were enriched CD4⁺/CD8⁺ T cells (CD2⁺>95%). Ovomucoid and ovalbumin induced IgE synthesis by a small fraction of B cells (1%) present in the ovalbumin and ovomucoid specific T cell lines.

Conclusions Human T cells recognized several egg white allergens and epitopes within the ovalbumin molecule. Specific IgE was produced in cultures stimulated with ovalbumin and ovomucoid. OA peptides 105–122 and 323–339 have no affinity to the specific IgE of the two patients; an observation which could be of particular interest regarding the mechanisms of peptide-based immunotherapy.

Keywords: allergen specific T cell lines, B cell epitope, cytokine profile, hens egg white allergens, ovalbumin 105–122, ovalbumin 323–339, ovomucoid, T cell epitopes.

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Introduction

The proteins of hen egg white frequently induce hypersensitivity symptoms among egg allergic individuals [1]. Hen egg is an essential ingredient in human as well

as animal food and is difficult to eliminate from the diet [1–3].

Among the many egg white proteins, four major allergens [4] have been detected: ovalbumin (OA, Gal d 2); ovomucoid (OM, Gal d 1); ovotransferrin (OTr, Gal d 3) and lysozyme (Lsz, Gal d 4) (allergen nomenclature revised, see references 26, 27). The complete primary structure and the secondary structure corresponding to the initiation region

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of mRNA of OA have been determined [5,6]. Furthermore, preliminary X-ray analysis of the two crystals of the molecule has been elucidated [7,8].

Several reports on the allergenicity of intact OA [9–13] and two OA peptides (1–10 and 323–339) were reported [14,15]. These peptides encompassed B cell epitopes which were recognized by specific IgE antibodies. OA 323–339 was also demonstrated to be a T cell epitope restricted by I-A^d molecule in mice [16,17]. Recently, an OA specific human T cell line (TCL) was reported that recognized OA 323–339 presented by HLA-DR10 [18].

Studies on T cell epitopes on various allergens are of importance considering that IgE-antibody formation is mostly T cell dependent. Knowledge of allergenic T cell epitopes are considered interesting regarding regulation of IgE antibody formation and may provide the development of antagonists blocking the T cell antigen receptor (TCR) [19–21]. For this purpose and because of the sparse information regarding human T cell epitopes for egg white proteins, we focused on well studied B cell epitopes of OA [9,15,22]. In previous investigations OA 105–122 showed strong affinity to rabbit IgG and to egg allergic specific IgE [23].

OM [13] and Lsz, major egg white allergens, were also investigated for T cell proliferation responses. In this paper we report the establishment of long-term TCL (>6 months in culture) which recognized OA, OM, Lsz and the synthetic peptides OA 105–122 and OA 323–339.

Materials and methods

Cell donors

RBa and ALi are two male, multiallergic patients expressing the HLA haplotypes A02 A19 B12 B17 DQ02 DQ03 DR07 and A01 A03 B08 DQ03 DR03 DR04 respectively, kindly determined at the Institute of Transplantation Immunology, Rikshospitalet, Oslo, Norway.

Radioallergosorbent tests [24] indicated high titres of serum total IgE in the serum of the two patients; 556 KU/l (RBa) and 8580 KU/l (ALi). Serum specific IgE to hen egg white was measured as CAP/RAST class 5 and 6 respectively.

One non-allergic individual (ØSk) was included as a control (S-IgE: 29 KU/l).

Culture medium

RPMI 1640 medium (Gibco, BRL, UK) supplemented with 10% c-inactivated AB⁺ normal human serum (Blood bank, University Hospital, Bergen, Norway), 1% 200 nM

L-glutamine, 2% 5000 IU/ml penicillin/streptomycin (Gibco, BRL, Eu. Div.) and 0.02% 2-mercapto-ethanol (Merck, Munich, Germany): complete (c)RPMI 1640/AB⁺ medium.

Peptides solid phase synthesis

Ovalbumin peptides 1–10, 11–19, 20–33, 34–46, 47–55, 56–70, 71–84, 85–92, 105–122, 132–148, 214–231, 323–339 and 368–385 were synthesized, purified to homogeneity and amino acid sequence ascertained by the methods currently used in our laboratory [11].

