

Toshiyuki Katsuki
Naoki Shimojo
Kiri Honma
Hajime Tsunoo
Yoichi Kohno
Hiroo Niimi

Department of Pediatrics,
Chiba University School of Medicine,
Chiba, and Meiji Institute of Health Science,
Odawara, Japan

Establishment and Characterization of Ovalbumin-Specific T Cell Lines from Patients with Egg Allergy

Key Words

Ovalbumin
T cell line
Restriction elements
Eptiope
Cytokine
Hen egg allergy

Abstract

In order to investigate T cell recognition of allergens in hen egg allergy, we have established 30 ovalbumin (OVA)-specific T cell lines (TCLs) from peripheral blood mononuclear cells of 6 patients with atopic dermatitis, who are positive for IgE antibodies to OVA and clinically allergic to hen egg, and characterized them for their cytokine production pattern. All TCLs we could study were mainly composed of CD4⁺ T cells. Most TCLs produced significant amounts of interleukin 4 (IL-4) and IL-5 but no or very little interferon γ on antigen stimulation, suggesting that these TCLs belong to TH2-type T cells. Restriction elements and epitope specificities were further studied on some TCLs. Antibody blocking of the proliferative responses of the TCLs to OVA indicated that HLA-DR are acting as the dominant restriction elements for these TCLs with minor contribution of HLA-DQ. By use of 187 overlapping synthetic peptides covering the whole sequence of OVA, at least 3 different T cell epitopes were identified.

Introduction

Food allergies are an abnormal immune response to foods and present various clinical features such as atopic dermatitis (AD), allergic rhinitis, bronchial asthma and allergic gastroenteritis. It has been demonstrated that IgE antibodies to food allergens are deeply involved in the development of food allergies including AD [1], and analysis of food-allergen-specific IgE antibodies, such as elucidation of antibody epitopes, has so far been a focus of study in food allergy. Elucidation of the T cell response to food allergens at the molecular level is pivotal since they mediate allergic inflammation as well as control allergen-specific IgE antibody production. In fact, establishment of T cell lines and clones specifically responding to aeroallergens facilitated

our understanding of allergen-specific T cell functions [2–4]. In contrast to aeroallergen, few food-allergen-specific T cell lines (TCLs) and clones have so far been described [5] and no comprehensive studies about cytokine production of food-antigen-specific T cells in patients with food allergy have been documented. This is partly due to difficulties in obtaining large blood samples from patients with food allergy, who usually are in their early infancy and/or present a weak peripheral blood mononuclear cell (PBMC) response to food allergens.

Hen egg allergy is the most frequently observed food allergy. Ovalbumin (OVA) is the most abundant and is also known as the most allergenic component in egg proteins [6]. Recently, by use of a better TCL-establishing method [7], we first reported the establishment and characterization

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34

© 1996 S. Karger AG, Basel
1018–2438/96/1094–0344 \$10.00/0

Correspondence to: Dr. Yoichi Kohno
Department of Pediatrics
Chiba University School of Medicine
1-8-1 Inohana, Chuo-ku
Chiba 260 (Japan)

