

Identification of human T cell epitopes in Japanese cypress pollen allergen, Cha o 1, elucidates the intrinsic mechanism of cross-allergenicity between Cha o 1 and Cry j 1, the major allergen of Japanese cedar pollen, at the T cell level

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Summary

Background Pollens from species of Cupressaceae family are one of the most important causes of respiratory allergies worldwide. In Japan, many patients with pollinosis have specific IgE to both pollens of Japanese cypress (*Chamaecyparis obtusa*) and Japanese cedar (*Cryptomeria japonica*). The sequences between Cha o 1 and Cry j 1, the major allergens of Japanese cypress and Japanese cedar pollens, respectively, are 80% identical.

Objective This study was undertaken to identify T cell epitopes in Cha o 1, and to elucidate the mechanism of cross-allergenicity between Cha o 1 and Cry j 1, at the T cell level.

Methods T cell epitopes in Cha o 1 were identified by the reactivity of T cell lines, generated from 19 patients, to stimulation with overlapping peptides. The subsets of T cell clones specific to rCha o 1 were determined according to their ability to produce IL-4 and IFN- γ . Peptide specificities of two T cell clones were determined by stimulation with the peptides from Cha o 1 and Cry j 1.

Results Four dominant and six subdominant T cell epitopes were identified in Cha o 1. While four T cell epitopes, p11-30, p211-230, p251-270 and p331-350, were common to Cha o 1 and Cry j 1, 4 T cell epitopes, p61-80, p71-90, p311-330 and p321-340, were considered to be unique to Cha o 1. The subsets of T cell clones were predominantly of T helper2-type. One T cell clone recognized p16-30, which is common to Cha o 1 and Cry j 1, but another recognized p321-330, which is unique to Cha o 1.

Conclusion Presence of both T cells reactive to T cell epitopes common to Cha o 1 and Cry j 1 and T cells specific to T cell epitopes unique to Cha o 1 in patients with pollinosis contributes to prolonged symptoms after the cedar pollen season in March and the following cypress pollen season in April.

Keywords allergen, Cha o 1, cross-allergenicity, Cry j 1, pollinosis, T cell clone, T cell epitope, T cell line

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Introduction

Pollens from species of the Cupressaceae family are one of the most important causes of respiratory allergies in the wide regions of North America, Mediterranean countries, Australia and Japan [1, 2]. Serum IgE from a large number of the patients with mountain cedar (*Juniperus ashei*) pollinosis is cross-reactive to the pollen proteins in Cupressaceae and Taxodiaceae species [3].

Pollinosis caused by Japanese cedar (Taxodiaceae family, *Cryptomeria japonica*) pollen is one of the most common allergic respiratory diseases in Japan [4]. Even after the pollen season for Japanese cedar in March, the symptoms of more than 70% of allergic patients persist through April. The

prolongation of the symptoms of pollinosis is thought to be because of exposure to the Japanese cypress (Cupressaceae family, *Chamaecyparis obtusa*) pollen, because of the presence of IgE in sera of patients that cross-reacts with components of pollens from Japanese cedar and Japanese cypress [5–7].

After purification of two major allergens, Cry j 1 and Cry j 2, from the pollen of Japanese cedar as well as Cha o 1 and Cha o 2 from Japanese cypress, complementary DNAs (cDNAs) encoding individual allergens have been cloned to deduce their primary structures [8–12]. The amino acid sequence of Cha o 1, consisting of 354 residues, showed 79–80% identity with that of Cry j 1 [11]. Therefore, it has been thought that there are T cells reactive to T cell epitopes that are common to Cha o 1 and Cry j 1 sequences. Several major T cell epitopes in Cry j 1 have been already identified [13–16]. Recently, the existence of a common T cell epitope at p213–228 in Cha o 1 and Cry j 1 has been experimentally demonstrated in mice [17]; however, little information is available for human T cell epitopes in Cha o 1.

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In this study, we identify dominant and subdominant T cell epitopes in Cha o 1 using T cell lines (TCLs) generated from peripheral blood mononuclear cells (PBMCs) of allergic patients by stimulation with overlapping peptides covering the entire sequence of Cha o 1. Two T cell clones (TCCs) specific to Cha o 1 are further characterized to confirm whether TCCs specific to individual T cell epitopes in Cha o 1 can react to the peptides from the same position in Cry j 1. Based on these observations, we try to confirm the existence of T cell epitopes common to Cha o 1 and Cry j 1 and those unique to either Cha o 1 or Cry j 1 and to discuss the intrinsic mechanism of cross-allergenicity between Cha o 1 and Cry j 1, at the T cell level.