Allergens

Crude hen egg white extract was prepared as described earlier [25], diluted in cRPMI/AB⁺ medium and used in final volume of 2.5% (v/v)/ml culture. OA, Gal d 2 (hen's egg type VII, A-7641, Sigma Chemical Co., St Louis, MO 63178, USA), the synthetic OA peptides, OM, Gal d 1 (Trypsin inhibitor from purified hen egg white ovomucoid Type III-0, No. T-2011, Sigma) and Lsz, Gal d 4 (from hen egg white, Grade VI, L-2879, Sigma) were used at final concentrations of 0.05 mg/ml. The allergen nomenclature was revised according to references 26 and 27. Analysis of the purities of the commercial egg white allergens was extensively studied [28].

Preparation of allergen specific T cell lines (TCL)

Peripheral blood mononuclear cells (PBMC) were collected from the interface after Ficoll Paque (Kabi Pharmacia, Uppsala, Sweden) centrifugation, washed and resuspended in cRPMI 1640/AB⁺ medium.

PBMC ($1\text{--}1.3 \times 10^6$ /well) were cultured in 24-well cell culture clusters (Costar, Cambridge) with or without allergen (0.05 mg/ml), final volume 2 ml. Five days after initiating the culture, rIL-2 (20 U/ml, Boehringer Mannheim, Germany) was added to each well. On day 7, each culture was collected, 200 µl in triplicate, cultured in 96-well cell culture clusters (Costar) and pulsed overnight by adding 20 µl, 0.05 mCi/ml [methyl-³H] thymidine (Amersham, Denmark).

Following stimulation the cultures were investigated for primary proliferation responses as measured by ³H-thymidine uptake. Cells from wells expanded by allergen-induced proliferation were isolated by Ficoll Paque centrifugation. Isolated T cell blasts were recultured in 24-well clusters and restimulated by phytohaemagglutinin PHA (0.8 µg/ml), rIL-2 (20 U/ml) and autologous irradiated (30 Gy) PBMC (1×10^5 /well). For maintaining the long-term TCL cultures the procedure was repeated each 14th.

day. The enriched allergen specific TCL were investigated for secondary proliferation responses 8 days after the first restimulation procedure.

Allergen specific TCL proliferation assay

Specific proliferation responses were tested 14 days after the last addition of PHA and IL-2. In 24-well plates, enriched T cells, harvested after Ficoll centrifugation (1×10^6 cells/well), added irradiated (30 Gy) autologous PBMC (1×10^5 cells/well) and specific allergen (0.05 mg/ml) respectively, were cultured for 3 days. Thereafter, 200 μ l of each culture were seeded in 96-well plates and added to 20 μ l, 0.05 mCi/ml [methyl- ^{3} H] thymidine. The radioactive uptake was determined the next day and cpm was given as mean of triplicate cultures. Cultures seeded in 24-well plates were harvested on day 4; pelleted cells were resuspended in complete medium and phenotype markers were stated by flow cytometry. The culture filtrates were stored at -20° for cytokine profile analysis.

When testing allergen specific responses to synthetic OA peptides, each specific TCL, harvested after Ficoll centrifugation, was cultured (8×10^4 cells/well) in 96-well plates, added irradiated autologous APC (10^4 – 10^5 cells/well) and the OA peptides, 0.05 mg/ml. The cells were pulsed overnight by [methyl ^{3} H] thymidine as described before. Cultures were harvested at day 5.

Phenotype analysis of TCL

Allergen stimulated TCL were analysed for the expression of CD4/CD8 and CD2/CD19 using monoclonal antibody T4RD/T8-FITC (Coulter 6603802) and T11RD1/B4-FITC (Coulter 6603801) respectively. The phenotypes were kindly analysed at the Immunochemical Laboratory (Professor Roald Matre), University Hospital, Bergen.

IgE determination

The procedure from Kabi Pharmacia Diagnostics AB (Uppsala, Sweden) was modified to allow quantitation of low levels of total IgE. Rabbit anti-human IgE, 100 μ g/ml, 300 μ l in PBS (sodium phosphate 1.19% w/v, sodium azide 0.01% w/v and Tween 20 1%, pH adjusted to 7), was coupled to polystyrene tubes 70×11 mm, and incubated overnight at 37°C . The tubes were washed three times with PBS, 2.5 ml. RPMI 1640 medium and 10% foetal calf serum (FCS) were added (300 μ l) to the tubes before 1 h incubation at 37°C . The tubes were washed as described, and could be stored at -20°C .