Received:
July 31, 1995
Accepted after revision:
October 11, 1995

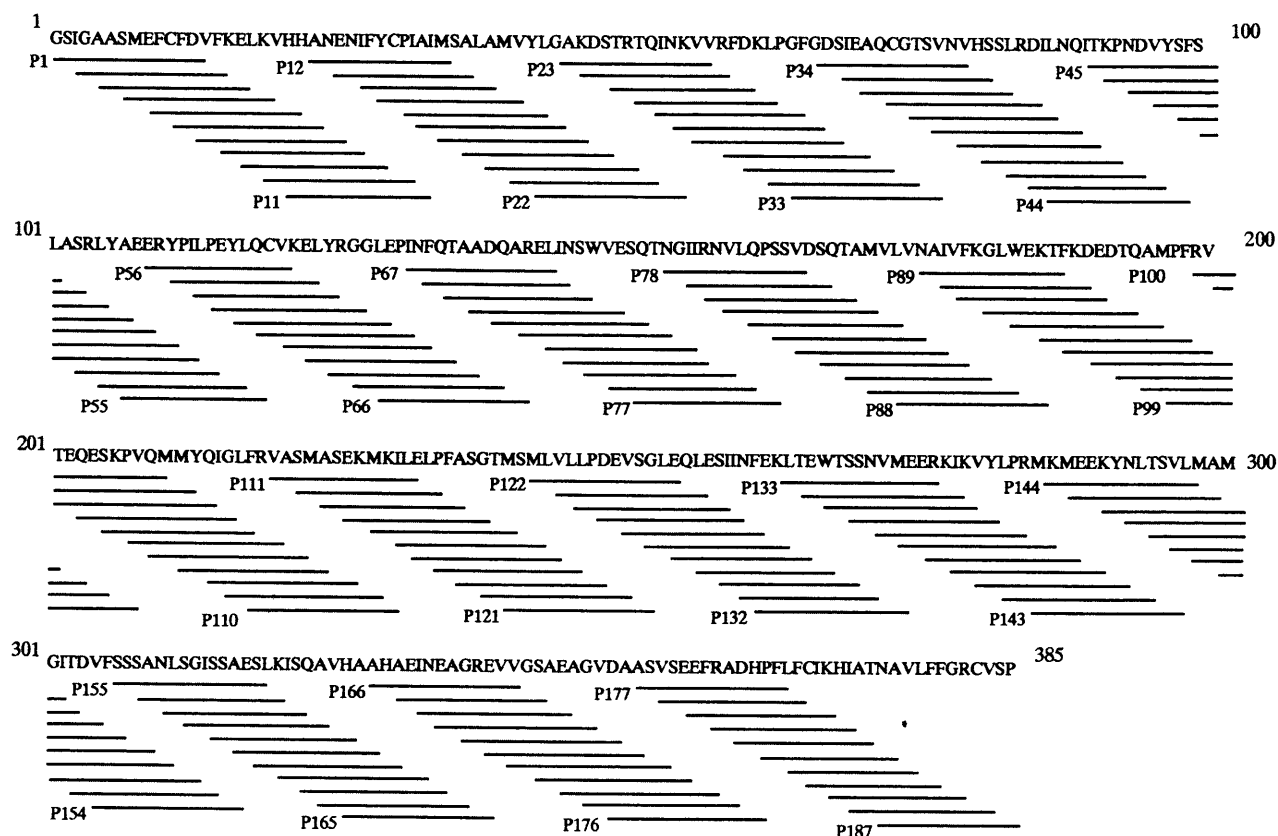


Fig. 1. Panel of 187 overlapping 13mer synthetic peptides covering the whole sequence of OVA. Peptides were synthesized by the 'multi-pin method'. Each peptide is offset by 2 residues from the preceding peptide, thus overlapping the preceding and the following one by 11 residues.

of an OVA-specific TCL from an AD patient with egg allergy [5]. This TCL produced significant amounts of interleukin (IL)-5 but no or very little IL-2 and interferon (IFN) γ on OVA stimulation, suggesting that the TCL might be involved in OVA-induced allergic reactions. In this report, in order to elucidate function of OVA-reacting T cells in egg allergy, we established more OVA-specific TCLs from several AD patients who were allergic to egg white and examined their cytokine production pattern. In addition, we further studied restriction elements for T cell responses and epitopes recognized by these TCLs.

Materials and Methods

Patients

Six AD patients (3 females and 3 males, age range 1–5 years, mean 2.8 years) were studied. All patients displayed hypersensitivity to egg white diagnosed on the basis of food elimination and challenge tests described by Sampson [1], elevated serum total IgE levels and posi-

tive scores in radioallergosorbent test (RAST) for egg white ($>2+$). High levels of IgE antibodies to OVA were also detected by ELISA (data not shown).

Antigen and Reagents

OVA (grade VII) and β -lactoglobulin were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Rhodamine-labeled anti-CD4 and FITC-labeled anti-CD8 antibodies for flow cytometry were purchased from Coulter (Miami, Fla., USA).

Overlapping Peptides

A panel of 187 overlapping 13mer peptides was synthesized by use of the multi-pin method (Chiron Mimotopes Pty. Ltd., Clayton, Australia) spanning the 385 residues of the OVA sequence, each peptide offset by two residues from the preceding peptide (fig. 1). Thus, each peptide overlapped the preceding and the following one by 11 residues. Because it was impractical to screen each peptide separately on each TCL for its ability to cause proliferation, we first investigated proliferation of the TCL in each of 17 pools of 11 sequential overlapping peptides, followed by testing of individual peptides from the most recognized pools when enough numbers of TCL become, available for further analysis.

