

Analysis of Helper T Cell Responses to Cry j 1-Derived Peptides in Patients with Nasal Allergy: Candidate for Peptide-Based Immunotherapy of Japanese Cedar Pollinosis

Keisuke Masuyama¹, Kazuaki Chikamatsu¹, Shuji Ikagawa², Tomokazu Matsuoka¹, Goro Takahashi¹, Takanori Yamamoto¹ and Shuichiro Endo¹

ABSTRACT

Background: Allergen specific immunotherapy is highly effective, but adverse events may occur during treatment. Peptide-based immunotherapy has been proposed as one of new strategies for reduction of allergic adverse reactions. We examined the possibility of candidate peptides for the development of peptide-based immunotherapy for Japanese cedar pollinosis.

Methods: Twelve Cry j 1-specific T-cell lines were established from peripheral blood mononuclear cells (PBMC) of 12 patients with Japanese cedar pollinosis. Using these T-cell lines, 37 Cry j 1-derived overlapping peptides were assessed for their proliferative responses and cytokine production.

Results: Four peptides corresponding to the Cry j 1 sequence were able to induce proliferative responses to more than one T-cell line: p61–80 (3/12; 25.0%); p115–132 (2/12; 16.6%); p206–225 (4/12; 33.3%); and p337–353 (5/12; 41.7%). Furthermore, T-cell lines generated from 11 of 12 donors (91.7%) responded to at least one of these four peptides. On the other hand, the pattern of cytokine production from Cry j 1-specific T-cell lines varied. Moreover, cytokine production patterns by stimulation with Cry j 1 peptide did not reflect those by stimulation with Cry j 1 protein.

Conclusions: Our results suggest four Cry j 1-derived peptides (p61–80, p115–132, p206–225 and p337–353) may be considered to be the immunodominant T-cell epitopes of the Cry j 1 molecule, and can be useful for the design of peptide-based immunotherapy for the management of Japanese cedar pollinosis.

KEY WORDS

Cry j 1, immunotherapy, nasal allergy, peptide, Th response

INTRODUCTION

Allergic rhinitis is characterized by allergen-specific IgE production and activation of effector cells including eosinophils, mast cells, and basophils. These events are under regulation by a distinct subset of T lymphocytes, T helper 2 (Th2) cells that preferentially produce interleukin (IL)-4, IL-5, and IL-13. Thus, allergic diseases have been recognized as an

inadequate peripheral regulation of allergen-specific T-cells in individuals.

Allergen immunotherapy to allergic individuals induces a state of clinical and immunological tolerance; therefore, allergen immunotherapy is recognized as a highly effective treatment for allergic rhinitis. Evidence has accumulated indicating that immunotherapy inhibits allergen-driven Th2 responses by immune deviation in favor of Th1 responses and/or by

¹Department of Otolaryngology-Head and Neck Surgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi and ²Shirafuji ENT Clinic, Kumamoto, Japan.

Correspondence: Keisuke Masuyama, Department of Otolaryngology-Head and Neck Surgery, Interdisciplinary Graduate School

of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan.

Email: mkeisuke@yamanashi.ac.jp

Received 18 May 2008. Accepted for publication 2 July 2008.

©2009 Japanese Society of Allergology

Table 1 Clinical characteristics of the pollen allergic donors

Patients	Age/Sex	RAST score	Symptomatic period (m)	Other allergens ^a	Therapeutic history ^b
AR-1	27M	4	60	M, R	ME
AR-2	36M	2	24	—	None
AR-3	50F	2	12	—	ME
AR-4	38M	3	60	M	ME
AR-5	37M	3	36	M	ME
AR-6	37M	4	72	—	ME
AR-7	25M	2	60	M, R	ME
AR-8	34M	3	48	M	ME
AR-9	33M	4	72	—	ME
AR-10	32M	3	36	M	ME
AR-11	48F	3	72	R	ME
AR-12	16M	2	24	R	ME

^a M; house dust mites, R; ragweed.^b ME; medications using oral antihistamines.

