Allergology International. 2009;58:237-245
DOI: 10.2332/allergolint.08-OA-0027

ORIGINAL ARTICLE

Recognition of T Cell Epitopes Unique to Cha o 2, the Major Allergen in Japanese Cypress Pollen, in Allergic Patients Cross-Reactive to Japanese Cedar and Japanese Cypress Pollen

Toshio Sone[1], Kazuo Dairiki[2], Keiko Morikubo[2], Kimiko Shimizu[2], Hajime Tsunoo[2], Takeshi Mori[2] and Kohsuke Kino[2]

ABSTRACT

Background: Pollens from species of the Cupressaceae family are one of the most important causes of respiratory allergies worldwide. Many patients with pollinosis have specific IgE to both allergens from Japanese cedar and Japanese cypress pollen. We set out to identify T cell epitopes in Cha o 2, the second major allergen of Japanese cypress pollen.

Methods: T cell lines (TCL) and T cell clones (TCC) specific to Cha o 2 were generated from allergic patients cross-reactive to Japanese cedar and Japanese cypress pollen. T cell epitopes in Cha o 2 were identified by responses of TCL stimulated with overlapping peptides. Abilities of IL-4/IFN-γ production by TCC were evaluated using enzyme immunoassay.

Results: Using TCL, 11 dominant and subdominant T cell epitopes were identified in Cha o 2. The subsets of TCC were predominantly of T helper 2-type. A T cell epitope p141–160 in Cha o 2 and corresponding peptide in Cry j 2 showed high homology. Although TCC PC.205.159 responded to stimulation with p141–160 in Cha o 2, it did not respond with corresponding peptide in Cry j 2, therefore, the T cell epitope was unique to Cha o 2.

Conclusions: Eleven T cell epitopes that were identified are unique to Cha o 2. Cha o 2 is a putative aeroallergen that can potentially sensitize human T cells. We concluded that generation of T cells specific to Cha o 2 in allergic patients acts as one of the causes of continuous allergic symptoms in April.

KEY WORDS

Cha o 2, cross-reactivity, Cry j 2, pollinosis, T cell epitope

INTRODUCTION

Pollens from species of the Cupressaceae family are one of the most significant causes of respiratory allergies in various geographic areas of North America, Australia, Africa, Japan and Mediterranean countries.[1,2] Serum IgE from allergic patients with pollinosis can be widely cross-reactive to tree pollen proteins in Cupressaceae and Taxodiaceae species.[3,4]

Pollinosis caused by Japanese cedar (Taxodiaceae family, *Cryptomeria japonica*) pollen is one of the most common allergic respiratory diseases in Japan.[5] Even after the pollen season, which is March for Japanese cedar, the symptoms of more than 70% of allergic patients persist through April. The prolongation of the symptoms of pollinosis is thought to be due to the exposure to Japanese cypress (Cupressaceae family, *Chamaecyparis obtusa*) pollen, since IgE in sera of patients cross-reacts with the components of pollens from Japanese cedar and Japanese cypress.[6-8] Serum

[1]Department of Allergy and Immunology, Faculty of Medicine, Saitama Medical University, Saitama and [2]Research and Development Center, Division of Research and Development, Meiji Dairies Corporation, Kanagawa, Japan.
Correspondence: Kohsuke Kino, PhD, Research and Development Center, Division of Research and Development, Meiji Dairies Corporation, 540 Naruda, Odawara, Kanagawa 250–0862, Japan.
Email: KOUSUKE_KINO@MEIJI–MILK.COM
Received 11 July 2008. Accepted for publication 28 September 2008.

IgE titers for Cry j 1 and Cry j 2 are consistently higher than Cha o 1 or Cha o 2, respectively.[7-9]

After purification of two major allergens, Cry j 1 and Cry j 2, from Japanese cedar pollen, as well as Cha o 1 and Cha o 2 from Japanese cypress, cDNAs encoding individual allergens have been cloned to predict their primary structures.[10-14] Several major T cell epitopes in Cry j 1,[15,16] Cry j 2[15-17] and Cha o 1[18] have already been identified. In our previous studies, we have demonstrated that 4 dominant and subdominant T-cell epitopes are common to Cry j 1 and Cha o 1 and the presence of T cells reactive to those T-cell epitopes in patients may play a role in cross-reactivity between Cry j 1 and Cha o 1. However, little information is available for human T cell epitopes in Cha o 2. Since the deduced amino acid sequence of Cha o 2, consisting of 464 residues, shows 74.3% identicality with that of Cry j 2,[14] it is expected to exist in both T cells reactive to T cell epitopes unique to Cha o 2 and common to both Cha o 2 and Cry j 2 in allergic patients.

In the present study, we examined the identification of dominant and subdominant T-cell epitopes in Cha o 2 using T cell lines (TCL) generated from peripheral blood mononuclear cells (PBMC) of allergic patients sensitized with Japanese cedar and Japanese cypress pollen by stimulation with overlapping peptides covered with Cha o 2 sequence. T cell clones (TCC) specific to Cha o 2 were also generated, and 3 were further examined to determine whether TCC specific to individual T cell epitopes in Cha o 2 would react to the stimulation with the corresponding positions of the peptides in Cry j 2. Based on these observations, we described how Cha o 2-specific $CD4^+$ T cells could be generated in allergic patients suffering from Japanese cedar pollinosis in terms of antigen presentation by B cells.