Generation of OVA-Specific TCLs

OVA-specific TCLs were generated by the method previously described [5]. Briefly, 2×10^5 PBMCs of atopic patients allergic to hen egg were incubated in 96-well round-bottom plates (Costar, Cambridge, Mass., USA) with 30 µg/ml OVA in 100 µl of RPMI 1640 medium (Gibco, Grand Island, N. Y., USA) containing 10% heat-inactivated human serum, 2×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin. Fresh 30 IU/ml recombinant rIL-2 (kindly provided by Takeda Chemical Industries, Osaka, Japan) was added on the 3rd day of the culture and rIL-2 was fed every 3–4 days for a total period of 20 days. On day 20, cells in the plate were washed to remove rIL-2 and resuspended in 100 µl of culture medium. Cells of each well were split in two adjacent wells. Autologous 100,000 Epstein-Barr-virus (EBV)-transformed lymphoblastoid cells that have been treated with mitomycin C (Kyowa Hakko, Tokyo, Japan) were added to each of the two wells as antigen-presenting cells and OVA to only one of them. Outgrowing cells in OVA-pulsed wells were transferred to a 24-well plate, followed by the stimulation with antigen-presenting cells and OVA.

Proliferation Assays

Cellular proliferation was measured using the tritiated methylthymidine ($[^3\text{H}]\text{TdR}$) assay, as has been described [5]. Briefly, 2×10^4 T cells were mixed with 5×10^4 mitomycin-C-treated autologous EBV-transformed lymphoblastoid cells and antigen in flat-bottomed 96-well plates (Costar) in a total volume of 200 µl. After 72 h of incubation at 37°C, 0.5 µCi $[^3\text{H}]\text{TdR}$ (New England Nuclear, Boston, Mass., USA) was added to each well. Sixteen hours later, the cells were harvested onto glass fiber strips, and the amount of incorporated $[^3\text{H}]\text{TdR}$ was measured by liquid scintillation counting. The results were expressed as the stimulation index or counts per minute (cpm; mean of the triplicate cultures $\pm$ SD):

$$\text{stimulation index} = \frac{\text{mean cpm in culture stimulated with antigen}}{\text{mean cpm in unstimulated culture}} \text{ or}$$

$$\Delta\text{cpm} = \text{mean cpm in culture stimulated with} - \text{antigen mean cpm in unstimulated culture.}$$

Determination of HLA Class II restriction

Proliferation inhibition experiments using anti-HLA-DR, anti-HLA-DP and anti-HLA-DQ monoclonal antibodies (mAbs) were performed to determine the HLA class II restriction of the TCLs. Ascites fluid of each antibody diluted 1:250 was added to the proliferation assay with OVA. After 72 h of incubation, $[^3\text{H}]\text{TdR}$ was added to each well. Sixteen hours later, the cells were harvested, and the amount of incorporated $[^3\text{H}]\text{TdR}$ was measured. Anti-HLA mAbs [anti-HLA-DR (HU-4), anti-HLA-DP (B7/21) and anti-HLA-DQ (HU-11 and HU-18)] were generous gifts from Drs. T. Sasazuki and T. Nishimura, University of Kyushu, Fukuoka, Japan. The appropriate dilution of the ascites was determined based on FACS analysis in which PBMCs were stained by serial dilutions of ascites from each mAbs. The results were expressed as percent inhibition:

$$\text{percent inhibition} = \frac{\Delta\text{cpm without anti-HLA mAb} - \Delta\text{cpm with anti-HLA mAb}}{\Delta\text{cpm without anti-HLA mAb}} \times 100.$$

Table 1. FACS analysis of OVA-specific TCLs

TCLs	CD4+ cells, %	CD8+ cells, %
J001	80.0	13.0
J002	95.0	2.0
J003	95.0	1.5
J004	66.3	10.1
J005	88.9	8.4
J006	92.3	1.2
J007	82.9	3.2
J008	93.2	2.9
J009	88.0	3.7

Determination of Cytokine in the Culture Supernatant

One million TCLs were stimulated with antigen in the presence of 2×10^6 autologous antigen-presenting cells in 24-well plates in a final volume of 2 ml. After 24-hour incubation, supernatants were harvested and cytokines secreted in the culture supernatant were measured using solid-phase sandwich ELISA. Commercial ELISA kits were purchased from Genzyme (Cambridge, Mass., USA) for IL-4 and IFN- γ . IL-5 was also measured by a commercial kit (Suntory, Kyoto, Japan). The sensitivity of the assays was 45 pg/ml for IL-4, 100 pg/ml for IFN- γ and 10 pg/ml for IL-5, respectively.

Results

Establishment of OVA-Specific TCLs

Thirty TCLs were established from 6 egg-allergic AD patients. The amounts of $[^3\text{H}]\text{TdR}$ incorporation in response to stimulation with OVA are shown as stimulation indices in figure 2. These TCLs underwent specific proliferation in response to OVA (stimulation index 3–42) but not to the control antigen, β -lactoglobulin (stimulation index <2), in the presence of autologous EBV-transformed lymphoblastoid cells, indicating that these TCLs were OVA specific. Flow cytometry analysis of selected TCLs showed that these TCLs were mainly composed of CD4+ T cells but not of CD8+ cells (table 1).