Culture filtrates or IgE standard solutions were added (300 μ l) to the coated test tubes and gently shaken overnight at room temperature (RT). As standards, Pharmacia IgE from 400–0.0188 KU/l, were used. All dilutions were made in RPMI 1640 including 10% FCS.

The tubes were washed, before 300 μ l Anti-IgE ^{125}I Phadebas RAST isotope (Kabi Pharmacia) was added. The radioactivity was adjusted to 70 000 cpm/300 μ l. Tubes were shaken overnight at RT, washed three times and counted in a gamma scintillation counter (1282 Compugamma, LKB, Wallack, Sweden) for 2 min. All samples were run in triplicates. Results were given as cpm and further interpolated as ng/ml IgE. The sensitivity of this assay was 50 pg/ml.

IL-4, IL-5, IFN- γ and TNF- α determinations

Estimation of IL-4, IFN- γ , IL-5 and TNF- α in culture filtrates of specific allergen-stimulated TCL were performed according to manufacturers' instructions, using commercial enzyme linked immunosorbent assay kits (human IL-4; human IFN- γ ; human IL-5; human TNF- α ; Quantikine, R & D Systems Europe, Abingdon, UK). The sensitivities of these assays were 3.0 pg/ml both for IL-4 and IFN- γ , 4.4 pg/ml for IFN- α and 1 pg/ml for IL-5. The specificities were documented by the manufacturer.

Results

Preparation of allergen specific TCL

Primary allergen specific proliferation responses of PBMC from two egg allergic patients are shown in Fig. 1. Both RBa and ALi responded upon stimulation with OA (46 172 cpm and 3919 cpm) and OM (32 729 cpm and 2769 cpm). Furthermore, PBMC of patient RBa proliferated when stimulated with the synthetic peptide OA 105–122 (29 635 cpm). TCL of patient ALi responded when stimulated with Lsz (6128 cpm) and OA 323–339 (4025 cpm). Cpm of control TCL (unstimulated TCL + irradiated APC) for patient RBa and ALi, were 24 660 cpm and 1465 cpm respectively. Results of Fig. 1 and Fig. 2 were given as ratios of proliferation index: stimulation induced cpm/control cpm.

Ficoll selected T cell blasts were restimulated by adding PHA and irradiated autologous APC as described (Materials and methods) and further investigated for their secondary responses. Especially cultures initially stimulated with the two synthetic peptides (OA 105–122, OA 323–339), showed very high secondary responses (>50 000 cpm) in both patients (results not shown).

When allergen specific proliferation responses were tested (Fig. 2), the results showed that TCL of patient RBa gave very high responses when stimulated with the allergen panel (hen's egg white extract, 224 674 cpm; OA, 248 012 cpm; OM, 141 197 cpm; Lsz, 199 426 cpm; OA 105–122, 235 704 cpm; OA 323–339, 244 760 cpm). Not only specific TCL derived from patient ALi, added OA 105–122 (7834 cpm), OA 323–339 (9996 cpm), but also OM (2687 cpm) and OA (3089 cpm) showed significant responses. Cpm of control TCL, patient RBa and ALi, were 4090 cpm and 384 cpm respectively.

Prior to specific allergen stimulation, the TCL cultures had not received PHA or IL-2 for the last 14 days.

PBMC from one non-allergic individual (S-IgE: 29 KU/l), initially included as a control, were stimulated with the panel of egg white allergens described and major allergens of cat (Fel d 1), Birch (Bet v 1), cod fish (Gad c 1) and mite (Der p 1). Primary responses (26000 cpm) and long-term TCL could only be established from cultures stimulated with Der p 1 (results not shown).