METHODS

SUBJECTS

Blood donors were recruited from the members of all research institutes of Meiji Dairies Corporation, and each gave informed consent. The Institutional Review Board approved the use of the sera and PBMC from all patients to pursue this study. All 19 donors (12 men and 7 women, aged 25–57 years) were patients with allergies who were sensitized with Japanese cedar and Japanese cypress pollen, as determined by diagnosis based on their case history and the presence of allergen-specific IgE, as measured by the Ala-STAT (Diagnostic Products, Los Angeles, CA, USA). All patients were on the panel for a study on the identification of T cell epitopes in Cha o 1, therefore, Ala-STAT scores for the patients have been described elsewhere.[18] PBMC from all subjects were reactive to the stimulation with rCha o 2 resulting in establishment of TCL specific to rCha o 2 as described below.

EXPRESSION OF rCha o 2 IN *Escherichia Coli*

The expression of rCha o 2 has been described elsewhere in a previous study.[14] All procedures were performed according to the guidelines for biosafety issued by the Ministry of Education, Culture, Sports, Science and Technology in Japan. In brief, a Cha o 2 cDNA fragment (pΔNchao2) was amplified from pBSch2–5, which included full length Cha o 2 cDNA (CHII-10), by polymerase chain reaction. The amplified Cha o 2 fragment was subcloned into pQE9 expression vector (Qiagen, Catsworth, CA, USA) through its *Bam*HI and *Pst* I sites. The plasmid was introduced in the host *E. coli*, M15 (pREP4), for expression. Protein synthesis was induced by adding 2 mM isopropyl-1-thio-β-D-galactopyranoside for an additional 4 hour culture. The His-tagged rCha o 2 was purified using a Ni^{2+}-NTA agarose. Purity of rCha o 2 was assessed at >90% as judged by sodium-dodecyl sulfate polyacrylamide gel electrophoresis after staining with coomassie brilliant blue R-250 followed by densitometry.

SYNTHESIS OF PEPTIDES

A panel of 46 overlapping peptides was synthesized according to the primary structure[14] of Cha o 2 using a solid phase peptide synthesizer, PSSM-8 (Shimazu, Kyoto, Japan), which uses the F-moc strategy. The 20-mer peptides were overlapped for 10 amino acids. Purification of the peptides was performed as described previously.[15] Synthesis of the overlapping peptides for Cry j 2 was described elsewhere.[15]

GENERATION OF Cha o 2-SPECIFIC TCL AND TCC

Allergen-specific, short-term TCL were generated from PBMC of 19 allergic patients. PBMC (4×10^6) were stimulated with 10 μg/ml rCha o 2 in 2 ml medium in each 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 experiments. After rCha o 2-stimulation, T cell blasts were further cultured in the complete medium in the presence of 20 U/ml rIL-2 (Bøehringer Manheim, Manheim, Germany) for an additional 12–14 days. TCL showing stimulation index (SI) >2 were used for determining T cell epitopes.

In addition, TCC were generated from 3 patients (PC, PV and PX) by the micromanipulation method as described elsewhere.[19] Culture medium containing T cell blasts after stimulation of PBMC with rCha o 2 for 8 days was 100-fold diluted with a complete medium and an aliquot of the cells were transferred to a culture dish 10 cm in diameter. A T cell blast was picked up using the tip of a micropipette microscopically and was transferred to 1 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), as antigen presenting cells (APC) in 0.2 ml complete medium containing 5 μg/ml rCha o 2 and 20 U/ml rIL-2, had been previously placed. After a 7-day cultivation of the cells, these were re-stimulated under the same conditions. Growing TCC appeared in about 40% of the wells and these were transferred to a 24-well culture plate and further stimulated twice with rCha o 2 in the presence of rIL-2. An aliquot of the TCC was used for a proliferation experiment in the absence of rIL-2. TCC showing SI >5 were used for the following experiments. The phenotypes of TCC were determined by flow cytometry using FACScan (Becton-Dickinson, Mountain View, CA, USA) after staining them with fluorescein isothiocyanate-conjugated anti-CD3, anti-CD4, anti-CD8, anti-T cell receptor (TCR)-αβ-1 and anti-TCR-γδ-1 monoclonal antibodies (BD Biosciences, San Jose, CA, USA).

T CELL PROLIFERATIVE RESPONSE

Proliferation of TCL and TCC was assayed by co-culturing the cells (2×10^4) with X-irradiated autologous EBV-B cells (5×10^4) as APC in 0.2 ml complete medium in 96-well flat-bottomed plates (Corning). rCha o 2 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 2 dose, respectively, and cells were incubated for 72 hours. Incorporation of [^{3}H]thymidine (Amersham Biosciences, Piscataway, NJ, USA) into the cells was performed according to the standard method as previously described.[15] All cultures were set up in triplicate (SEM <10%). The value was converted to stimulation index (SI) and was further expressed as positivity index (PI), introduced by Counsell *et al.*,[20] which is the product of the mean SI and the frequency in the population of SI ≥2. The PI was used to measure the strength and frequency of responses of T cells to peptides relative to the responses to the rCha o 2.

MEASUREMENT OF LYMPHOKINES

TCC (5×10^5) were co-cultured with 1×10^6 X-irradiated autologous EBV-B cells in 1 ml of RPMI-1640 supplemented with complete medium in the presence of 0.5 μM, each peptide containing T cell epitopes for 24 hours. After gentle centrifugation, culture supernatant was obtained. The amounts of IFN-γ and IL-4 in the supernatant were measured using sandwich-enzyme immunoassay (EIA) kits for IFN-γ (≥15 pg/ml, R&D Systems, Minneapolis, MN, USA) and IL-4 (≥3 pg/ml, Endogen, Cambridge, MA, USA), respectively. TCC with a ratio of IFN-γ/IL-4 of >10 were classified as T helper 1-type (Th1) cell. Those with a ratio of 0.1–10 were classified as Th0 cells, and those with a ratio <0.1 were classified as Th2 cells.