Materials and methods

Subjects

All blood donors were recruited from the members of Research Institutes of Meiji Dairies Corporation, and gave informed consent. The Institutional Review Board approved use of the sera and PBMCs from all patients to pursue this study. The nineteen donors (12 male and seven female aged 23–55 years) were allergic patients who have been sensitized with Japanese cedar and Japanese cypress pollen, as determined by diagnosis made on the basis of their case histories and the presence of allergen-specific IgE, as measured by the Ala-STAT (Diagnostic Products, Los Angeles, CA, USA). The values were expressed in International Units (IU) and were further divided into classes 1–4 according to the manufacturer's definition. In addition, proliferative responses of all PBMCs from the patients were induced by stimulation with 10 µg/mL rCha o 1 and Cry j 1 after 7-day culture as measured by the [³H]thymidine incorporation assay as described previously [15]. Human leucocyte antigen (HLA) class II-genotypes of 10 patients, P. A.—P. Q., were described elsewhere [15].

Expression of rCha o 1 protein in Escherichia coli

We constructed a p-ΔNChao1 from pCHA-1 [11], encoding residues 1–354 of Cha o 1. The p-ΔNChao1 fragment was subcloned into pQE9 expression vector (Qiagen, Crawshaw, CA, USA) through their *Bam*HI and *Pst*I sites (PΔChao1). PΔChao1 was introduced into the host *E. coli*, M15 (pREP4), for expression. Protein synthesis was induced by the addition of isopropyl-1-thio-β-D-galactoside. The His-tagged rCha o 1 was purified by Ni²⁺-NTA agarose (Qiagen) column chromatography.

Synthesis of peptides

A panel of 35 overlapping peptides was synthesized according to the Cha o 1 sequence deduced from a cDNA encoding Cha o 1 [11], by a solid phase peptide synthesizer, PSSM-8 (Shimadzu, Kyoto, Japan), which uses the F-moc strategy. The 20-mer peptides had overlapping of 10 amino acids with each other. Purification of the peptides was performed as described previously [15]. Synthesis and purification of several overlapping peptides from Cry j 1 were reported elsewhere [15].

Generation of Cha o 1-specific T cell lines and T cell clones

Allergen-specific, short-term TCLs were generated from PBMCs of 19 patients. PBMCs (4×10^6) were stimulated with 10 µg/mL rCha o 1 in 2 mL of medium in one well of a 24-well flat-bottomed plate (Corning, Corning, NY, USA) for 8 days. RPMI-1640 medium supplemented with 15% human AB serum and antibiotics (streptomycin and penicillin (Life Technologies, Grand Island, NY, USA)) (complete medium) was used throughout the experiment. After rCha o 1-stimulation, T cell blasts were further cultured in complete medium in the presence of 20 U/mL rIL-2 (Boehringer Mannheim, Mannheim, Germany) for an additional 12–14 days. TCLs with a stimulation index (SI) > 3 were used for the determination of T cell epitopes.

In addition, TCCs were generated from three patients by the micromanipulation method, as described elsewhere [16]. Briefly, culture medium containing T cell blasts after stimulation of PBMC with rCha o 1 for 8 days was 100-fold diluted with complete medium and an aliquot of the cells were transferred to 10 cm diameter culture dish. One T cell blast was picked up using a tip of a micropipette under the microscope and was transferred to one well of a 96-well round-bottomed plate to which 2×10^5 X-irradiated autologous Epstein–Barr virus transformed B cell lines (EBV-B cells) in 0.2 mL complete medium containing 10 µg/mL rCha o 1 and 20 U/mL rIL-2 had been previously placed. After 7-day cultivation of the cells, these were re-stimulated under the same conditions. Growing TCCs appeared in about 40% of the wells, and these were transferred to a 24-well culture plate and further stimulated twice with rCha o 1 in the presence of rIL-2. An aliquot of the TCCs was used for proliferation experiment in the absence of rIL-2. TCCs with a SI > 5 were used for the following experiments. The phenotypes of TCCs were determined by flow cytometry using a FACScan (Becton-Dickinson, Mountain View, CA, USA) after staining them with FITC-conjugated anti-CD3, anti-CD4, anti-CD8, anti-TCR-αβ-1 and anti-TCR-γδ-1 monoclonal antibodies (all from BD Biosciences, San Jose, CA, USA).

T cell proliferative response Proliferation of TCLs and TCCs was assayed by co-culturing the cells (2×10^4) with X-irradiated autologous EBV-B cells (5×10^4) as antigen-presenting cells (APCs) in 0.2 mL complete medium in 96-well flat-bottomed plates (Corning). rCha o 1 and synthetic peptides were added to each well to a final concentration of 10 µg/mL and 0.5 µM, corresponding to a 20 µg/mL rCha o 1 dose, respectively, and the cells were incubated for 72 h. Incorporation of [³H]thymidine (Amersham Biosciences, Piscataway, NJ, USA) into the cells were assessed by the standard method as previously described [16]. All cultures were set up in triplicate (SEM < 10%). The value was converted to SI and was further expressed as a positivity index (PI, introduced by Counsell et al. [18]), which is the product of the mean SI and the frequency in a population of TCLs with SI ≥ 2.