inducing T regulatory responses which produce the inhibitory cytokines such as IL-10 and transforming growth factor (TGF)- β .¹⁻³ Recently, intended as a novel strategy for allergen immunotherapy, the use of peptides which retain immunogenicity but are of insufficient length to cross-link IgE on the surface of mast cells, thereby reducing the risk of inducing anaphylaxis, has been introduced.^{4,5} Actually, Oldfield *et al.* have demonstrated that overlapping Fel d 1-derived T-cell peptides produced hyporesponsiveness to rechallenge with peptides as well as whole allergen, both clinically and in terms of *in vitro* T cell reactivity.^{6,7} Moreover, treatment using these peptides showed significant improvements in clinical outcome measurements such as the allergen-induced nasal and bronchial reactions, and asthma/rhinitis quality of life.⁸

Japanese cedar pollinosis caused by exposure to *Cryptomeria japonica* pollen is one of the most prevalent allergic diseases in Japan, and two major allergens, Cry j 1 and Cry j 2, have been isolated and specific IgE have been detected in patients suffering from pollinosis.^{9,10} To date, a number of HLA class II-restricted Cry j 1 and Cry j 2 epitopes defined by CD4⁺ T cell have been identified.¹¹⁻¹⁴ Among these epitopes, Hirahara *et al.* have reported the development of a hybrid peptide in which 3 dominant T-cell determinants in Cry j 1 and 4 in Cry j 2 have been considered to be potential therapeutic agents for Japanese cedar pollinosis.¹⁵ They chose p212–224, p235–247 and p312–330 from among T cell epitopes identified in the Cry j 1 sequence. Similarly, Sone *et al.* have also reported an artificial polypeptide which contains two (p108–120 and p211–225) and three T cell epitopes chosen from Cry j 1 and Cry j 2 sequences, respectively.¹² As expected, this polypeptide was able to stimulate more efficiently PBMC obtained from allergic patients as compared with individual peptides. To explore the possibility of other candidate

peptides for the development of peptide-based immunotherapy for Japanese cedar pollinosis, we investigated helper T cell responses to overlapping peptides derived from Cry j 1 sequence using Cry j 1-specific T cell lines obtained from patients with Japanese cedar pollinosis.

METHODS

SUBJECTS

Peripheral blood mononuclear cells were obtained from twelve patients whose diagnosis was based on clinical symptoms, positive skin prick tests, and radioallergosorbent test (RAST) scores against Japanese cedar pollen. The study was approved by the Institutional Review Board at the University of Yamanashi, University Hospital. Written informed consent was obtained from each individual. Details of the clinical data are shown in Table 1.

PURIFICATION OF Cry j 1 AND PEPTIDE SYNTHESIS

Cry j 1, a major allergen of *Cryptomeria japonica* (Japanese cedar) pollen, was purified as described.^{9,16} Briefly, dry pollen was defatted with ether, placed in 0.125 M ammonium bicarbonate, and stirred for 2 days at 4°C. Solid ammonium sulphate was added to the extract to 100% saturation. The precipitate was dissolved in 0.01 M Tris-HCL, pH 7.8, and dialyzed against the same buffer. A sample of this preparation was applied to a DEAE-cellulose column (Whatman, Madison, U.K.) and eluted with the same buffer. The eluate was dialyzed against 0.01 M acetate buffer (pH 5.0) and applied to a CM-cellulose column (Whatman). The column was eluted first with 0.01 M acetate buffer, and then with 0.1 M phosphate buffer, pH 7.0. The second elute was concentrated and purified by molecular sieving HPLC (Bio Sil TSK-250; BioRad, Hercules, CA). A fraction thus obtained contained 41 and 46 kDa proteins as determined by SDS-PAGE