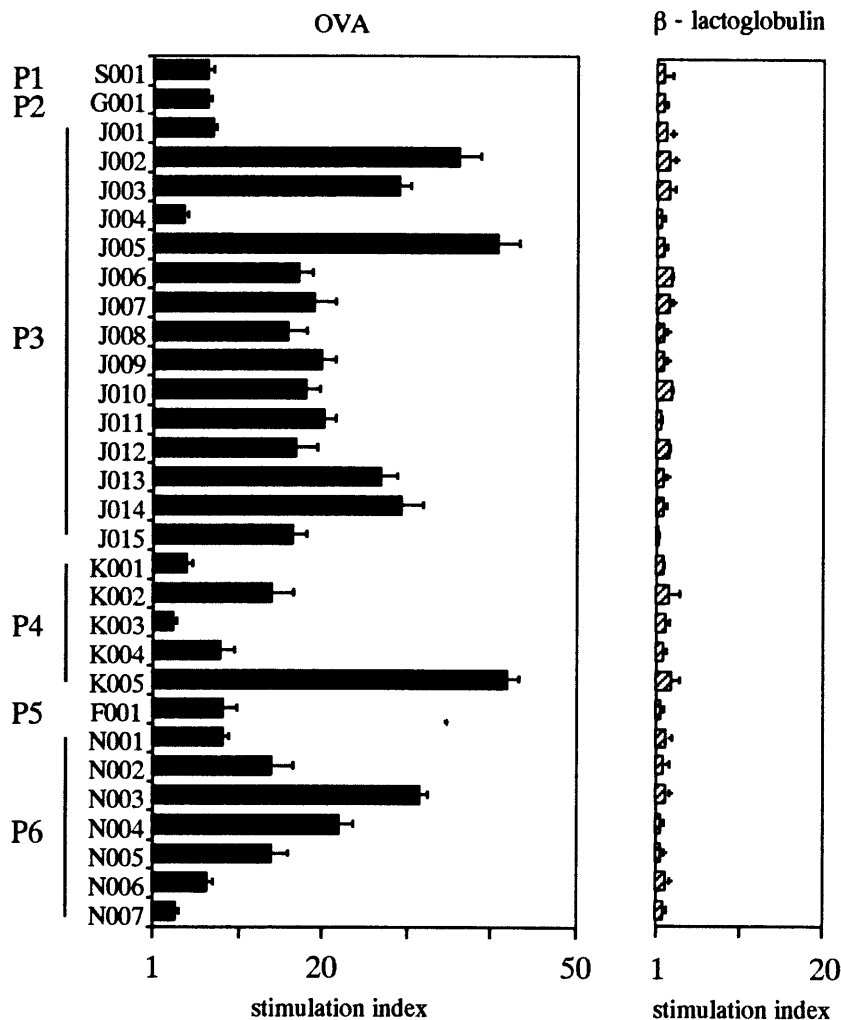
Cytokine Production in OVA-Specific TCLs

In response to stimulation with OVA, 28 out of 30 TCLs secreted detectable amounts of IL-4 and 16 out of 23 TCLs secreted IL-5 into the culture medium (fig. 3). IFN- γ was undetectable for all of the 30 TCLs.

HLA Restriction of OVA-Specific TCLs

We next studied HLA restriction of the TCLs. Since growth of OVA-specific TCLs was not so good, it was im-

Fig. 2. Proliferative responses of 30 OVA-specific TCLs established from 6 egg-allergic AD patients. The stimulation index of the TCL-proliferative response to a control antigen, β -lactoglobulin, was less than 2. cpm values were 271–2,025 for unstimulated cultures and 1,323–44,550 for cultures with OVA.



possible to examine all TCLs for their HLA restriction. Ten TCLs that showed relatively good growth were studied. The addition of anti-HLA-DR mAb markedly inhibited OVA-specific proliferation of 7 out of 10 TCLs, and the addition of anti-HLA-DQ mAb inhibited the OVA-specific proliferation of the 3 remaining TCLs. On the other hand, anti-HLA-DP mAb had virtually no effect (table 2).