Measurement of lymphokines TCCs (5×10^5) were co-cultured with 1×10^6 X-irradiated autologous EBV-B cells in 1 mL of complete medium in the presence of 0.5 µM of a particular peptide for 24 h. After gentle centrifugation,

culture supernatant was obtained. The amounts of IFN- γ and IL-4 in the supernatant were measured using a sandwich-enzyme immunoassay kits for IFN- γ (≥ 15 pg/mL, R&D Systems, Minneapolis, MN, USA) and IL-4 (≥ 3 pg/mL, Endogen, Cambridge, MA, USA), respectively. Definition of the subsets of T helper (T_H)-type was as follows: T_H2 if the IL-4/IFN- γ secretion ratio was >10 ; T_H1 if the IFN- γ /IL-4 secretion ratio was >10 ; T_H0 if the secretion ratio was $10 > \text{IL-4/IFN-}\gamma > 0.1$.

Results

Levels of immunoglobulin E antibody to pollen allergen

The levels of IgE specific to Japanese cypress and Japanese cedar pollens in sera from 19 patients are denoted in Table 1. The values of mean $\pm$ SEM were as follows: 3.92 ± 6.53 IU/mL (2.05 ± 0.94 for the class) for Japanese cypress pollen and 48.34 ± 27.40 IU/mL (3.90 ± 0.31) for Japanese cedar pollen. The average serum IgE level specific to Japanese cypress pollen was 12-fold lower than that of Japanese cedar pollen among the patients. In addition, IgE levels specific to Japanese cypress pollen were lower than those specific to Japanese cedar pollen in the same patient serum without exception. These observations were consistent with the previous reports [6, 7]. Since the seven patients (P. I., P. J., P. K., P. Q., P. S., P. Y. and Q. A.) who had low IgE values of Japanese cypress pollen in Table 1 used one of four T cell epitopes unique to Cha o 1 such as p61-80, p71-90, p311-330 and p321-340, they suffered from pollinosis by Cha o 1 allergen apparently.

Mapping of T cell epitopes

Proliferative responses of all PBMCs from the patients upon stimulation with rCha o 1 and Cry j 1 were observed (data not

shown). The observations directly indicated the presence of both T cells reactive to Cha o 1 and Cry j 1 in the blood of patients.

Cha o 1-specific TCLs were generated from PBMCs of 19 patients. TCLs reactive to rCha o 1 with a SI >3 were examined to identify T cell epitopes by stimulation with overlapping peptides from Cha o 1. In preliminary experiments, proliferative responses of the TCLs stimulated with rCha o 1 were higher than those stimulated with 20-mer overlapping peptides, since TCLs were reactive to several T cell epitopes derived from rCha o 1 but those stimulated with 20-mer overlapping peptides responded to a T cell epitope situated in one peptide. Thus, TCL reactivity reaching to SI ≥ 2 by stimulation with a peptide was considered to be a positive response. TCLs could recognize several positions of the overlapping peptides (Fig. 1). Three peptides, p61-80,