Table 2 HLA class II genotypes of the pollen allergic donors

Patients	DR		DQ		DP
AR-1	DRB1 * 1403	DRB3 * 0301	DQA1 * 0501	DQB1 * 0301	DPB1 * 0202
	DRB1 * 1501	DRB5 * 0101	DQA1 * 0102	DQB1 * 0602	DPB1 * 0402
AR-2	DRB1 * 0405	DRB4 * 0101	DQA1 * 0301	DQB1 * 0401	DPB1 * 0501
	DRB1 * 0410	DRB4 * 0101	DQA1 * 0301	DQB1 * 0402	DPB1 * 0301
AR-3	DRB1 * 0101		DQA1 * 0101	DQB1 * 0501	DPB1 * 0501
	DRB1 * 1502	DRB5 * 0102	DQA1 * 0103	DQB1 * 0601	DPB1 * 0901
AR-4	DRB1 * 0405	DRB4 * 0101	DQA1 * 0301	DQB1 * 0401	DPB1 * 0501
	DRB1 * 0901	DRB4 * 0101	DQA1 * 0301	DQB1 * 0303	
AR-5	DRB1 * 0901	DRB4 * 0101	DQA1 * 0301	DQB1 * 0303	DPB1 * 0501
	DRB1 * 1502	DRB5 * 0102	DQA1 * 0103	DQB1 * 0601	DPB1 * 0901
AR-6	DRB1 * 0405	DRB4 * 0101	DQA1 * 0301	DQB1 * 0401	DPB1 * 0501
	DRB1 * 1401	DRB3 * 0202	DQA1 * 0101	DQB1 * 0502	
AR-7	DRB1 * 1403	DRB3 * 0101	DQA1 * 0501	DQB1 * 0301	DPB1 * 0201
	DRB1 * 1302	DRB3 * 0301	DQA1 * 0102	DQB1 * 0604	DPB1 * 0901
AR-8	DRB1 * 1405	DRB3 * 0202	DQA1 * 0101	DQB1 * 0503	DPB1 * 0202
	DRB1 * 0803		DQA1 * 0103	DQB1 * 0601	DPB1 * 0201
AR-9	DRB1 * 0405	DRB4 * 0101	DQA1 * 0301	DQB1 * 0401	DPB1 * 0201
	DRB1 * 1302	DRB3 * 0301	DQA1 * 0102	DQB1 * 0604	DPB1 * 0501
AR-10	ND		ND		ND
AR-11	DRB1 * 1502	DRB5 * 0102	DQA1 * 0103	DQB1 * 0601	DPB1 * 0402
	DRB1 * 1302	DRB3 * 0301	DQA1 * 0102	DQB1 * 0604	DPB1 * 0501
AR-12	DRB1 * 1502	DRB5 * 0102	DQA1 * 0103	DQB1 * 0601	DPB1 * 0201
	DRB1 * 1402	DRB3 * 0202	DQA1 * 0501	DQB1 * 0301	DPB1 * 0901

ND; not determined.

analysis (data not shown).

Peptides were synthesized from the amino acid sequence of Cry j 1 reported by Griffith *et al.*¹⁷ and Sone *et al.*,¹⁸ by utilizing a solid-phase simultaneous multiple peptide synthesizer PSSM-8 (Shimadzu, Kyoto, Japan) based on Fmoc strategy with PyBOP-HOBt-NMM (1 : 1 : 1.5). Peptides overlapping by 6 to 15 amino acids and covering the entire Cry j 1 molecule were produced.¹³ All peptides were purified by C18 reverse-phase high-performance liquid chromatography (Millipore Corp., Bedford, MA).

HLA CLASS II TYPING

Genotypes of HLA class II (DR, DQ, DP) alleles of the pollen allergic donors were determined by hybridization of HLA-DR, DQ, and DP genes amplified by polymerase chain reaction with sequence-specific oligonucleotide probes, as described previously.¹³

ESTABLISHMENT OF Cry j 1-SPECIFIC T-CELL LINES

T-cell lines were generated by stimulating 1×10^5 PBMC with Cry j 1 (2.5 µg/ml) in RPMI-1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% pooled, heat-inactivated normal human serum in each of sixty wells of 96-well flat-bottomed culture plates (Nunc, Roskilde, Den-

mark). After 7 days, irradiated (30 Gy) autologous PBMC at 1×10^5 /well, human rIL-2 (10 U/ml), human rIL-4 (10 U/ml), and Cry j 1 (2.5 µg/ml) were added to culture wells with T-cell blasts and maintained for an additional 7 days. Antigen-induced proliferation of the T-cell blasts was assayed, and the culture wells exhibiting Cry j 1-specific proliferation were transferred and propagated in a 24-well plate.

PROLIFERATION ASSAYS

T cells were co-cultured with irradiated (30 Gy) autologous PBMC (1×10^5 /well) in the presence of Cry j 1 (1.25 µg/ml) in 96-well flat-bottomed plates for 72 hours and pulsed with 1 µCi/well of [³H] TdR for the last 24 hours, and the incorporated radioactivity was measured by liquid scintillation counting. Stimulation indices (S.I.) were calculated by comparing the antigen-containing wells with those containing medium alone. In the case where synthesized peptides were used as antigen, PBMC (1.5×10^5 /well) were cultured with various overlapping peptides (2.5 µM) in culture medium for 2 hours at 37°C, after which excess peptides and nonadherent cells were removed by gently washing the plates three times with RPMI-1640 media containing 3% human serum. The remaining adherent cells were irradiated at 30 Gy and used as antigen presenting cells (APC).