Epitope Mapping by Use of Synthetic OVA Peptides

Four OVA-specific TCLs were screened for their ability to respond to each of the 17 peptide pools. Figure 4a shows a typical set of data from the pool scan of one OVA-specific TCL, S001. TCL S001 proliferated almost exclusively in response to pool 100–110. Among the 11 peptides in the pool, S001 most strongly responded to peptide P101 (OVA201–

213). Lower responses were also observed in response to the overlapping peptides P100 (OVA199–211), P102 (OVA203–215) and P103 (OVA205–217). No significant proliferation was detected in response to any other peptide. Figure 4b shows another typical set of data from the pool scan of an OVA-specific TCL, F001. Since the OVA-specific TCL F001 recognized two pools, pool 122–132 and pool 133–143, the individual peptides that fall partially within both pools, P127–P137, were tested individually. TCL F001 clearly responded to peptide P131 (OVA261–273). A proliferative response of similar magnitude was observed in response to peptides P132 (OVA263–275) and P133 (OVA265–277). These results indicate that TCL F001 recognizes an epitope located at position 261–277 within the OVA molecule. Two other OVA-specific TCLs (G001 and

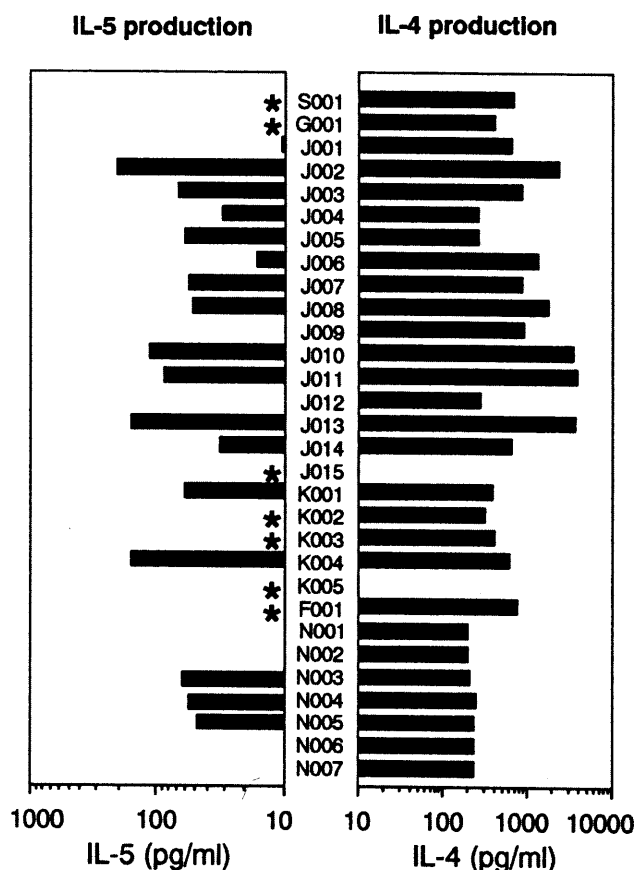


Fig. 3. IL-4 and IL-5 production of OVA-specific TCLs in the culture supernatant. * = Not tested. Supernatant was harvested 24 h after OVA stimulation and cytokines were measured by a commercial sandwich kit. EBV-transformed lymphoblastoid cells were used as antigen-presenting cells. IFN- γ was undetectable for all TCLs.

J015) were scanned for their responses to each of the 17 peptide pools. TCL G001 recognized pool 100–110 (OVA199–231) while TCL J015 responded to pool 1–11 (OVA1–33). Further analysis of the T cell epitope detected by the individual peptides could not be done because of the poor growth of these TCLs. A summary of the epitope mapping is shown in table 3. The results indicate that at least 3 different OVA epitopes are recognized by T cells from AD patients with egg allergy.