RESULTS

MAPPING OF T CELL EPITOPES

rCha o 2-specific TCL were generated from PBMC of 19 patients. Identification of T cell epitopes in rCha o 2 was examined by the reactivity of TCL to the stimulation with a panel of overlapping peptides from the sequence of Cha o 2 in the presence of autologous EBV-B cells as APC. All TCL recognized rCha o 2 (SI ≥2) (Fig. 1). These were also reactive to several regions of the overlapping peptides except for p1–20, p221–250, p331–350 and p381–464 located in the C-terminal region of Cha o 2. As for the overlapping peptides in Cha o 2, two peptides, p141–160 and p361–380 were recognized by more than one-half of the TCL. However, it was somewhat difficult to judge the degree of importance of other T cell epitopes which were identified. To evaluate the relative reactivity of each overlapping peptides in comparison with rCha o 2, 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.[20] In this manner, the responses of 19 TCL to each peptide could be compared with those to rCha o 2 (Fig. 2). The border values of PI for dominant and subdominant T cell epitopes were >155 (50% SI × 50% frequency) and >99 (40% SI × 40% frequency), respectively. The ranking of peptides in descending order of the PI was as follows: p361–380, p41–60, p141–160, p371–390, p351–370 and p151–170 (6 dominant T-cell epitopes) and p341–360, p91–110, p81–100, p131–150 and p191–210 (5 subdominant T-cell epitopes).

COMPARISON OF T CELL EPITOPES BETWEEN Cha o 2 AND Cry j 2

The PI for Cry j 2 peptides was calculated from the values of SI and the frequency of T cell epitopes in the population of TCL as described elsewhere.[15] Since 10 (PA-PQ) of the 19 allergic patients are the same blood donors from which TCL specific to Cry j 2 have already been generated,[15] their TCL responses to the T cell epitopes in Cha o 2 can be directly compared with those of Cry j 2 to determine whether the identified T cell epitopes were unique to Cha o 2 or common to Cha o 2 and Cry j 2. The dominant and subdominant T cell epitopes identified in Cha o 2 and Cry j 2 sequences were found to localize in 4 limited regions, p81–100, p141–170, p191–210 and p341–370 (Fig. 3). Among them, at least 10 successive amino acid residues at position p151–162 (LMNSPEFHLVFG) in Cha o 2 were identical among Cha o 2 and Cry j 2, while the other 3 peptides were different, accompanied with one or more residue substitution. It can therefore be said that the former peptide is a candidate for T cell epitopes common to Cha o 2 and Cry j 2 and the latter contains T cell epitopes unique to either Cha o 2 or Cry j 2.

The presence of 3 linear IgE epitopes at positions

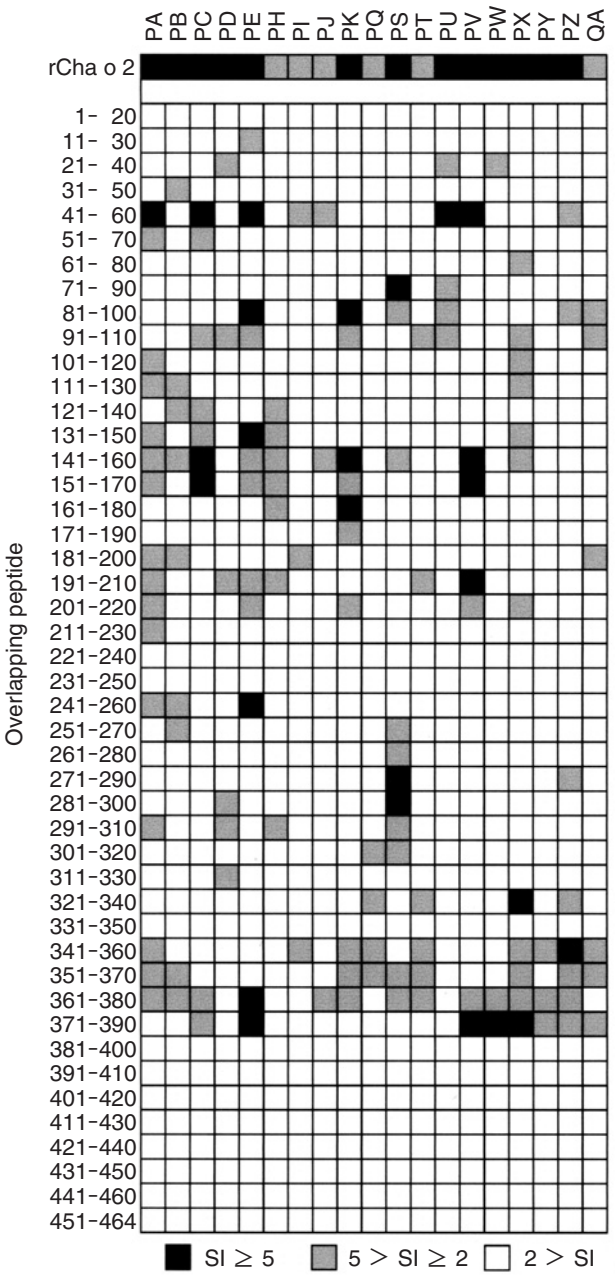

Fig. 1 Mapping of T cell epitopes in Cha o 2. TCL from 19 allergic patients were generated by stimulation with rCha o 2. Proliferation of TCL was tested with denoted overlapping 20-mer peptides in Cha o 2 in the presence of X-irradiated autologous EBV-B cells as APC. The values of incorporated [^{3}H]thymidine into TCL were converted to SI. The values of SI are shown.

p115–123, p274–289 and p293–301 in Cry j 2 has been reported.[21] Amino acid sequences of 2 peptides, p274–289 and p293–301, were found to be identical among Cry j 2 and Cha o 2, respectively.