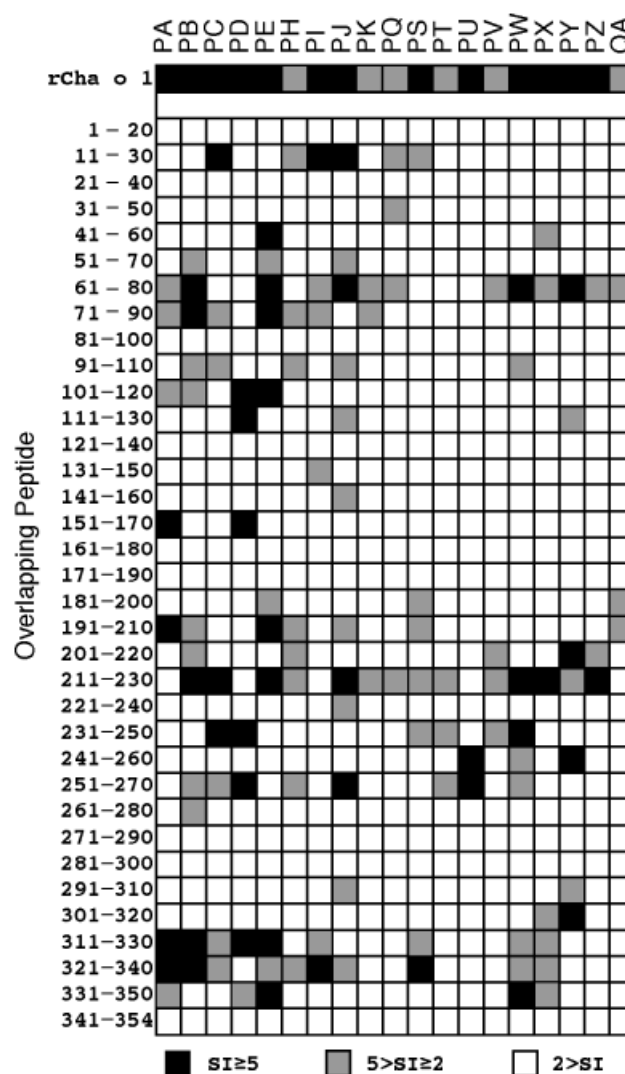


Fig. 1. Mapping of T cell epitopes in Cha o 1. T cell lines (TCLs) from 19 allergic patients were generated by stimulation with rCha o 1. Proliferation responses of TCLs with denoted overlapping 20-mer peptides in Cha o 1 in the presence of X-irradiated autologous Epstein-Barr virus transformed B cell lines as antigen-presenting cells were examined. The amounts of [^{3} H]thymidine incorporated into TCLs were converted to stimulation index (SI). To overview responsibilities of the TCLs, the values of SI were simply divided into three groups at the threshold values of five and two.

Table 1. Levels of IgE antibody to pollen allergen in sera of patients using Ala-STAT

Patient	Japanese cypress		Japanese cedar	
	IU/mL	Class	IU/mL	Class
P. A.	5.0	3	65.3	4
P. B.	4.0	3	69.2	4
P. C.	2.1	2	49.9	4
P. D.	4.5	3	61.5	4
P. E.	5.0	3	61.4	4
P. H.	1.8	2	27.1	4
P. I.	1.1	1	14.5	3
P. J.	1.0	1	40.8	4
P. K.	1.3	1	13.0	3
P. Q.	1.0	1	21.7	4
P. S.	1.2	1	48.1	4
P. T.	4.0	3	27.3	4
P. U.	2.6	2	104.7	4
P. V.	31.0	4	113.5	4
P. W.	2.8	2	47.6	4
P. X.	4.3	3	55.5	4
P. Y.	1.2	1	23.1	4
P. Z.	1.9	2	23.9	4
Q. A.	1.0	1	34.6	4

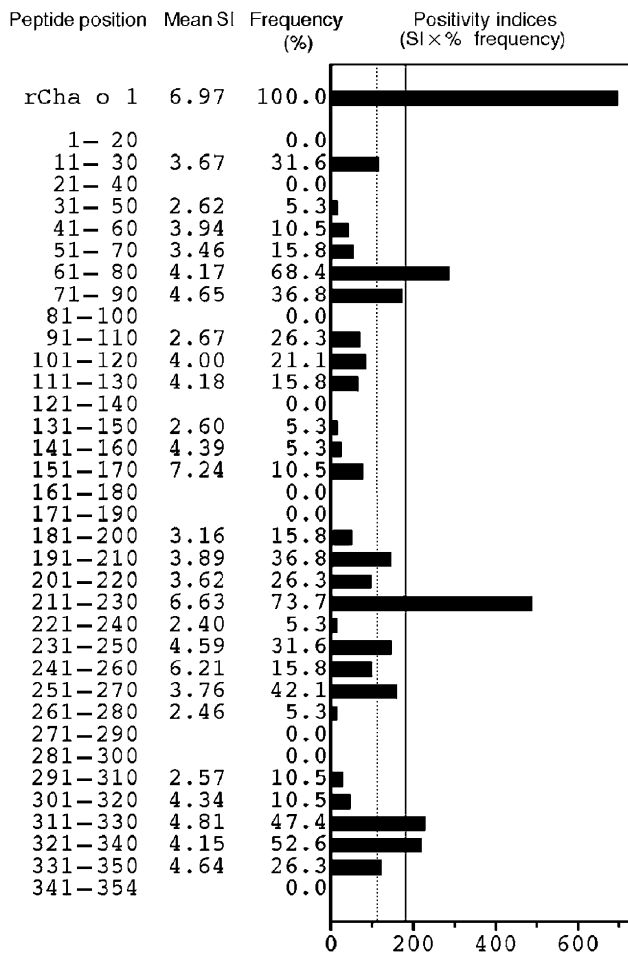


Fig. 2. A schematic representation of the positions of dominant and subdominant T cell epitopes evaluated by positivity index (PI). PI is the product of the mean stimulation index (SI) and the frequency in a population where $SI > 2$, denoted in Fig. 1. The solid and dotted lines indicate the threshold PI values of 174 ($50\% SI \times 50\% frequency$) and 112 ($40\% SI \times 40\% frequency$), respectively, to distinguish dominant and subdominant T cell epitopes.

p211-230 and p321-340, were recognized to the extent of more than 50% of the TCLs.