Table 2. HLA restriction elements for 10 OVA-specific TCLs

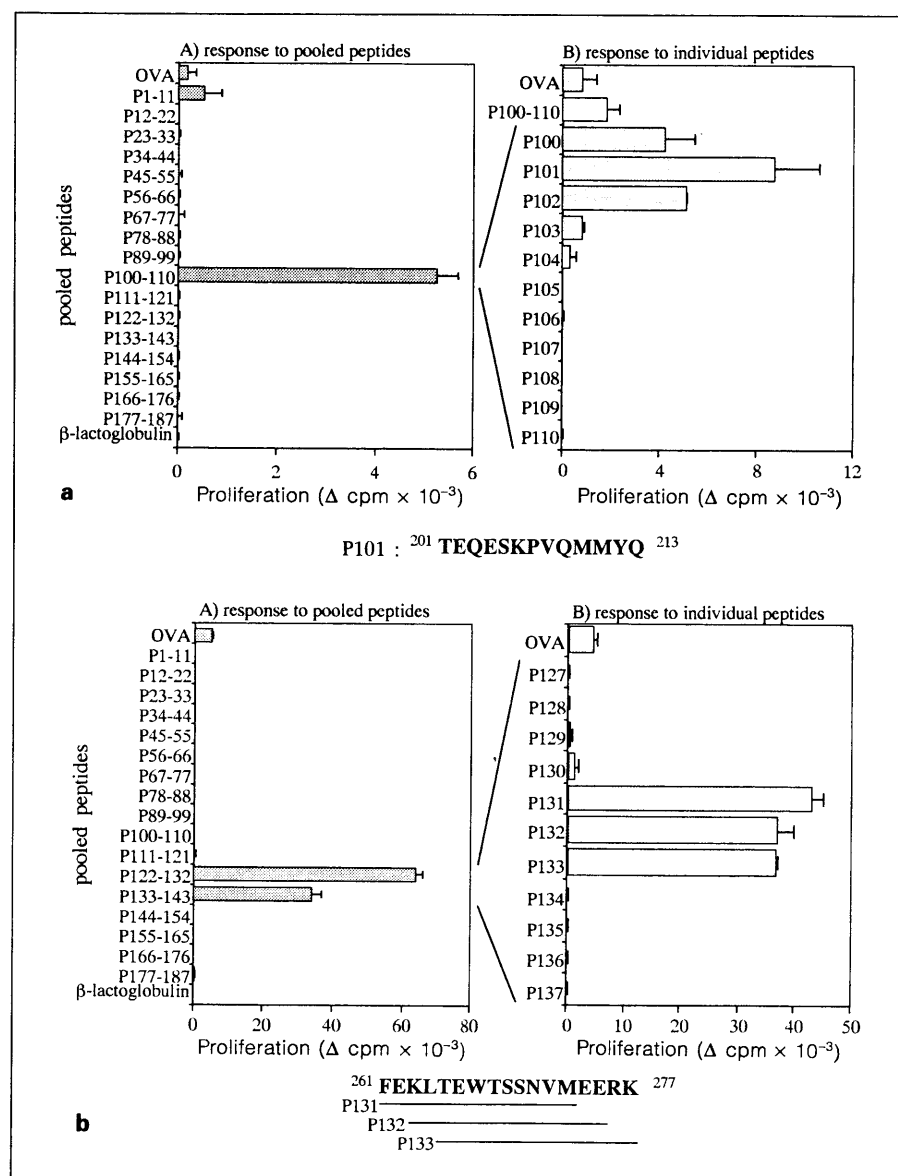
TCLs		Inhibition, %			HLA restriction
		anti-HLA-DR	anti-HLA-DQ	anti-HLA-DP	
P1	S001	54	88	27	DQ
P2	G001	92	0	0	DR
P3	J006	51	91	13	DQ
	J008	95	13	10	DR
	J010	95	13	14	DR
	J011	90	38	38	DR
	J013	94	0	10	DR
	J015	92	22	0	DR
P4	K004	95	5	0	DR
P5	F001	11	87	0	DQ

Figures indicate percent inhibition of the proliferative response of TCLs by anti-HLA class II mAbs. Figures in italics are considered as significant inhibition.

Discussion

Mouse CD4⁺ helper T cells are distinguished by their cytokine production phenotype and divided into two distinct and mutually exclusive subsets [8]. TH1 cells produce IL-2 and IFN- γ , whereas TH2 cells secrete IL-4, IL-5, IL-6 and IL-10, which promote B cell responses, especially IgE production. Although the presence of defined TH1 and TH2 subsets in humans has not been definitely established, it has been demonstrated that most aeroallergen (house dust mite etc.)-specific T cell clones established from PBMCs of atopic patients belong primarily to the TH2 subset [2–4]. In contrast to aeroallergens, no food-allergen-specific TCLs or clones have so far been reported except in our earlier study [5]. The results of this study reveal that OVA-specific TCLs conform to the TH2 subset, producing large amounts of IL-4 and IL-5 but no detectable amounts of IFN- γ . The dominance of the TH2 type among OVA-specific T cells from patients with egg allergy appears not to be due to the protocol used to establish TCLs, since purified protein derivative of *Mycobacterium-tuberculosis*-specific TCLs derived from the same patients by the same method produced high levels of IFN- γ but very little IL-4 on purified protein derivative stimulation (data not shown). Taken together, the results suggest that OVA-specific T cells in patients with egg allergy may play an important role in allergic inflammation including IgE antibody production. Our study re-

Fig. 4. Reactivity of OVA-specific TCLs to synthetic OVA peptides. TCLs were first tested for its proliferative response to 17 pools of 11 peptides, then to individual peptides in the presence of autologous EBV-transformed lymphoblastoid cells in 3-day culture. **a** Reactivity of OVA-specific TCL S001 to synthetic OVA peptides. **b** Reactivity of OVA-specific TCL F001 to synthetic OVA peptides. Concentrations of OVA or OVA peptides were 25 or 5 µg/ml, respectively.



vealed that OVA-specific TCLs consist of CD4⁺ T cells, confirming our previous observation [5]. Of interest, αS1-casein-specific TCLs, which were established from PBMCs of patients with milk allergy by the same method, contained a substantial proportion of CD8⁺ T cells although CD4⁺ cells comprise a major population [Nakajima et al., submitted]. This raises the possibility that different food antigens may induce different T cell subpopulations and a more detailed analysis is required for the establishment of functions of CD4 and CD8 T cells in food allergy.