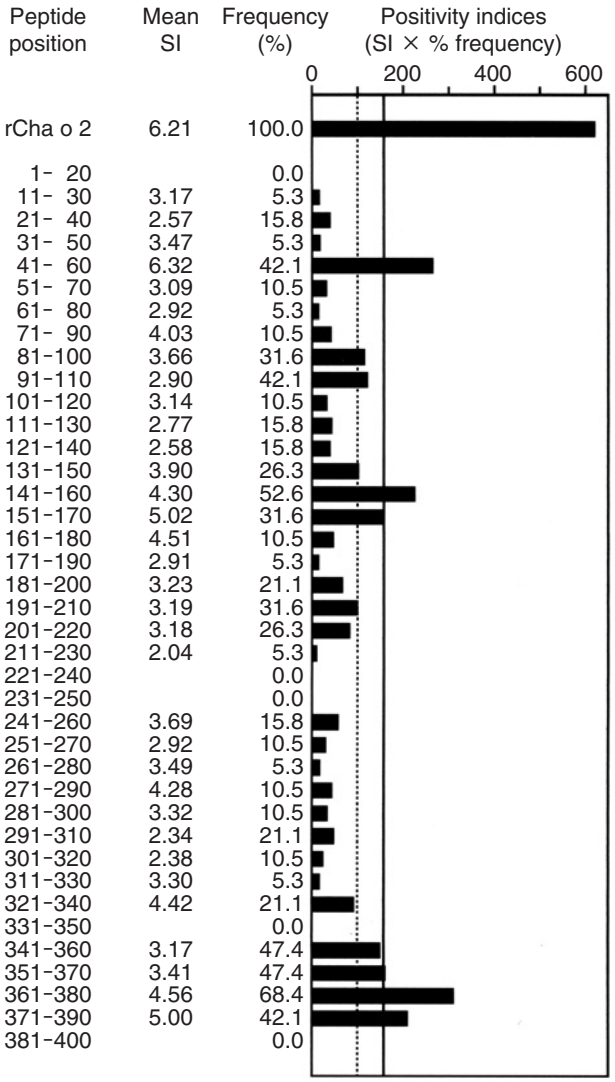

Fig. 2 A schematic representation of the positions of dominant and subdominant T cell epitopes evaluated by Positivity Indices (PI). PI is the product of the mean SI and the frequency in the population showing SI $\geq$ 2, denoted in Fig. 1. The solid and dotted lines indicate the threshold PI values of 155 (50% SI × 50% frequency) and 99 (40% SI × 40% frequency), respectively, to distinguish dominant and subdominant T cell epitopes.

CHARACTERIZATION OF TCC WITH SPECIFICITY FOR Cha o 2

TCC specific to rCha o 2 were generated from PBMC of 3 patients, PC, PV and PX. In total, 12 TCC specific to rCha o 2 (SI >5) were used for the following experiments. The phenotypes of all TCC determined by flow cytometry were CD3$^+$, CD4$^+$, CD8$^-$, Tαβ and Tγδ (unpublished data).

Individual TCC were reactive to one or two neighboring overlapping peptides of Cha o 2 and secreted IFN-γ and IL-4 measured by EIA (Table 1). Among 12 TCC, 10 (83%) were classified as Th2 cells, 2 (17%)