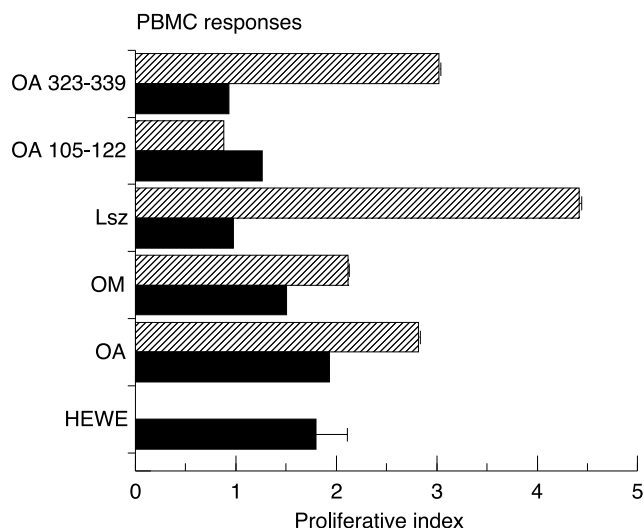


Fig. 1. Primary allergen specific proliferation responses of PBMC from two allergic patients. PBMC ($1\text{--}1.3 \times 10^6$ cells/well) were stimulated with a panel of hen egg white proteins/solid phase synthetic peptides at final concentrations of 0.05 mg/ml, while the crude hen egg white extract concentration was 2.5% v/v/ml. Proliferation responses were assayed on day 8 and given as cpm of triplicate cultures (see Results in text). Control cultures are with no addition of allergens. Results are given as ratios of proliferation index: allergen induced proliferation, cpm/spontaneous proliferation of control, cpm. Patient RBa (■): significant responses were obtained when hen egg white extract (HEWE), OA, OM and OA 105–122 were used as allergens. Patient ALi (▨): significant responses were obtained using OA, OM, Lsz and OA 323–339 as stimulants. HEWE was not tested on this patient.

Allergen specific TCL and responses to synthetic OA peptides

Thirteen synthetic OA peptides were tested for their potential to induce proliferation responses, using long-term cultures of allergen specific TCL from the two patients, shown in Fig. 3, a and b. Cultures stimulated with PHA, IL-2 or PHA+ IL-2 were also investigated (all results are not included in Fig. 3). In Fig. 3 the antigen specific proliferation responses are given as cpm. Very low cpm (<300 cpm) of control wells, similar to background counts for unstimulated TCL + irradiated APC, were subtracted.

For patient RBa, Fig. 3a, most allergen specific TCL and cultures which only had received PHA together with IL-2, showed responses to OA peptide 34–46. The peptide was not recognized by TCL stimulated with OA 323–339, Lsz, PHA or IL-2. Furthermore, TCL with specificity for OA 323–339 recognized several other OA peptides, especially OA 132–148, OA 214–231 and OA 323–339 but also OA 105–122, intact OA and hen's egg white extract.

In patient ALi, Fig. 3b, cultures specific for OA, OM, OA 323–339 as well as TCL stimulated with PHA and/or

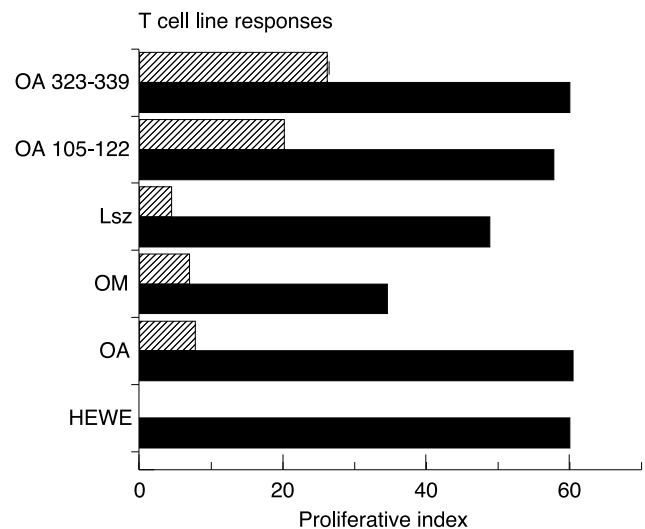


Fig. 2. Allergen specific proliferation responses of TCL. TCL (1×10^6 cells/ml) were stimulated with specific allergen (0.05 mg/ml), and added irradiated (30 Gy) autologous APC (10^5 cell/well). Responses were measured on day 4 and given as mean cpm of triplicate cultures (see Results in text). Results are given as ratios of proliferation index: allergen induced proliferation, cpm/spontaneous proliferation of control, cpm. Patient RBa (■): very high T cell responses could be observed for the six allergen preparations tested. Patient ALi (▨): the two synthetic peptides in particular, but also OM and OA, gave significant specific responses, as compared to control. Hen egg white extract (HEWE) was not tested on this patient.