Our results support the observation that IgE-mediated reactions to OVA are important in the pathogenesis of AD in patients with egg allergy [1]. However, the results of this

Table 3. Peptide reactivity of 4 OVA-specific TCLs tested

Patient	T cell line	Reactive peptides	Reactive sequences
P1	S001	P101	201–213
P2	G001	P100–110 ¹	198–231
P3	J015	P1–11 ¹	1–33
P5	F001	P131–133	261–277

¹ Pooled peptides. Note that P101 is included in the pooled peptides P100–110, indicating that at least 3 different OVA epitopes exist.

study are at odds with a recent report by Kondo et al. [9] which showed that PBMCs from egg-allergic AD patients produce IL-2 and IFN- γ on OVA stimulation. Based on their data, they suggested that TH1-type T cells are involved in the development of AD. The reason for the discrepancy between their results and ours, in which very little or no production of IFN- γ by OVA-specific TCLs was detected, is not clear at present. One possibility is the difference in the patient subpopulations. We studied AD patients with high-titer RAST scores to egg white and IgE antibodies to OVA detected by ELISA. These patients demonstrated a pruritic erythematous morbilliform rash followed by exacerbation of eczematous lesions on egg challenge. In contrast, Kondo et al. [9] investigated cytokine production from PBMCs of AD patients presenting only a late cutaneous response to egg challenge. The important role of TH2 cells in AD has directly been established by recent findings that house-dust-mite-specific TH2-type T cells can be successfully cloned from affected skin as well as from peripheral blood in atopic patients with AD [10–12]. Although Kondo et al. [9] claimed the involvement of TH1-type T cells, they also found an increase in CD23 expression on PBMCs after OVA stimulation. It has been well established that the expression of CD23 on B cells and macrophages is enhanced by IL-4. Thus, the results strongly suggest that TH2-type cytokines such as IL-4 might be produced, although the IL-4 content of the culture supernatant was not measured. All of these studies, including our own, support the dominant role of TH2-rather than TH1-type T cells in the pathogenesis of AD. The role of TH1 cells in AD awaits immunohistochemical examination of the cytokines produced by infiltrating T cells in the affected skin of AD patients with little or no allergen-specific IgE antibodies.

Several investigators have reported that the non-food-allergen (such as house dust mite)-specific T cell response is restricted mainly by HLA-DR molecules [13, 14], although other reports have shown diverse HLA restriction, including HLA-DR, -DQ and -DP [15–17]. Our results indicate that HLA-DR acts as the dominant restriction element for OVA-specific TCLs with a minor contribution from HLA-DQ. There has been one study on the HLA restriction of T cell response to OVA reported by Yoshizawa [18], which suggests that DQ and DP are the main restriction elements for OVA recognition. They investigated HLA restriction by antigen-induced IL-2 responsiveness of PBMCs but did not measure cytokine production. In contrast, we established OVA-specific TCLs based on antigen-induced proliferation, suggesting that the difference between their results and ours reflects the difference in the system used. Alternatively, different types of HLA molecules may be involved in the acti-

vation of IL-4- and/or IL-5-producing T cells versus IL-2-responding T cells. Since most OVA-specific T cells produce IL-4 and/or IL-5 upon OVA stimulation, our results at least indicate that HLA-DR is a main restriction element for the production of allergic-reaction-inducing cytokines from T cells.

OVA has been studied as a model protein for T cell recognition and the murine T cell epitope has been defined [19]. In this study, we prepared 187 overlapping peptides spanning the entire sequence of OVA and evaluated the epitope specificity of some OVA-specific TCLs. We demonstrate 3 distinct T cell epitopes which seem to differ among patients. Recently, we found that OVA323–339 is also a T cell epitope recognized by an OVA-specific TCL [5], indicating the existence of at least 4 distinct OVA T cell epitopes in AD patients with egg allergy. Although the study of Yssel et al. [20] suggested that multiple T cell epitopes may be present in *Dermatophagoides pteronyssinus* major allergen Der p I, O'Hehir et al. [21] have reported the existence of major epitopes in house dust mite allergen. If there exist a few major OVA epitopes, new strategies for immune intervention in allergic diseases may involve interfering specifically with the interaction between antigen-presenting cells, allergen and T lymphocytes. The use of peptides that contain the T cell epitopes of OVA may be promising in improving the effective inactivation of OVA-specific T cells. In fact, we have already shown that a peptide analog of OVA323–339 could partially inhibit the activation of OVA323–339-specific TCLs by competing with the relevant OVA peptide binding to HLA class molecules [5]. Much more detailed information about the T cell epitopes of OVA and HLA restriction elements is required to make such therapies operational. We are currently trying to find the major OVA epitopes by in vitro study of the secondary PBMC proliferative response to OVA peptides in addition to establishing more OVA-specific TCLs.