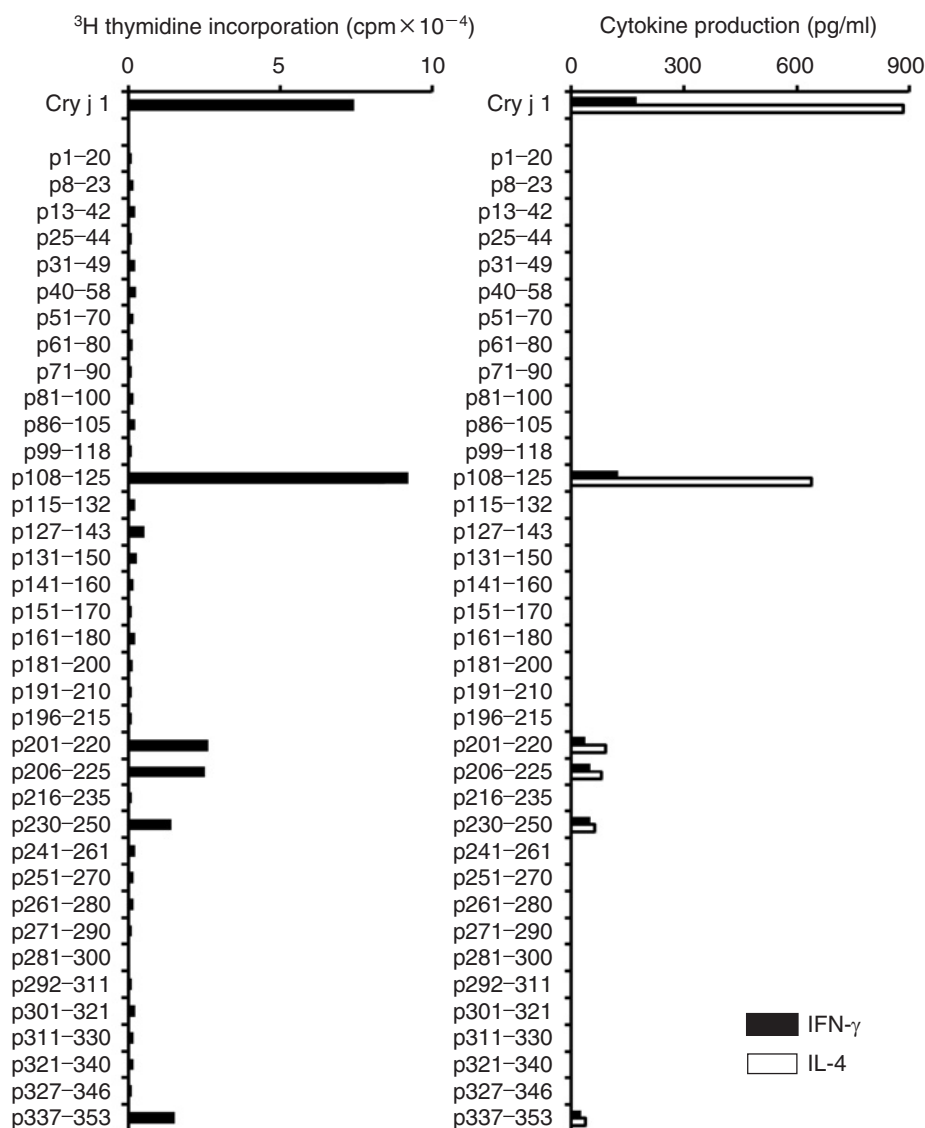


Fig. 1 Proliferative response and cytokine production by a Cry j 1-specific T cell line established from patient AR-1. Cry j 1-specific T-cell line established from patient AR-1 responded to 5 different peptides; p108–125, p201–220, p206–225, p230–250, and p337–353.