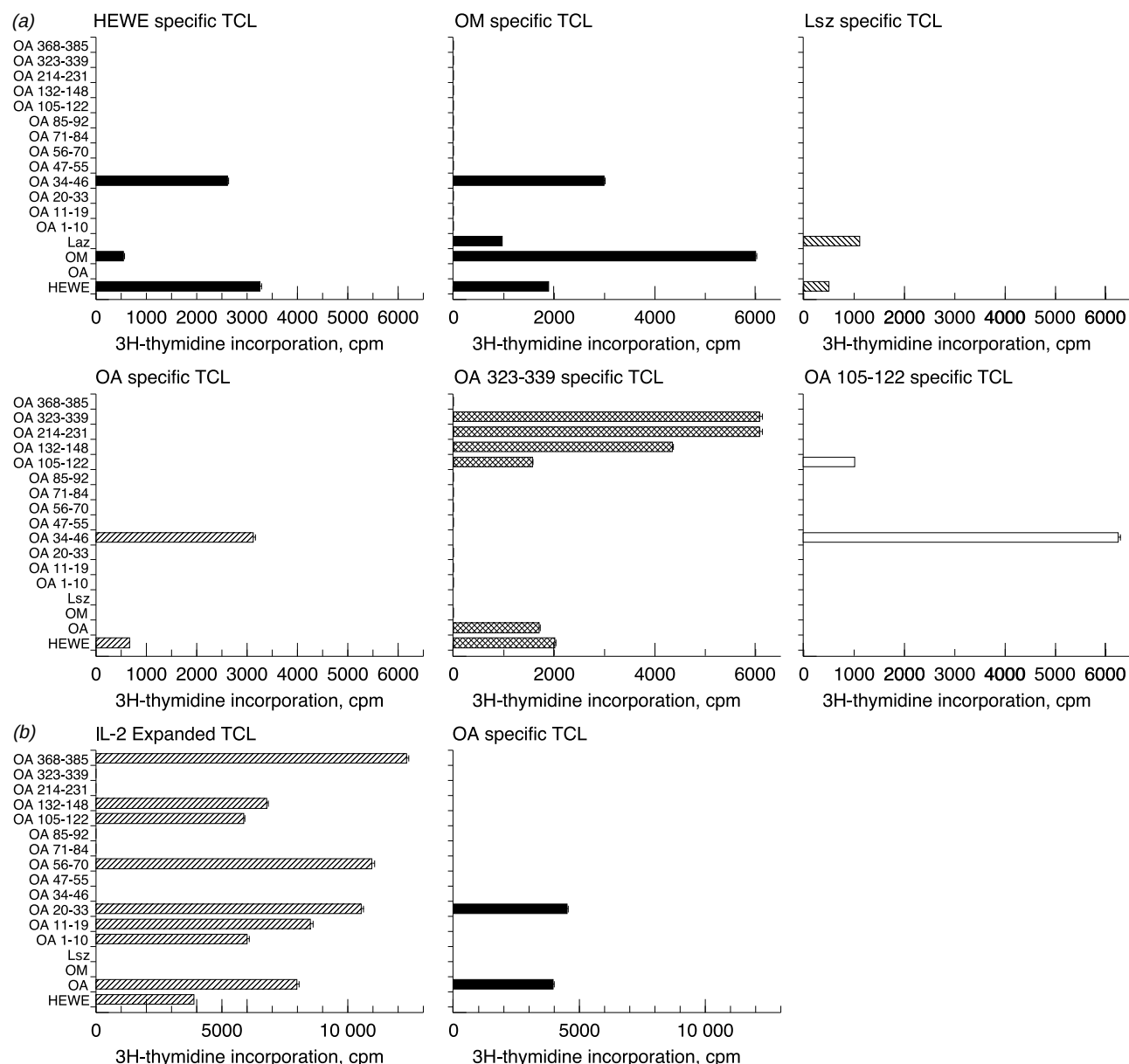


Fig 3. Long-term cultures of allergen specific TCL established from two egg allergic patients and proliferation responses obtained when testing 13 different synthetic OA peptides (0.05 mg/ml). (a) Patient RBA: peptide OA 34–46 triggered proliferation of most TCL. OM specific TCL recognized Lsz. TCL specific for OA 323–339 recognized peptides OA 323–339, OA 214–239, OA 132–148 and OA 105–122 as well as intact OA and hen egg white extract (HEWE). (b) Patient ALi: TCL cultured in the presence of IL-2 only, recognised 7 of the 13 OA peptides; OA 1–10, 11–19, 20–33, 56–70, 105–122, 132–148, and 368–385. Intact OA and crude egg white extract were also recognized by these cells. Most of the allergen specific TCL recognized OA 20–33, as demonstrated by the OA specific TCL. Control (TCL + irradiated APC) values were generally very low (<300 cpm) and similar to background counts. Therefore, these cpm values were subtracted from the antigen induced cpm responses, shown in this figure.

IL-2, responded to OA peptide 20–33. Furthermore, IL-2 expanded TCL responded vigorously to 7 out of 13 OA peptides; 1–10, 11–19, 20–33, 56–70, 105–122, 132–148, 368–385, intact OA and crude egg white extract. Generally (for both patients), control wells (TCL + APC)

showed responses <300 cpm. However, control wells of the IL-2 expanded TCL showed high spontaneous proliferation (>10 000 cpm, irradiated autologous APC control <200 cpm). Proliferative responses of IL-2 expanded TCL, and of the OA specific TCL are shown in Fig 3b.

Table 1. Phenotyping of long-term, allergen specific T cell lines (TCL). TCL, generated from two hen egg white allergic patients, RBa and ALi, were stimulated with specific allergen (0.05 mg/ml) or PHA (0.8 µg/ml) + IL-2 (20 U/ml) and investigated for CD4⁺ and CD8⁺ expression on day 5