Acknowledgement

We are grateful to Drs. T. Sasazuki and T. Nishimura for their kind gifts of anti-HLA mAbs. We thank Ms. Margaret Dooley Ohto for reviewing the manuscript. This study was supported by a Grant-in Aid for Scientific Research on Priority Area from the Ministry of Education, Science and Culture (No. 06265203).

References

- 1 Sampson HA: The role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1993;91:549-551.
- 2 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;144:4651-4656.
- 3 Parronchi P, Macchia D, Piccinini MP, et al: Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show different profile of cytokine production. *Proc Natl Acad Sci USA* 1991;88:4538-4542.
- 4 Wierenga EA, Snoek M, Jansen HM, Bos JD, van Lier RAW, Kapsenberg ML: Human atopy-specific types 1 and 2 T helper cell clones. *J Immunol* 1991;147:2942-2949.
- 5 Shimojo N, Katsuki T, Coligan JE, et al: Identification of the disease-related T cell epitope of ovalbumin and epitope-targeted T cell inactivation in egg allergy. *Int Arch Allergy Immunol* 1994;105:155-161.
- 6 Yunginger JW: Food antigens; in Metcalfe DD, Sampson HA, Simon RA (eds): *Food Allergy Adverse Reactions to Foods and Food Additives*. Oxford, Blackwell Scientific Publications, 1991, pp 36-51.
- 7 Pette M, Fujita K, Wilkinson D, et al: Myelin autoreactivity in multiple sclerosis: Recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple sclerosis patients and healthy donors. *Proc Natl Acad Sci USA* 1990;87:7968-7972.
- 8 Mosmann TR, Cerwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-2357.
- 9 Kondo N, Fukutomi O, Agata H, et al: The role of T lymphocytes in patients with food-sensitive atopic dermatitis. *J Allergy Clin Immunol* 1993;91:658-668.
- 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;97:389-394.
- 11 van Reijsen FC, Bruijnzeel-Koomen CA, Kalthoff FS, et al: Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* 1992;90:184-193.
- 12 Sager N, Feldmann A, Schilling G, et al: House dust mite-specific T cells in the skin of subjects with atopic dermatitis. *J Allergy Clin Immunol* 1992;89:801-810.
- 13 O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR: MHC class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988;64:627-631.
- 14 van Neerven RJJ, van 't Hof W, Ringrose JH, et al: T cell epitopes of house dust mite major allergen Der p II. *J Immunol* 1993;151:2326-2335.
- 15 Carballido JM, Carballido-Perrig N, Kägi MK, et al: T cell epitope specificity in human allergic and nonallergic subjects to bee venom phospholipase A₂. *J Immunol* 1993;150:3582-3591.
- 16 Higgins JA, Lamb JR, Marsh SGE, et al: Peptide-induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite). *J Allergy Clin Immunol* 1992;90:749-756.
- 17 van Neerven RJJ, van de Pol MM, van Milligan FJ, Jansen HM, Aalberse RC, Kapsenberg ML: Characterization of cat dander-specific T lymphocytes from atopic patients. *J Immunol* 1994;154:4203-4210.
- 18 Yoshizawa I: HLA restriction in OVA-induced IL-2 responsiveness to lymphocytes from patients with hen-egg allergy. *J Saitama Med School* 1992;19:57-69.
- 19 Shimonkevitz R, Colon S, Kappler JW, Marrack P, Grey HM: Antigen recognition by H-2-restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. *J Immunol* 1984;133:2067-2074.
- 20 Yssel H, Johnson KE, Schneider PV, et al: T cell activation-inducing epitopes of the house dust mite allergen Der p I: Proliferation and lymphokine production patterns by Der p I-specific CD4⁺ T cell clones. *J Immunol* 1992;148:738-745.
- 21 O'Hehir RE, Verhoef A, Panagiotopoulou E, et al: Analysis of human T cell response to the group II allergen of *Dermatophagoides* species: Localization of major antigenic sites. *J Allergy Clin Immunol* 1993;92:105-113.