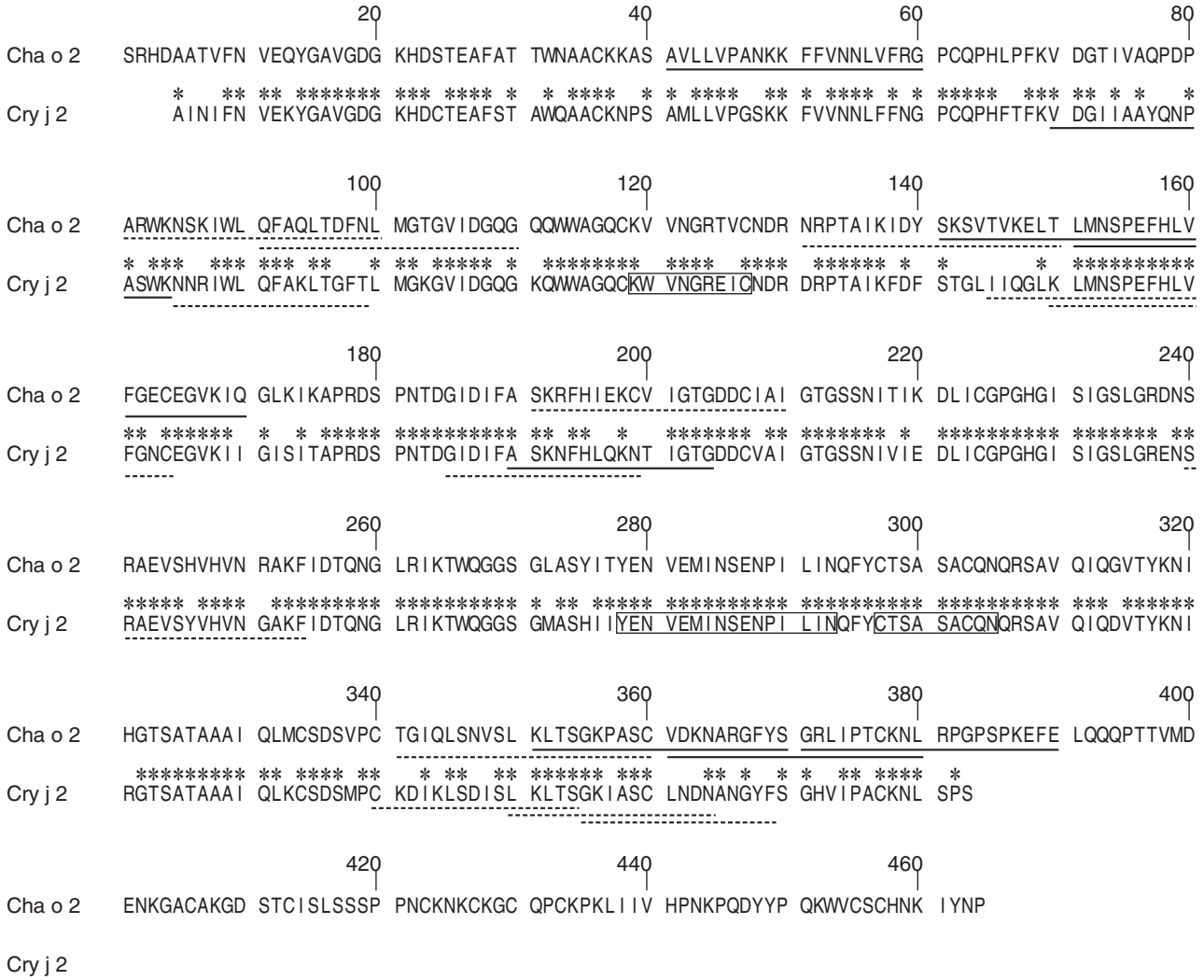

Fig. 3 Positions of dominant and subdominant T cell epitopes in Cha o 2 and Cry j 2. The asterisks indicate identical amino acid residues between Cha o 2 and Cry j 2. The solid and dotted underlines indicate the position of dominant and subdominant T cell epitopes, respectively. The PI for Cry j 2 peptides was calculated from the values of SI and the frequency in the population described elsewhere.[15] Boxed sequences in Cry j 2 indicate the position of linear IgE epitopes previously reported.[21]

were Th0 cells and none (0%) were Th1 cells.

Three TCC were further examined to determine whether they could recognize T cell epitopes in the sequence of Cry j 2 (Fig. 4). Two TCC, PV.202.5 and PC.205.101, were reactive to Cha o 2 p41–60 and p361–380, respectively, however these TCC could not respond to the stimulation with the corresponding overlapping peptides in Cry j 2. While a TCC PC.205.159 could recognize Cha o 2 p141–160, it showed no response to the stimulation with three peptides, p136–150, p141–155 and p146–160 in Cry j 2. Therefore, although 3 TCC reacted to individual peptides containing T cell epitopes in Cha o 2, they did not react to corresponding peptides in Cry j 2. These findings suggest that all dominant and subdominant T cell epitopes identified by the reactivity of TCL and TCC were unique to Cha o 2.

DISCUSSION

The explanation of the mechanism of cross-reactivity of allergens at the T cell level was undertaken using the relationship between birch pollen allergy and food allergy. Patients with birch pollen allergy sensitized with major allergen Bet v 1 frequently develop hypersensitivity to foods containing Mal d 1, such as apples,[22] Api g 1 in celery[23] and so on[24] especially in European countries. These reactions are caused by the presence of Bet v 1-specific IgE which are capable of binding to these food proteins, in addition to the presence of T cells reactive to major T cell epitopes in Bet v 1 and cross-reactive to homologous T cell epitopes in food allergens. Maintenance and/or generation of such T cells in patients with birch pollinosis by stimulation with homologous T cell epitopes in food

Table 1 Characterization of Cha o 2-specific TCC

Name of TCC	Epitope position	IFN-γ (pg/ml)	IL-4 (pg/ml)	Th subset
PC.203.22	121－140	1070	1370	Th0
PC.205.159	141－160	＜15	1990	Th2
PC.205.134	151－170	＜15	973	Th2
PC.204.26	361－380	77	32	Th0
PC.205.101	361－380	＜15	1010	Th2
PV.202.5	41－60	＜15	1540	Th2
PV.204.11	151－170	＜15	1200	Th2
PV.206.4	201－220	＜15	1970	Th2
PX.201.30	201－220	＜15	5450	Th2
PX.201.47	321－340	＜15	802	Th2
PX.201.14	351－370	＜15	1200	Th2
PX.201.8	371－390	＜15	402	Th2

allergens are postulated to be relevant to the onset of such food allergies.[24]

Almost all patients suffering from Japanese cedar pollinosis have IgE in their sera with specificity to Cry j 2 and Cha o 2.[9] In the present study, we identified T cell epitopes in Cha o 2 to study the mechanism of cross-reactivity between Japanese cedar pollinosis and Japanese cypress pollinosis at the T cell level. Individual TCL specific to Cha o 2 recognized multiple T cell epitopes in Cha o 2. Analysis of the reactivity of TCL to the stimulation with each overlapping peptide from sequences of Cha o 2 provided us with information on the existence of 11 dominant and subdominant T cell epitopes in Cha o 2. The C-terminus of Cry j 2 is identified as sera at position 379, since the C-terminal region of the precursor protein undergoes processing during maturation.[12] Simultaneously, Cha o 2 is thought to undergo the same cleavage, resulting in difficulty in identifying T-cell epitopes at position 381 to 464 in the C-terminal region of the Cha o 2.