T cell lines	RBa: CD4 ⁺ /CD8 ⁺ (%)	ALi: CD4 ⁺ /CD8 ⁺ (%)
Control	41/ 40	nv
Hen egg white extract	61/ 27	nd
OA	60/ 26	88/6
OM	40/ 44	92/3
Lsz	35/ 49	nv
OA 105-122	37/ 49	90/3
OA 323-339	32/ 53	89/1
IL-2	17/ 80	60/10
PHA+IL-2	3/ 87	44/35

nd = not determined, nv = non-viable.

Similarly, for both patients, each TCL recognized their own peptide specificity and most cultures responded to hen egg white extract. OM specific T cells recognized Lsz. Stimulation responses after the addition of allergens/peptides and mitogens were tested at least twice against each culture and reproducible results were found.

Flow cytometry

CD4/CD8 ratios of each allergen specific TCL are described in Table 1. The phenotype markers CD4, CD8, CD2 and CD19 were investigated on day 4, following allergen specific stimulation and harvesting of culture filtrates for cytokine analysis. Allergen stimulated TCL of patient RBa were rich in CD8⁺ T cells, in contrast to allergen specific TCL of patient ALi which were mostly CD4⁺ (>88%). In both patients, proportion of CD8⁺ T cells appeared to increase when IL-2 and PHA + IL-2 were used as stimulants. In all cultures studied CD2⁺ was >95%.

Cytokine production

IL-4, IFN- γ , IL-5 and TNF- α were measured in culture filtrates of TCL stimulated with specific allergens, IL-2, PHA and PHA + IL-2. Culture filtrates from TCL added irradiated autologous APC were used as controls. Results are depicted in Table 2.

None of the cytokines were detected in filtrates from control cultures with the exception of low amounts of IL-5, in cultures derived from patient RBa.