To evaluate relative reactivities of overlapping peptides in comparison with rCha o 1, PI was calculated to give scores to each peptide according to the frequency of the positive response as well as the magnitude of the positive response [18]. In this way, the response of 19 TCLs to each peptide could be compared with those to rCha o 1 (Fig. 2). The threshold values of PI for determining dominant and subdominant T cell epitopes were > 174 ($3.48 SI \times 50\% frequency$) and > 112 ($2.8 SI \times 40\% frequency$), respectively. The ranking of the peptides in order of PI values was as follows; p211-230, p61-80, p311-330 and p321-340 (dominant T cell epitopes) and p71-90, p251-270, p231-250, p191-210, p331-354 and p11-30 (subdominant T cell epitopes).

Comparison of the positions of T cell epitopes between Cha o 1 and Cry j 1

The PI for Cry j 1 peptides was calculated from the values of SI and the frequency of T cell epitopes in population of

TCLs, as described elsewhere [15]. The dominant and subdominant T cell epitopes identified in Cha o 1 and Cry j 1 sequences were found to localize in six limited regions: p16-30, p66-90, p191-225, p231-245, p251-270 and p316-345 (Fig. 3). Among these regions, at least 10 successive amino acid residues at positions p16-30, p192-203, p211-225, p251-270 and p336-345 were identical in Cha o 1 and Cry j 1, while those at positions p66-90, p231-245, 316-340 were different in that there was one or more residue substitution between them. Therefore, the former peptides are candidates for T cell epitopes common to Cha o 1 and Cry j 1 and the latter peptides are candidates for T cell epitopes unique to either Cha o 1 or Cry j 1.

It has been reported that four dominant and subdominant T cell epitopes, p16-30, p214-222 (core sequence), p251-265 and p337-346 (core sequence) identified in Cry j 1 are presented to $CD4^+$ T cells by HLA class II-DQ6 [16], -DP5 [16,19], -DQ6 [16] and -DR52 [13] molecules, respectively. The amino acid sequences of these T cell epitopes at the same positions are identical between Cha o 1 and Cry j 1 (Fig. 3). HLA-class II-genotypes of 10 patients, P. A.—P. Q., were described elsewhere [15]. Five patients, P. C., P. H., P. I., P. J. and P. Q., from whom TCLs were reactive to p11-30, possessed HLA class II-DQ6 molecule. Six patients, P. B., P. C., P. E., P. H., P. K. and P. Q., from whom TCLs reacted to p211-230, shared HLA class II-DP5 molecule. Among five patients, from whom TCLs were reactive to p251-270, 4 (P. B., P. C., P. H. and P. J., except for P. D.) possessed HLA class II-DQ6 molecule. Among three patients, from whom TCLs reacted to p331-350, 2 (P. A. and P. E., except for P. D.) shared HLA class II-DR52 molecule (Fig. 1). These observations support the existence of four common T cell epitopes, p16-30, 214-222, p251-265 and p337-346 between Cha o 1 and Cry j 1.

Characterization of T cell clones with specificity for Cha o 1

TCCs specific to rCha o 1 were generated from PBMCs of three patients (P. A., P. C. and P. W.). Finally, 15 TCCs specific to rCha o 1 ($SI > 5$) were used for following experiments. The phenotypes of all these TCCs, determined by flow cytometry, were $CD3^+$, $CD4^+$, $CD8^-$, $TCR\alpha\beta^+$ and $TCR\gamma\delta^-$ (data not shown).

Each TCC was caused to react with one peptide or two neighbouring overlapping peptides of Cha o 1 (Table 2). The amounts of IFN- γ and IL-4 produced by TCCs stimulated with a peptide including a T cell epitope were measured by enzyme immunoassay (Table 2). Among 15 TCCs, 5 (33%) were classified as T_H2^- , 8 (53%) were T_H0^- and 2 (13%) were T_H1^- type.