QUANTIFICATION OF IL-4 AND IFN- γ IN SUPERNATANT OF THE T CELL LINES

Culture supernatants of the same T cell lines evaluated for proliferative responses, after stimulation by APC plus antigen, were collected immediately before the addition of [³H] TdR and stored in aliquots at –80°C until the lymphokine concentrations were determined. IL-4 ELISA and IFN- γ ELISA (MEDGENIX, Fleurus, Belgium) were used to quantify IL-4 and IFN- γ in the supernatants according to the manufacturer's instructions.

RESULTS

ESTABLISHMENT OF Cry j 1-SPECIFIC T-CELL LINES

We generated Cry j 1-specific T-cell lines from PBMC obtained from all 12 pollen allergic donors (Table 1, 2). After 3 x in vitro stimulation, the outgrowing T cells were tested for their proliferative responses in the presence of Cry j 1 protein and irradiated autologous PBMC. Moreover, these Cry j 1-specific T cell lines were tested for their production of IFN- γ and IL-4. As expected, these T-cell lines produced various amounts of IFN- γ and/or IL-4 in response to Cry j 1 presented by autologous PBMC. Figure 1 shows the

Table 3 Proliferative responses to Cry j 1-derived peptides of Cry j 1-specific T-cell lines obtained from the pollen allergic donors^a.

Peptide	Patients												Total Responses
	AR-1	AR-2	AR-3	AR-4	AR-5	AR-6	AR-7	AR-8	AR-9	AR-10	AR-11	AR-12	
p13–32		++											1/12
p61–80			+							+		+++	3/12
p71–90								+					1/12
p81–100			+										1/12
p86–105			+										1/12
p108–125	+++												1/12
p115–132		+			+++								2/12
p127–143				++									1/12
p201–220	+++												1/12
p206–223	+++			+			+				+		4/12
p230–250	+++												1/12
p261–280			+										1/12
p271–290								+++					1/12
p301–321				++									1/12
p337–353	+++			++		+++			++		+		5/12

^a +; 5 ≤ S.I. < 50, ++; 50 ≤ S.I. < 100, +++; 100 ≤ S.I.

representative data obtained from patient AR-1.

PROLIFERATIVE RESPONSES TO Cry j 1-DERIVED OVERLAPPING PEPTIDES

Next, proliferative responses of each established T-cell lines against autologous PBMC pulsed with Cry j 1-derived overlapping peptides were investigated. As shown in Figure 1, Cry j 1-specific T-cell line established from patient AR-1 responded 5 different peptides; p108–125, p201–220, p206–225, p230–250, and p337–353. Similarly, all established Cry j 1-specific T-cell lines were tested for proliferative responses to Cry j 1-derived overlapping peptides. Table 3 summarizes the proliferative responses to Cry j 1-derived overlapping peptides. Among a panel of 37 overlapping peptides corresponding to the amino acid sequence of Cry j 1, 15 peptides were able to stimulate at least one T-cell line tested. These peptides represent a widely dispersed set of epitopes from the N- to the C-terminus of the Cry j 1 molecule. It is highly likely that these antigenic peptides include the T-cell epitopes of Cry j 1. Notably, 4 peptides were able to induce proliferative responses to more than two T-cell lines: p61–80 (3/12; 25.0%); p115–132 (2/12; 16.6%); p206–225 (4/12; 33.3%); and p337–353 (5/12; 41.7%). Furthermore, T-cell lines generated from eleven of twelve donors (all except AR-8) responded to at least one of these four peptides. Accordingly, these results suggest that these 4 peptides may be immunodominant T-cell epitopes of Cry j 1.

CYTOKINE PRODUCTION BY STIMULATION WITH Cry j 1 AND Cry j 1-DERIVED OVERLAPPING PEPTIDES

Nine of the 12 established T-cell lines were assessed for cytokine production by stimulation with Cry j 1 protein. However, the pattern of cytokine production from Cry j 1-specific T-cell lines varied. Two T-cell lines obtained from AR-1 and AR-2, produced IL-4 predominantly (Th2 pattern), whereas 4 (AR-6, -7, -8, and -9) produced IFN- γ predominantly (Th1 pattern), and 3 (AR-3, -4, and -5) produced IFN- γ and IL-4 equally (Th0 pattern), as shown in Table 4. Patients' RAST scores, the length of their clinical experience, and their therapeutic history did not appear to bear any relationship to their pattern of cytokine production (data not shown).