Filtrates collected from TCL (patient RBa only) stimulated with hen's egg white extract, OA and OM, showed significant values of IL-4 (375, 165 and 62 pg/ml respectively). IL-4 could also be detected in cultures added IL-2 (82 pg/ml) and PHA + IL-2 (16 pg/ml) (RBa) and in OA 323-339 (21, 28 pg/ml) and OA 105-122 (21,

Table 2. Cytokine (IL-4, IL-5, IFN- γ , TNF- α) content in culture filtrates from allergen specific T cell lines established from PBMC of two allergic patients, RBa and ALi. The TCL were stimulated with specific allergen, 0.05 mg/ml, and the cell filtrates were harvested on day 5. TCL stimulated with IL-2 (20 U/ml), PHA (0.8 µg/ml) or PHA + IL-2 were also investigated. Samples were run in duplicate in a quantitative sandwich enzyme immunoassay. Cytokine concentrations were expressed as pg/ml

T cell lines	Cytokines (patient RBa)				Cytokines (patient ALi)			
	IL-4	IL-5	IFN- γ	TNF- α	IL-4	IL-5	IFN- γ	TNF- α
Control	0	11.7	0	0	0	0	0	0
Egg white extract	375	>1500	480	42	nd	nd	nd	nd
OA	165	>1500	750	69	0	12	69	15
OM	62	555	420	34	0	345	660	54
Lsz	0	>1500	276	5805	0	23	0	0
OA 105-122	21	990	840	6405	21	111	84	0
OA 323-339	21	1080	660	63	28	54	0	0
IL-2	82	>1500	262	38	nd	nd	nd	nd
PHA+IL-2	16	156	930	48	nd	nd	nd	nd
PHA	0	0	54	0	nd	nd	nd	nd

nd = not determined; >1500 pg/ml IL-5 indicate greater concentrations than the highest standard.

Table 3. Levels of IgE in culture filtrates collected from specific allergen stimulated (0.05 mg/ml) TCL derived from two hen egg white allergic patients RBa and ALi. The culture filtrates were harvested on day 5. IgE is given as ng/ml and % binding.

T cell lines	RBa		ALi	
	IgE (ng/ml)	% IgE (binding)	IgE (ng/ml)	% IgE (binding)
Control	0.792	0.48	1.008	0.56
Hen egg white extract	0.864	0.53	0.936	0.56
OM	2.664	1.1	2.442	1.03
Lsz	0.828	0.51	1.116	0.56
OA	1.296	0.66	1.296	0.68
OA 105–122	0.828	0.51	0.864	0.51
OA 323–339	0.936	0.54	0.936	0.55

21 pg/ml) stimulated cultures, patient RBa and ALi respectively. IFN- γ was found in all filtrates tested, ranging from 54 to 930 pg/ml, except Lsz and OA 323–339 specific filtrates of patient ALi. IL-5 was found at very high concentration in all specific allergen stimulated (555–1500 pg/ml) and IL-2 (1500 pg/ml) stimulated TCL filtrates of patient RBa. Significant amounts of IL-5 were also present in the filtrates collected from allergen specific TCL of patient ALi (12–345 pg/ml). TNF- α was found in moderate amounts (15–69 pg/ml) in most of the culture filtrates tested, in both patients.

IgE in filtrates of allergen specific TCL

Filtrates collected from OA and especially OM specific TCL (both patients) showed IgE values significantly higher than in unstimulated TCL, as shown in Table 3. Specific allergen induced IgE secretions, given as ng/ml, were 2.664 and 2.442 (OM), 1.296 and 1.296 (OA) versus 0.792 and 1.008 (unstimulated control), for patients RBa and ALi respectively.

Discussion

Little is reported on human T cell epitopes reactive to any of the major food allergens. Despite knowledge of the complete primary structure of OA, only in the murine system were OA 323–339 and OA 258–281 reported to encompass T cell epitopes [16,17,29]. An OA specific TCL, derived from a hen egg allergic patient, recognized OA 323–339 [18]. Long-term TCL cultures specific for OM, Lsz and the synthetic peptide OA 105–122 are reported in the present study.

The synthetic peptides OA 323–339 and OA 105–122 were used as stimulants because of their amphipathic nature, and thus predicted to be immunogenic for T helper cells [30]. The observation that CD4⁺ T cell lines and clones could be divided into T helper sub-populations according to their cytokine profiles has been shown for inhalation allergens [31–34]. Cytokine detection (IL-4, IL-5, INF- γ and TNF- α) was performed to identify T cell sub-populations involved in antigen recognition. Our results indicated a striking difference in the amount produced and overall cytokine profile detected in the allergen stimulated TCL, when comparing the two patients. In patient RBa, most of the cultures produced IL-4, and very high amounts of IL-5. IL-5 is a pro-inflammatory cytokine that promotes production and function of eosinophils and provides B cell help [35].