Four T cell epitopes common to Cha o 1 and Cry j 1, p16-30, p214-222, p251-265 and p337-346, have thoroughly identical amino acid sequences at the same positions between both allergens. However, a T cell epitope peptide p11-30 examined in this study contains two amino acid substitutions at positions 12 and 15 between Cha o 1 and Cry j 1 (Fig. 3). To eliminate possibility of the presence of another T cell epitope in p11-30, one TCC PC101.17 specific for p11-30 was examined with p11-25 and p16-30 peptides derived from Cry j 1 sequence (Fig. 4). The TCC could react to p16-30 peptide

		20		40		60		80
Cha o 1	DNPIDSCWRG	DANWDQNRMK	LADCAVGFGS	SAMGGKGGAF	YTVTSSDDDP	VNPAPGTLRY	GATRERSLWI	IFSKNLNIKL
Cry j 1	DNPIDSCWRG	DSNWAQNRMK	LADCAVGFGS	STMGGKGGDL	YTVTNSDDDP	VNPAPGTLRY	GATRDRPLWI	IFSGNMNIKL
		100		120		140		160
Cha o 1	NMPLYIAGNK	TIDGRGAEVH	IGNGGPCLFM	RTVSHVILHG	LNHGCSNTSV	SGNVLISEAS	GVVPVHAQDG	DAITMRNVT
Cry j 1	KMPMYIAGYK	TFDGRGAQVY	IGNGGPCVFI	KRVSNVILHG	LHLYGCSTSV	LGNVLINESF	GVEPVHPQDG	DALTILRTATN
		180		200		220		240
Cha o 1	VWIDHNSLSD	SSDGLVDVTL	ASTGVTISNN	HFFNHKKVML	LGHSDIYSDD	KSMKVTVAFN	QFGPNAGQRM	PRARYGLIHV
Cry j 1	IWIDHNSFSN	SSDGLVDVTL	SSTGVTISNN	LFFNHKKVML	LGHDDAYSDD	KSMKVTVAFN	QFGPNCGQRM	PRARYGLVHV
		260		280		300		320
Cha o 1	ANNNDPWSI	YAIGGSSNPT	ILSEGNSTFA	PNDSKKKEVT	RRVGCESPST	CANWVWRSTQ	DSFNNGAYFV	SSGKNEGTNI
Cry j 1	ANNNDPWTI	YAIGGSSNPT	ILSEGNSTFA	PNESYKKQVT	IRIGCKTSSS	CSNWVWQSTQ	DVFYNGAYFV	SSGKYEGGNI
		340						
Cha o 1	YNNNEAFKVE	NGSAAPQLTK	NAGVLTCLLS	KPCS				
Cry j 1	YTKKEAFNVE	NGNATPQLTK	NAGVLTCSLS	KRC				

Fig. 3. T cell epitopes identified in Cha o 1 and Cry j 1. The asterisks indicate identical amino acid residues between Cha o 1 and Cry j 1. The solid- and dotted-underlines indicate the position of major T cell epitopes and subordinate T cell epitopes, respectively. The PI for Cry j 1 peptides was calculated from the values described previously [15].

Table 2. Characterization of Cha o 1-specific TCCs

TCC	Epitope position	IFN- γ (pg/mL)	IL-4 (pg/mL)	T _H -type
PA.101.2	61–80	226	299	T _H 0
PA.101.7	71–90	1160	381	T _H 0
PA.101.14	71–90	<15	117	T _H 0
PA.101.42	71–90	367	200	T _H 0
PA.101.20	311–330	<15	1510	T _H 2
PA.101.17	321–340	920	4	T _H 1
PA.101.6	331–350	2030	199	T _H 1
PC.101.17	11–30	<15	236	T _H 2
PC.101.19	71–90	529	1060	T _H 0
PC.101.23	71–90	1360	1100	T _H 0
PC.101.3	91–110	186	392	T _H 0
PC.101.20	91–110	30	625	T _H 2
PW.101.15	61–80	<15	598	T _H 2
PW.101.3	91–110	<15	251	T _H 2
PW.101.10	211–230	<15	5	T _H 0

TCC, T cell clones; T_H, T helper.

alone, indicating that a T cell epitope was situated in p16–30 of which sequence is identical between Cha o 1 and Cry j 1.

To demonstrate the presence of T cell epitopes unique to Cha o 1 but not to Cry j 1, one TCC PA101.17 was examined with two peptides derived from Cha o 1 and 4 peptides from Cry j 1 (Fig. 4). The TCC could recognize both p311–330 and p321–340 in Cha o 1, indicating with certainty that the core sequence of the T cell epitope was situated in p321–330. However, this TCC could not react to four overlapping peptides at positions p311–340 in Cry j 1, indicating that T cell epitope unique to Cha o 1 existed. Thus, T cells having the ability to recognize T cell epitopes unique to Cha o 1 exist in the blood of allergic patients.

Discussion

The cross-allergenicity between Cha o 1 and Cry j 1 has been demonstrated by the presence of IgE in sera from patients with pollinosis that is cross-reactive with Cha o 1 and Cry j 1 [6, 7]. In general, induction of allergen specific IgE by plasma cells is caused by successive immune responses after activation of CD4⁺ T cells specific to the allergen during exposure to a certain allergen. In the present study, we have identified dominant and subdominant T cell epitopes in Cha o 1 to clarify the intrinsic mechanism of cross-allergenicity between Cha o 1 and Cry j 1, at the T cell level.