Next, the cytokine production by stimulation with 37 overlapping peptides was also assessed. Similar to proliferation responses, each T-cell line except AR-5, produced cytokines by stimulation with identical peptides that showed proliferative responses (data not shown). However, cytokine production patterns by stimulation with Cry j 1 peptide did not reflect those by stimulation with Cry j 1 protein (Table 4). Although most T-cell lines reactive with Cry j 1-derived peptides produced some IL-4 irrespective of the amount of IFN- γ production. Interestingly, however, the T-cell lines obtained from patients AR-3 and AR-8 secreted IFN- γ but failed to produce IL-4 in recognition of the peptides, p86–105 and p271–290, respectively. For further characterization of these two peptide specific T-cell lines, these T-cell lines were incubated with peptide-pulsed APC during various peri-

Table 4 Cytokine production profiles of Cry j 1-specific T-cell lines obtained from the pollen allergic donors

Antigens	Patients								
	AR-1	AR-2	AR-3	AR-4	AR-5	AR-6	AR-7	AR-8	AR-9
Cry j 1 protein	174/877 ^a	437/1410	39/34	550/509	393/316	665/131	59/20	204/46	49/15
p13–32		82/127							
p61–80			269/46						
p71–90								37/ < 10	
p81–100			39/32						
p86–105			73/ < 10						
p108–125	116/638								
p115–132		< 8/109			480/218				
p127–143				23/118					
p201–220	36/87								
p206–223	51/80			18/27	95/ < 10		27/12		
p230–250	51/58								
p261–280			57/35						
p271–290								91/ < 10	
p301–321				127/115					
p337–353	44/36			127/214		131/98			203/93

^a The values indicate IFN- γ /IL-4 production, respectively.

ods (24, 48, and 72 hours), and at which times culture supernatants were collected for the determination of cytokine concentrations. These two T-cell lines failed to produce IL-4, while producing large amounts of IFN- γ and undergoing considerable proliferation throughout these three incubation periods (data not shown). Moreover, cultures using various peptide concentrations (2.5, 5, and 10 μ M) were also performed, however; the same patterns of cytokine production were observed (data not shown). These results suggest that p86–105 and p271–290 are likely to be Cry j 1-derived Th1 epitopes.

DISCUSSION

Allergen-specific immunotherapy is a highly effective treatment in patients with IgE-dependent diseases including insect venom anaphylaxis and allergic rhinitis. However, the administration of natural allergen extracts might occasionally give rise to adverse events due to severe IgE mediated reactions, therefore; several new strategies have been developed to reduce the potential to cross-link IgE on mast cells while still containing the relevant T-cell epitopes. As a new approach, synthetic peptides corresponding to T-cell epitopes of the allergen have been evaluated in various studies for peptide-based immunotherapy. More recently, a clinical immunotherapy trial that used synthetic peptides of allergens was also reported.^{8,19,20} One of the advantages of peptide-based immunotherapy may be related to the insufficient length to cross-link IgE on the surface of mast cells, thereby eliminating the risk of induction of anaphylaxis. Another advantage is that immunotherapy can be accomplished within a shorter period of time us-

ing a relatively high-dose injection of antigenic peptides. Fellrath *et al.* have designed a double-blind, placebo-controlled phase I clinical trial in patients hypersensitive to bee venom using 3 long synthetic overlapping peptides mapping the whole sequence of phospholipase A2, a major bee venom allergen, and demonstrated that there was peptide-specific T-cell hyporesponsiveness and an increase of IL-10 and IFN- γ secretion by stimulation with peptides in the peptide group.²⁰ On the other hand, Kay A.B. and colleagues have demonstrated that 12 synthetic overlapping peptides encompassing most of the T-cell epitopes of the major cat allergen Fel d 1 can induce T cell tolerance in animal experiments and these peptides have been shown to decrease the amount of proliferation as well as cytokine production in cat allergic patients.^{6,7} Moreover, this treatment has also been reported to have potential for inhibiting upper and lower airway outcome measurements in a pilot study.⁸ Thus, peptide-based immunotherapies have been shown to be effective for bee venom and cat allergen.