All cultures studied (patient RBa) produced IFN- γ , generally >5-fold the concentrations of IL-4. TNF- α , a multi-functional cytokine, produced by all T subtypes as well as other cells and considered as a potent contributor to allergic inflammation [36,37], could also be detected. TCL of patient ALi produced relatively low amounts of all cytokines tested, which was not due to a lower number of cells. The TCL secretion of INF- γ was higher than IL-5. Furthermore, IL-4 could only be detected in filtrates of the two OA-peptide specific TCL, following stimulation with respective synthetic peptides.

Mitogen stimulation (PHA with or without IL-2) had no detectable effects on the production of IL-4 and IL-5, and differed from allergen mediated activation. It is possible that PHA stimulation of T cells requires a specific co-stimulatory signal for IL-5 production, since normal PBMC produced IL-5 after stimulation with PHA + PMA [38]. Recently it was reported that production of IL-5 was closely related to IL-2 production [39].

Our results indicated that allergen specific TCL of patient RBa contained a mixture of T helper sub-populations, although the majority of cytokines secreted were TH2-like (IL-4, IL-5). This was in contrast to the TH1/TH0-like profile observed for patient ALi (mostly INF- γ). The high proportion of CD8⁺ T cells present in TCL cultures of patient RBa probably contributed to cytokine profile. Although the significance of CD8⁺ T cells in atopy has been little investigated, recent observations revealed that CD8⁺ T cells derived from rats [40] and humans [41,42] have the potential to produce TH1 and TH2 cytokines. Furthermore, an OA specific, H-2K^d restricted, CD8⁺ T cell clone was shown to produce a soluble factor which suppressed antibody production in an OA specific manner [43]. Total serum IgE of patient RBa was much lower than in patient ALi (556 versus 8580 KU/l). It is possible that the relatively high proportion of CD8⁺ T cells in patient RBa contributed to some downregulation of IgE synthesis.

When measuring IgE secreted by allergen specific stimulated TCL, in both patients, only TCL stimulated with OA and especially OM showed significantly higher amounts of IgE when compared to controls. The amount of B cells (CD19⁺) present in the stimulated TCL was <1%; indeed only small fractions of human B cells are required for IgE response [44]. Interestingly, allergen specific IgE in serum from both patients, showed binding to intact molecules of OA and OM, but not to the peptides OA 105–122 and OA 323–339 (results not shown). This implies that these two synthetic peptides, though interactive at the T cell level, have no affinity to specific IgE at the B cell level. Indeed, this could be an important observation regarding experimental peptide-based immunotherapy [20].

OM specific TCL recognized Lsz. Both immunoautoradiographic analysis [4] and high performance liquid chromatography (HPLC) revealed that the commercial OM-preparation used was contaminated by Lsz [28].

To investigate potential T cell epitopes of OA molecule, individual TCLs were cultured in the presence of a panel of 13 synthetic OA peptides. Again, the two patients differed markedly in their responses to different OA peptides. Only OA peptides 34–46 and 20–33 were recognized by most TCL cultures of patient RBa and ALi respectively. Accordingly, the mechanisms of allergen stimulation seem to have individual differences, as TCL from patient RBa specific for OA 323–339, recognized several other OA peptides (OA 105–122, OA 132–148 and OA 214–231), while IL-2 expanded TCL from patient ALi, which had not been exposed to allergen *in vitro*, recognized seven OA peptides (1–10, 11–19, 20–33, 56–70, 105–122, 132–148, 368–385) (Fig. 3).

Despite the low number of allergic patients investi-

gated, the data demonstrated that various human T cell populations have the potential to recognize several regions of the OA molecule.

For further investigations, overlapping peptides covering the whole OA molecule will be tested on a larger number of egg allergic and non-allergic individuals. A comparison between allergic and non-allergic T cell responses towards these allergens could reveal the 'disease related' epitopes. By increasing our knowledge of T and B cell epitopes, explaining immunotherapeutic mechanisms at the level of APC, allergen and T cell interactions could be achievable.

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