It has been clearly demonstrated that T cell epitopes situated in antigenic peptide are presented by APCs via various types of HLA class II-DR, -DQ and -DP molecules carrying antigenic peptides to CD4⁺ T cells bearing TCR $\alpha\beta$. Crystallographic investigation of a complex of a human TCR $\alpha\beta$, an antigenic peptide and an HLA-class II molecule has clearly elucidated that the residues of positions at No.-1 (P-1), P2, P3, P5, P7 and P8 in the antigenic peptide contact with TCR and those at P1, P4, P6 and P9 bind to the cleft of the HLA class II molecule as anchors [20, 21]. Thus, it is generally accepted that the antigenic peptides are composed of at least 10 residues and likely no-substitution of amino acids within the peptide is permitted if the T cell epitope in the antigenic peptide bound to HLA class II molecules is completely presented to TCR $\alpha\beta$ [22–24].

According to the criteria, dominant and subdominant T cell epitopes in Cha o 1 and Cry j 1 (Fig. 3) can be classified into two groups: one comprises antigenic peptides containing a T cell epitope common to Cha o 1 and Cry j 1 and the other comprises peptides containing a T cell epitope unique to either Cha o 1 or Cry j 1.

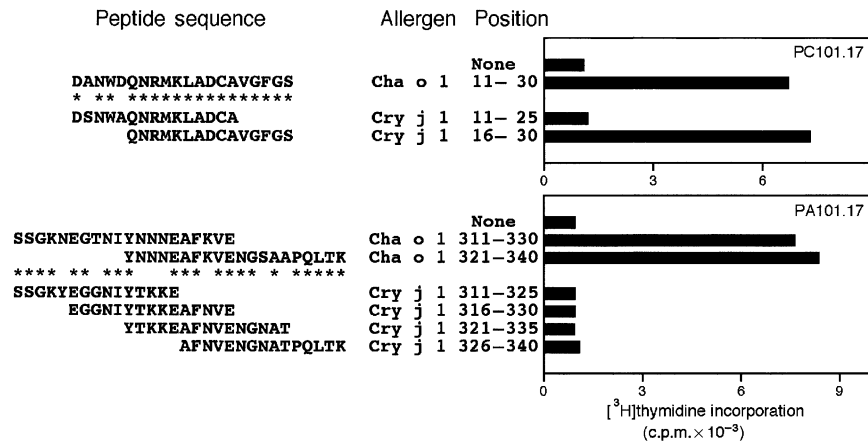


Fig. 4. Proliferative responses of T cell clones (TCCs) stimulated with peptides in *Cha o 1* and *Cry j 1*. Proliferation of two TCCs was tested with 20-mer and 15-mer peptides from *Cha o 1* and *Cry j 1*, respectively, in the presence of X-irradiated autologous Epstein-Barr virus transformed B cell lines as antigen-presenting cells. Proliferation assay of the TCCs is described in 'Materials and methods'.

In a former group, amino acid sequences of four dominant and subdominant T cell epitopes, p16-30, p214-222 (core sequence), p251-265 and p337-346 (core sequence) are thoroughly identical between *Cha o 1* and *Cry j 1* (Fig. 3). Nine patients among 10 (except P. D. among P. A.—P. Q.), from whom TCLs are capable of responding to the stimulation with at least one of those T cell epitopes, possess limited HLA class II-types such as DQ6 [16], DP5 [16, 19] and DR52 [13], having a potency to present appropriate T cell epitopes, as described above. In addition, presence of the lymph node cells reactive to a T cell epitope p213-224 common to *Cry j 1* and *Cha o 1* in B10.S mice immunized with *Cha o 1* has been clearly demonstrated [17]. Therefore, four T cell epitopes common to *Cha o 1* and *Cry j 1* apparently exist in *Cha o 1* and *Cry j 1* sequences.

Two other antigenic peptides p191-210 and p231-250 in *Cha o 1* show relatively high sequence identity with those in *Cry j 1*, with only a few amino acid substitutions, each other. At present, it is difficult to decide whether these T cell epitopes are common to *Cha o 1* and *Cry j 1* or unique to either *Cha o 1* or *Cry j 1*, and will remain so until the core sequences of the epitopes are determined.

In the second group, dominant and subdominant antigenic peptides at p61-80, p71-90, p311-330 and p321-340 in *Cha o 1* are less similar to those in *Cry j 1*. We experimentally demonstrated that a TCC PA.101.17 could recognize p321-330 whose sequence is unique to *Cha o 1* (Fig. 4). Three other T cell epitopes are considered to be unique to *Cha o 1*, based on the properties of antigenic peptides situated in HLA-class II-TCR $\alpha\beta$ complex, as mentioned above.