In the present study, we demonstrated that four peptides of Cry j 1 (p61–80, p115–132, p206–225 and p337–353) activated more than one T-cell line. Furthermore, T-cell lines generated from eleven out of twelve donors reacted with at least one of these four peptides. Therefore, a mixture of these four peptides may be a useful cocktail for peptide-based immunotherapy for patients sensitive to Cry j 1. To date, Hirahara *et al.* and Sone *et al.* have reported on the benefit of the hybrid or polypeptides consisting of Cry j 1-derived T cell epitopes for the development of peptide-base immunotherapy.^{12,15} These hybrid pep-

tides or polypeptides would have superior potential for inducing T-cell proliferative responses as compared with a mixture of the T-cell determinants. However, the clinical efficacy in immunotherapy using these peptides has yet to be reported. Hirahara *et al.* and Sone *et al.* have selected a total 4 different peptides, p108–120, p211–225, p235–247, and p312–330 from T cell-epitopes identified in the Cry j 1 sequence. In our study, p206–225 inducing T-cell proliferative responses in 4/12 (33.3%) was the identical peptide which they selected. Alternatively, 2 other peptides, p61–80 and p337–353 have also been shown to induce proliferative responses in relative high frequency, 3/12 (25.0%) and 4/12 (41.7%), respectively. Hori *et al.* have demonstrated a significant positive association between Japanese cedar pollinosis and HLA-DPB1*0501 (79.2% in patients versus 60.6% in controls).¹⁴ Furthermore, they found that p214–222 was the minimal antigenic site of the immunodominant peptide for the HLA-DPB1*0501 restricted T cells. Similar to p206–225, the p61–80 peptide has also been reported to be presented in the context of HLA-DPB1*0501 alleles as T-cell defined epitope.¹¹ HLA-DPB1*0501 is one of the most frequently expressed HLA-DP common alleles in the Japanese population, therefore; these two peptides may be useful for peptide-based immunotherapy. It is noteworthy that T cell lines generated from some DPB1*0501 negative donors were able to proliferate in response to p61–80 or p206–225 peptides, suggesting that these two peptides may be recognized in the context of any other HLA class II. With respect to another peptide, p337–353, the restriction element remains unknown. Ikagawa *et al.* showed that Cry j 1 p335–346 was presented in the context of HLA-DRA + DRB3*0301, however; the frequency of HLA-DRB3*0301 is less than 20% in the Japanese population.¹³ Therefore, the p337–353 peptide may also be an immunodominant T cell epitope that is presented by multiple HLA class II molecules. Another possibility is that the p337–353 peptide may be more immunogenic among Cry j 1-derived T cell epitopes. Thus, for development of a broadly applicable peptide-based immunotherapy, universal T cell epitopes that are immunogenic in individuals of many HLA haplotypes should be identified, selected, and used.

In general, it is accepted that PBMC of allergic patients preferentially induce Th2 rather than Th1 responses under stimulation with certain allergens; however, proteins or longer peptides containing multiple epitopes may induce various T-helper responses, and the extent of T-helper responses to each epitope may also vary. Actually, Sone *et al.* indicated that the p191–205 peptide presented by HLA-DRB1*0901 and HLA-DQB1*0602 molecules could preferentially induce Th2 and Th0, respectively.¹¹ Gardner *et al.* demonstrated that high dose allergen stimulation of T cells induced expansion of IFN- γ + T cells, apoptosis

of CD4+IL-4+ T cells and T cell anergy in house dust mite allergic patients.²¹ Thus, other factors such as HLA class II allele, the allergic status of the donor, type of antigens and their concentration, might also influence immune responses in individuals. Interestingly, Cry j 1-specific T-cell lines obtained from AR-3 and AR-8 showed Th1 responses to p86–105 and p271–290, respectively. These T-cell lines secreted IFN- γ , but not IL-4 regardless of the time course and antigen concentrations. Th1 cells have inhibitory effects on Th2 function and have been shown to prevent airway inflammation in mouse models.²² Alternatively, Szabo *et al.* have demonstrated that Th1-associated transcription factor, T-bet not only induced Th1 development but also actively suppressed Th2 differentiation in vitro.²³ Thus, allergen-specific Th1 cells appear to have a suppressive function to Th2 responses to allergen, however; it remains unclear how allergen-specific Th1 cells are interrelated to other regulatory T cell subsets including naturally occurring CD4+CD25+ T cells, Tr1, and Th3 cells, in allergic disease. Furthermore, there is little information regarding the relation between the nature of the peptide and the clinical efficacy in peptide-based immunotherapy. A better understanding of the nature of peptides and the specificity of Cry j 1-specific T cell responses in allergic patients is necessary to better design, and develop more effective peptide-based immunotherapy in the future.