The length of the antigenic peptides containing T cell epitope eluted from human leukocyte antigen (HLA) class II-DR, -DQ, and -DP molecules needs at least 10 amino acid residues.[25,26] Crystallographic investigation of a human αβTCR complex and HLA-class II molecule has clearly elucidated that the side chains of P-1, P2, P3, P5, P7, and P8 in the antigenic peptide are in contact with TCR and that the side chains of P1, P4, P6, and P9 are the anchor residues to binding HLA class II molecule.[27] Therefore, it can be said that most of the antigenic peptide are certainly important for specific presentation of the antigenic peptide by HLA-class II molecule and its recognition by TCR in the HLA-class II/TCR complex. Substitution in the antigenic peptide at each of the positions contacted by the TCR at positions P-1, P2, P3, P5, P7, and P8 created antagonist ligands.[27-29] Therefore, no substitution of the amino acids throughout antigenic peptides may permit complete stimulation of T cells reactive to the peptide.

According to the interpretation of complete activation of T cells via TCR and HLA-class II molecule/antigenic peptide complex, 10 dominant and subdominant T cell epitopes identified in Cha o 2, except for p151–162, are believed to be unique to Cha o 2. To confirm whether p151–162 is a common or unique T cell epitope between Cha o 2 and Cry j 2, we generated Cha o 2-specific TCC from 3 allergic patients. Finally, 12 TCC of which the majority belong to the Th2 subset judging from the production of IL4/IFN-γ ratio were obtained. PC.205.159 alone recognized a peptide p141–160 in Cha o 2. The TCC was reactive to p141–160 in Cha o 2 but not to the stimulation with corresponding peptides derived from Cry j 2 (Fig. 4). Looking at these results, our study has shown that the 11 dominant and subdominant T cell epitopes which were identified are unique to Cha o 2.

The onset mechanism of Japanese cypress pollinosis still remains controversial. The incidence of allergic patients sensitized with pollens from both Japanese cedar and Japanese cypress, Japanese cedar alone, and Japanese cypress alone, diagnosed by RAST or skin test, have been 77%, 23% and 0%, respectively.[6] Therefore, none of the allergic patients sensitized with cypress pollen alone exists. It is thought that allergic patients are first sensitized with Japanese cedar pollen and secondly with Japanese cypress pollen.

In a previous study, we clearly demonstrated the presence of 4 common T cell epitopes at p11–30, p211–230, p251–270 between Cha o 1 and Cry j 1.[18] T cells specific to common T cell epitopes maintain activated conditions during the cypress pollen season in April. These may take part in the formation of other activated T cells specific to Cha o 1.[18] On the contrary, T cells specific to Cha o 2 can recognize T cell epitopes which are unique to Cha o 2 and none are common to Cha o 2 and Cry j 2. The mechanism of the formation of Cha o 2-specific $CD4^+$ T cells in aller-