It is generally accepted that the ratio of the subsets of T_H2 : T_H0 : T_H1 -type of TCCs generated from PBMCs of allergic patients by stimulation with purified pollen allergens such as *Cry j 1* [16], *Cry j 2* [16], *Bet v 1* [25, 26], *Lol p 1* [27], *Phl p 1* [28], *Phl p 5* [29] and *Art v 1* [30] is roughly 2 : 2 : 1. The ratio of T_H2 : T_H0 : T_H1 -type among 15 TCCs with specificity to *rCha o 1* in this study was evaluated to 5 : 8 : 2 (Table 2). The somewhat increased number of T_H0 -type may be because of the presence of three T_H0 -type TCCs specific to p71-90, generated from patient P. A. The ratio of T_H2 : T_H1 -type in the TCCs reveals the predominance of T_H2 -type, suggesting

that T_H2 -type T cells play a central role in the immunopathogenesis of the pollinosis.

An onset mechanism of Japanese cypress pollinosis remains still controversial. Epidemiological approach has been indicating that the incidence of allergic pollinosis sensitized with pollens from both Japanese cedar and Japanese cypress, Japanese cedar alone and Japanese cypress alone, diagnosed by RAST or skin test, were 77%, 23% and 0%, respectively [5]. Namely, none of patients sensitized with Japanese cypress pollen alone exist. Serum IgE levels specific to Japanese cypress pollen are usually lower than those specific to Japanese cedar pollen (Table 1) [6]. Then, allergenicity of Japanese cypress pollen seems to be weaker than that of Japanese cedar pollen. Usually, healthy individuals firstly suffer from Japanese cedar pollinosis and secondly Japanese cypress pollinosis. We propose a hypothesis to elucidate an onset mechanism of Japanese cypress pollinosis as follows. $CD4^+$ memory T cells specific to T cell epitopes common to *Cry j 1* and *Cha o 1* in patients with Japanese cedar pollinosis are thought to continue the production of T_H2 -type cytokines not only in cedar pollen season in March but also in cypress pollen season in April. The continuous production of such cytokines in patients may lead plasma cells to the direction to up-regulate the production of IgE cross-reactive to *Cry j 1* and *Cha o 1* and may lead the activation of $CD4^+$ naïve T cells specific to *Cha o 1* during sensitization by Japanese cypress pollen. These events may result in the generation of new $CD4^+$ memory T cells reactive to T cell epitopes unique to *Cha o 1* (Fig. 1). Indeed, TCLs from 17 patients, except for P. T. and P. U., suffered from Japanese cedar and cypress pollinosis reacted to one of four T cell epitopes, p61-80, p71-90, p311-330 and p321-340 of which individual sequences are unique to *Cha o 1* (Fig. 1). In addition, TCLs specific to *Cha o 2* generated from two patients, P. T. and P. U., reacted to p361-380 and p41-60 unique to *Cha o 2*, the second major allergen of Japanese cypress pollen, respectively (T. Sone et al., personal communication). Therefore, generation of $CD4^+$ memory T cells specific to *Cha o 1* in patients seems to be critical for the onset of Japanese cypress pollinosis. Activation of both $CD4^+$ memory T cells reactive to T cell epitopes common to *Cha o 1* and *Cry j 1* and those unique to *Cha o 1*

may be necessary to induce symptoms of Japanese cypress pollinosis, resulting in the prolongation of the symptoms of pollinosis after cedar pollen season in March and following cypress pollen season in April. The hypothesis is supported in part by the explanation of cross-allergenicity between Bet v 1, a major birch pollen allergen and Mal d 1, a major apple allergen [31], and Bet v 1 and Api g 1, a major allergen in celery [32], at the T cell level.

Patients suffering from pollinosis caused by the exposure to tree pollens such as cypress and juniper have been increasing in diverse geographic areas [1–3]. Primary structures of Jun a 1 (*J. ashei*) [33], Jun v 1 (*J. virginiana*) [34], Cup a 1 (*Cupressus arizonica*) [35] and Cup s 1 (*C. sempervirens*) [34], deduced from the cDNA sequences are now available. These allergens, including Cha o 1 and Cry j 1, have a high degree of sequence identity, up to 81–96% [1, 36]. Comparing the major T cell epitopes in Cha o 1 and Cry j 1, p212–230 are identical and p61–90 and p311–340 are highly similar. Therefore, these regions may be postulated to be antigenic peptides possessing major T cell epitopes in the Group 1 of pollen allergen common to the species in Cupressaceae family.

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