Taken together, our data indicated that four Cry j 1-derived peptides (p61–80, p115–132, p206–225 and p337–353) may be considered to be the immunodominant T-cell epitopes of the Cry j 1 molecule, and can be useful for the design of broadly applicable efficacious peptide-based immunotherapy for the management of Japanese cedar pollinosis.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by a Health Science Research Grant from the Ministry of Health, Labour and Welfare.

We thank Drs. M. Suzuki (Ajinomoto Co., Kawasaki, Japan) and K. Hama (Ono Pharmaceuticals, Osaka, Japan) for kindly supplying the human recombinant IL-2 and IL-4, respectively.

REFERENCES

1. Nelson HS. Allergen immunotherapy: where is it now? *J Allergy Clin Immunol* 2007;**119**:769-77.
2. Durham SR. Allergen immunotherapy (desensitization) for allergic diseases. *Clin Med* 2006;**6**:348-51.
3. Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. *J Allergy Clin Immunol* 2004;**113**:1025-34.
4. Larche M. Peptide therapy for allergic diseases: basic mechanisms and new clinical approaches. *Pharmacol Ther* 2005;**108**:353-61.

5. Larche M. Update on the current status of peptide immunotherapy. *J Allergy Clin Immunol* 2007;**119**:906-9.
6. Oldfield WLG, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001;**167**:1734-9.
7. Oldfield WLG, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;**360**:47-53.
8. Alexander C, Tarzi M, Larche M *et al*. The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. *Allergy* 2005;**60**:1269-74.
9. Yasueda H, Yui Y, Shimizu T, Shida T. Isolation and partial characterization of major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* 1983;**71**:77-86.
10. Sakaguchi M, Inouye S, Tanai M, Ando S, Usui M, Matuhasi T. Identification of the second major allergen of Japanese cedar pollen. *Allergy* 1990;**45**:309-12.
11. 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.
12. Sone T, Morikubo K, Miyahara M *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.
13. Ikagawa S, Matsushita S, Chen Y-Z, Ishikawa T, Nishimura Y. Single amino acid substitutions on a Japanese cedar pollen allergen (Cry j 1)-derived peptide induced alterations in human T cell responses and T cell receptor antagonism. *J Allergy Clin Immunol* 1996;**97**:53-64.
14. Hori T, Kamikawaji N, Kimura A *et al*. Japanese cedar pollinosis and HLA-DP5. *Tissue Antigens* 1996;**47**:485-91.
15. 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.
16. Matsushita S, Muto M, Suemura M, Saito Y, Sasazuki T. HLA-linked nonresponsiveness to *Cryptomeria japonica* pollen antigen. I. Nonresponsiveness is mediated by antigen-specific suppressor T cell. *J Immunol* 1987;**138**:109-15.
17. Griffith IJ, Lussier A, Garman R *et al*. Cloning of Cry j 1, the major allergen of *Cryptomeria japonica* (Japanese cedar) [abstract]. *J Allergy Clin Immunol* 1993;**91**:339.
18. Sone T, Komiyama N, Shimizu K *et al*. Cloning and sequencing of cDNA coding for Cry j 1, a major allergen of Japanese cedar pollen. *Biochem Biophys Res* 1994;**199**:619-25.
19. Muller U, Akdis CA, Fricker M *et al*. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;**101**:747-54.
20. Fellrath JM, Kettner A, Dufour N *et al*. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol* 2003;**111**:854-61.
21. Gardner LM, O'Hehir RE, Rolland JM. High dose allergen stimulation of T cells from house dust mite-allergic subjects induces expansion of IFN- γ + T cells, apoptosis of CD4+IL-4+ T cells and T cell anergy. *Int Arch Allergy Immunol* 2004;**133**:1-13.
22. Cohn L, Homer RJ, Niu N *et al*. T helper 1 cells and interferon γ regulate allergic airway inflammation and mucus production. *J Exp Med* 1999;**190**:1309-17.
23. Szabo SJ, Sullivan BM, Stemann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in Th1 lineage commitment and IFN- γ production in CD4 and CD8 T cells. *Science* 2002;**295**:338-42.