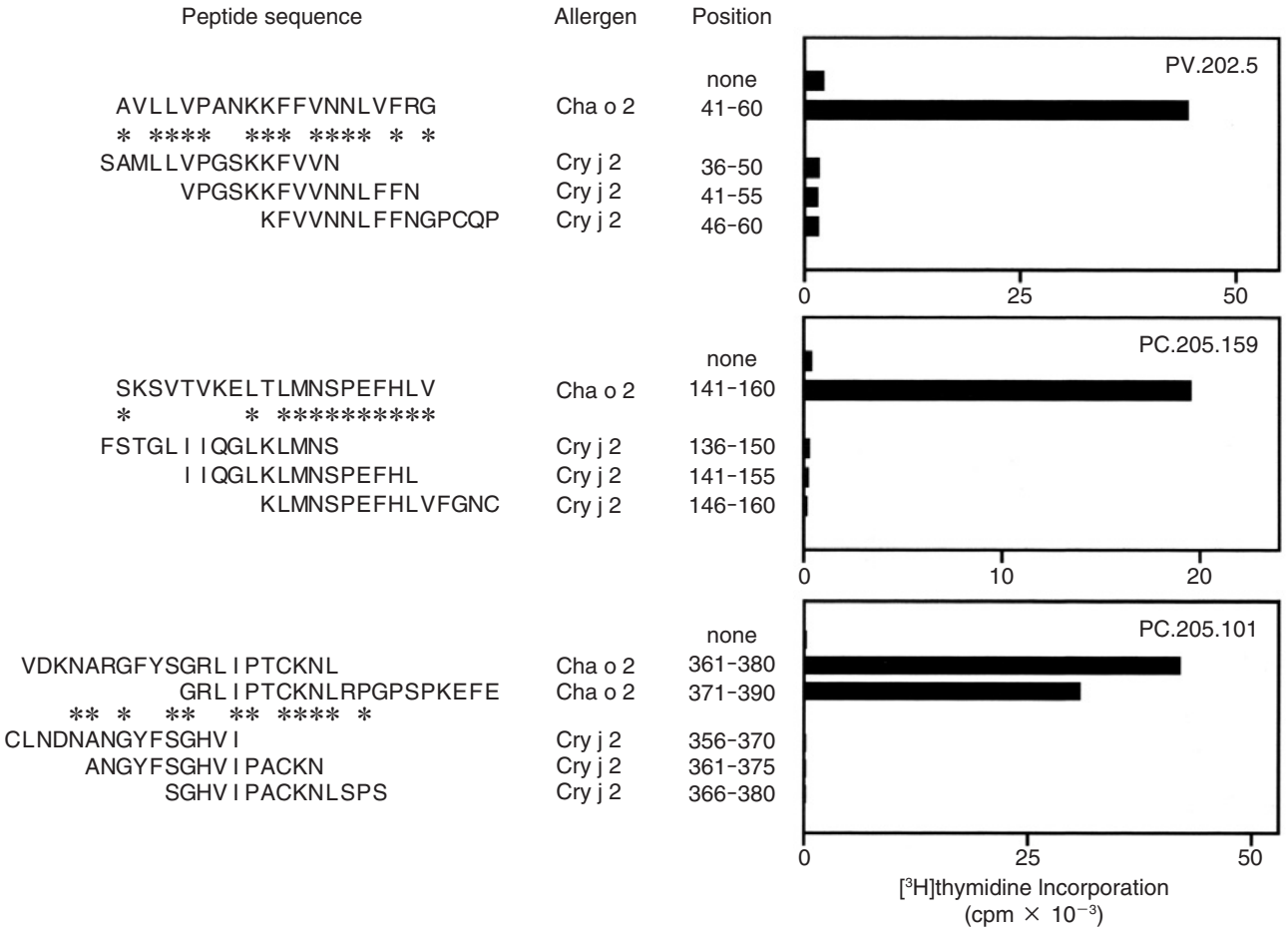

Fig. 4 Proliferative responses of TCC stimulated with peptides in Cha o 2 and Cry j 2. Proliferation of 3 TCC was tested with 20-mer and 15-mer peptides from Cha o 2 and Cry j 2, respectively, in the presence of X-irradiated autologous EBV-B cells as APC. Proliferation assay of the TCC is described in the Methods section. Cultures are set up in triplicates and the mean value is indicated (SEM < 10%).

gic patients who suffer from Japanese cedar pollinosis remain unresolved. We propose the possibility that B cells in allergic patients may possess a critical role for the generation of $CD4^+$ T cells unique to Cha o 2 in allergic patients. The capacity of B cells to present antigen to $CD4^+$ T cells is well established.[31,32] In addition, B cells are capable of participating in the priming of naïve T cells.[33,34] Furthermore, B cells take up IgE combined with its specific antigen or allergen via low affinity receptor for IgE (CD23a), instead of via B cell receptor, on its surface and efficiently presents antigen to $CD4^+$ T cells in mice in vivo[35-37] and in humans in vitro using EBV-B cells bearing CD23a as APC and allergen-specific TCC.[38,39] Interestingly, amino acid sequences of 2 linear IgE epitopes, p274–289 and p293–301, identified[21] in Cry j 2 are identical to those in Cha o 2 at p278–293 and p197–305, respectively (Fig. 3). After the Japanese cedar pollen season in March, IgE specific to p274–289 or p293–301 in Cry j 2 remains in the blood of allergic patients even in April. The IgE can bind to linear IgE epitopes in Cha o 2, resulting in the elevation of IgE uptake combined with Cha o 2 by naïve B cells via CD23a. Thereafter, naïve T cells specific to Cha o 2 may activate and clonally expand toward the formation of Cha o 2-specific $CD4^+$ effector T cells by antigen presentation via HLA-class II molecules on B cells after capturing IgE-Cha o 2 complex.

In general, the induction of Japanese cypress pollinosis is relevant to several immunopathological factors such as: (1) allergic patients pre-sensitized with Japanese cedar pollen, (2) the presence of T cells capable of reacting to common T cell epitopes in Cry j 1 and Cha o 1, (3) generation of IgE-producing B cells capable of binding to linear common B cell epitopes in Cry j 2 and Cha o 2, and (4) generation of $CD4^+$ effector T cells with specificity to T cell epitopes specific to either Cha o 1 or Cha o 2.

In conclusion, our results clearly indicate that T cell epitopes identified in our study are unique to Cha o 2, but are not common to Cry j 2. It is apparent that the generation of T cells specific to Cha o 2 in pa-

tients sensitized with Japanese cedar as well as Japanese cypress pollen directly plays a direct role in the prolongation of allergic symptoms in April.

REFERENCES

1. Di Felice G, Barletta B, Tinghino R, Pini C. Cupressaceae pollinosis: identification, purification and cloning of relevant allergens. *Int Arch Allergy Immunol* 2001;**125**:280-9.
2. Weber RW. Patterns of pollen cross-allergenicity. *J Allergy Clin Immunol* 2003;**112**:229-39.
3. Schwietz LA, Goetz DW, Whisman BA, Reid MJ. Cross-reactivity among conifer pollens. *Ann Allergy Asthma Immunol* 2000;**84**:87-93.
4. Pham NH, Baldo BA, Bass DJ. Cypress pollen allergy. Identification of allergens and crossreactivity between divergent species. *Clin Exp Allergy* 1994;**24**:558-65.
5. Yasueda H, Yui Y, Shimizu T, Shida T. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* 1983;**71**:77-86.
6. Namba H, Saitou K, Sahashi N *et al*. Relationship between pollen counts of *Cryptomeria japonica* and Cupressaceae and the severity of allergic symptoms. *Allergol Int* 2001; **50**:133-42.
7. Ito H, Nishimura J, Suzuki M *et al*. Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann Allergy Asthma Immunol* 1995;**74**:299-303.
8. Ito H, Suzuki M, Mamiya S *et al*. Analysis of the allergenic components of Hinoki cypress (*Chamaecyparis obtusa*) pollen by immunoblotting with the sera from patients with Japanese cedar pollinosis. *Allergol Int* 1996;**45**: 181-6.
9. Yasueda H, Saito A, Sakaguchi M *et al*. Identification and characterization of a group 2 conifer pollen allergen from *Chamaecyparis obtusa*, a homologue of Cry j 2 from *Cryptomeria japonica*. *Clin Exp Allergy* 2000;**30**:546-50.
10. Sone T, Komiyama N, Shimizu K, Kusakabe T, Morikubo K, Kino K. Cloning and sequencing of cDNA coding for *Cry j* I, a major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;**199**:619-25.
11. Komiyama N, Sone T, Shimizu K, Morikubo K, Kino K. cDNA cloning and expression of *Cry j* II, the second major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;**201**:1021-8.
12. Namba M, Kurose M, Torigoe K *et al*. Molecular cloning of the second major allergen, *Cry j* II, from Japanese cedar pollen. *FEBS Lett* 1994;**353**:124-8.
13. Suzuki M, Komiyama N, Itoh M *et al*. Purification, characterization and molecular cloning of Cha o 1, a major allergen of *Chamaecyparis obtusa* (Japanese cypress) pollen. *Mol Immunol* 1996;**33**:451-60.
14. Mori T, Yokoyama M, Komiyama N, Okano M, Kino K. Purification, identification, and cDNA cloning of Cha o 2, the second major allergen of Japanese cypress pollen. *Biochem Biophys Res Commun* 1999;**263**:166-71.
15. Sone T, Morikubo K, Miyahara *et al*. T cell epitopes in Japanese cedar (*Cryptomeria japonica*) pollen allergens: choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for the management of Japanese cedar pollinosis. *J Immunol* 1998;**161**:448-57.
16. Hirahara K, Tatsuta T, Takatori T *et al*. Preclinical evaluation of an immunotherapeutic peptide comprising 7 T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens. *J Allergy Clin Immunol* 2001; **108**:94-100.
17. Hashiguchi S, Hino K, Taniguchi Y *et al*. Immunodominance of seven regions of a major allergen, Cry j 2, of Japanese cedar pollen for T-cell immunity. *Allergy* 1996; **51**:621-32.
18. Sone T, Dairiki K, Morikubo K *et al*. 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. *Clin Exp Allergy* 2005;**35**:664-71.
19. Sone T, Morikubo K, Shimizu K, Komiyama N, Tsunoo H, Kino K. Peptide specificity, HLA class II restriction, and T-cell subsets of the T-cell clones specific to either Cry j 1 or Cry j 2, the major allergens of Japanese cedar (*Cryptomeria japonica*) pollen. *Int Arch Allergy Immunol* 1999;**119**:185-96.
20. Counsell CM, Bond JF, Ohman JL, Greenstein JL, Garman RD. Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. *J Allergy Clin Immunol* 1996;**98**:884-94.
21. Tamura Y, Kawaguchi J, Serizawa N *et al*. Analysis of sequential immunoglobulin E-binding epitope of Japanese cedar pollen allergen (Cry j 2) in humans, monkeys and mice. *Clin Exp Allergy* 2003;**33**:211-7.
22. Fritsch R, Bohle B, Vollmann U *et al*. Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. *J Allergy Clin Immunol* 1998;**102**:679-86.
23. Bohle B, Radakovics A, Jahn-Schmid B, Hoffman-Sommergruber K, Fischer GF, Ebner C. Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery:evidence at the T cell level. *Eur J Immunol* 2003;**33**:3303-10.
24. Bohle B. The impact of pollen-related food allergens on pollen allergy. *Allergy* 2007;**62**:3-10.
25. Rammensee H-G, Friede T, Stevanovic S. MHC ligands and peptide motifs: first listing. *Immunogenetics* 1995;**41**: 178-228.
26. Rammensee H-G, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 1999;**50**:213-9.
27. Hennecke J, Carfi A, Wiley DC. Structure of a covalently stabilized complex of a human alphabeta T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. *EMBO J* 2000;**19**:5611-24.
28. De Magistris MT, Alexander J, Coggeshall M *et al*. Antigen analog-major histocompatibility complexes act as antagonists of the T cell receptor. *Cell* 1992;**68**:625-34.
29. Scott Weber K, Donermeyer DL, Allen PM, Kranz DM. Class II-restricted T cell receptor engineered *in vitro* for higher affinity retains peptide specificity and function. *Proc Natl Acad Sci USA* 2005;**102**:19033-8.
30. Donermeyer DL, Scott Weber K, Kranz DM, Allen PM. The study of high-affinity TCRs reveals duality in T cell recognition of antigen: specificity and degeneracy. *J Immunol* 2006;**177**:6911-9.
31. Macaulay AE, Dekruyff RH, Goodnow CC, Umetsu DT. Antigen-specific B cells preferentially induce $CD4^+$ T cells to produce IL-4. *J Immunol* 1997;**158**:4171-9.
32. Constant SL. B lymphocytes as antigen-presenting cells for $CD4^+$ T cells priming in vivo. *J Immunol* 1999;**162**: 5695-703.
33. Rodríguez-Pinto D, Moreno J. B cells can prime naïve $CD4^+$ T cells in vivo in the absence of other professional

antigen-presenting cells in a CD154-CD40-dependent manner. *Eur J Immunol* 2005;**35**:1097-105.
34. Crawford A, MacLeod M, Schumacher T, Corlett L, Gray D. Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. *J Immunol* 2006; **176**:3498-506.
35. Kehry MR, Yamashita LC. Low-affinity IgE receptor (CD23) function on B cells: Role in IgE-dependent antigen focusing. *Proc Natl Acad Sci USA* 1989;**86**:7556-60.
36. Westman S, Gustavson S, Heyman B. Early expansion of B cells after primary immunization with antigen complexed with IgE. *Scand J Immunol* 1997;**46**:10-5.
37. Getahun A, Hjelm F, Heyman B. IgE enhances antibody and T cell responses in vivo via CD23$^+$ B cells. *J Immunol* 2005;**175**:1473-82.
38. van der Heiden FL, van Neerven RJJ, van Katwijk M, Bos JD, Kapsenberg ML. Serum-IgE-facilitated allergen presentation in atopic disease. *J Immunol* 1993;**150**:3643-50.
39. Bheekha Escura R, Wasserbauer E, Hammershumid F, Pearce A, Kidd P, Mudde GC. Regulation and targeting of T-cell immune responses by IgE and IgG antibodies. *Immunology* 1995;**86**:343-50.