

Analysis of sequential immunoglobulin E-binding epitope of Japanese cedar pollen allergen (Cry j 2) in humans, monkeys and mice

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Summary

Background Japanese cedar (*Cryptomeria japonica*; CJ) pollinosis has been reported to occur naturally in Japanese monkeys (*Macaca fuscata*) as well as in humans. Most human patients and monkeys with pollinosis have specific IgE for Cry j 2, a major allergen of CJ pollen.

Objective The main purpose of this study was to identify IgE B cell epitopes of Cry j 2 using a synthetic peptide in humans, monkeys and mice.

Methods We synthesized 38 overlapping peptides that span the entire length of Cry j 2. We examined the B cell epitopes of Cry j 2 that are recognized by IgE in the sera of human patients and monkeys with pollinosis and immunized mice using synthetic peptides of Cry j 2. We also examined the reaction of Cry j 2-specific mouse monoclonal IgG antibodies to the peptides. Furthermore, we conducted a histamine release assay with leucocytes from a pollinosis patient using human serum albumin (HSA) conjugated with the peptides as a B cell epitope.

Results We found that 16 of the 20 pollinosis patients who had specific IgE to Cry j 2 also exhibited IgE reaction with some Cry j 2 peptides. Of these 16 patients, 10 exhibited IgE reaction with Cry j 2 peptide no. 13 (¹²¹GQCKWVNGREICNDRDPTA¹⁴⁰). Five of the seven monkeys with CJ pollinosis exhibited a reaction with peptide no. 13. Furthermore, IgE in mice immunized with Cry j 2 and two mouse monoclonal IgG antibodies reacted with peptide no. 13. Peptide no. 13-conjugated HSA showed the release of histamine from basophils. Furthermore, to determine the minimum epitope in peptide no. 13, we conducted an enzyme-linked immunosorbent assay inhibition test. The core of the epitope in humans, monkeys and mice was ¹²⁴KWVNGREI¹³¹.

Conclusion We found that ¹²⁴KWVNGREI¹³¹ is an important B cell epitope recognized by IgE in humans, monkeys and mice.

Keywords allergen, epitope, IgE, Japanese cedar pollen

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Introduction

Japanese cedar (*Cryptomeria japonica*; CJ) pollinosis is a typical type I allergy induced by CJ pollen and one of the most common allergic diseases in Japan [1, 2]. In areas where there are CJ trees, approximately 30% of the general population have specific IgE for CJ allergen and approximately 10% of the population suffer from the symptoms [1, 2]. Furthermore, the natural occurrence of CJ pollinosis in Japanese monkeys (*Macaca fuscata*) has been reported [3, 4]. These monkeys with CJ pollinosis show

symptoms similar to those of humans: tear secretion, eye redness, sneezing and rhinorrhoea.

Two major allergens, Cry j 1 and Cry j 2, have been isolated [5, 6]. Cry j 2 has been characterized as a major allergen that is completely different from Cry j 1 in terms of immunophysicochemical properties and N-terminal amino acid sequences [6]. The cDNA nucleotide sequence of Cry j 2 has been determined [7, 8]. We found that most humans [9] and monkeys [10] with CJ pollinosis symptoms have anti-Cry j 2 IgE.

Epitope mapping using synthetic peptides has been demonstrated to be a suitable tool for determining some B cell epitopes of some allergens [11–13]. Understanding the mechanisms of allergen-specific immune reactions in allergic diseases is expected to offer researchers a means of establishing methods for more efficient immunotherapies or more appropriate treatment of allergic diseases. No previous report has addressed the analysis

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of sequential epitope mapping of Cry j 2 using synthetic peptides.

In this study, we investigated the determinants of the epitopes of Cry j 2 recognized by IgE in human patients and monkeys with pollinosis and immunized mice using synthetic peptides.

Materials and methods

Antigen

Cry j 2, a major allergen in CJ pollen, was purified as described previously [6].

Peptides

Thirty-eight overlapping peptides that span the entire length of Cry j 2 were synthesized by means of a solid-phase technology using a solid-phase peptide synthesizer, Symphony (Protein Technologies, Inc., Tucson, AZ, USA) based on the primary sequence of Cry j 2 (Table 1). In addition, we prepared synthetic peptides which was considered to be the candidate epitope (Table 2).

Sera from pollinosis human patients and monkeys

Sera were obtained from 20 human patients and seven monkeys with CJ pollinosis and high titres of specific IgE for crude CJ pollen allergen and Cry j 2 [more than CAP class 3 (3.5 UA/mL) using the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden)]. The patients and monkeys were selected on the basis of clinical symptoms of seasonal allergic rhinitis. As a negative control, 20 human patients with mite allergy and high titres of specific IgE for mite allergens had no anti-CJ IgE.

Sera from immunized mice

Ten female BDF1 mice, 8 weeks of age, were purchased from Japan SLC Inc. (Shizuoka, Japan) and immunized with Cry j 2. The mice were immunized by intraperitoneal (i.p.) injection of 10 µg of Cry j 2 mixed with 2 mg of aluminium hydroxide gel (Pierce, Rockford, IL, USA). The mice were boosted six times with 1 µg of Cry j 2 and 2 mg of aluminium hydroxide gel at 2-week intervals. Two weeks after the final immunization, serum samples were obtained from the immunized mice.

Depletion of IgG from serum

IgG produced in the mice after immunization with Cry j 2 and an aluminium hydroxide gel adjuvant may compete with IgE for immobilized antigens in the enzyme-linked immunosorbent assay (ELISA). To avoid such interference, we eliminated IgG in the sera of the mice with the use of a protein G column (Zymed, San Francisco, CA, USA) [14].

Mouse monoclonal IgG antibodies

We used three monoclonal IgG antibodies for Cry j 2 (24 [15], N26 [16] and S1) in this study. One monoclonal IgG antibody (S1) was prepared as previously described [16].

Table 1. Overlapping synthetic peptides of Cry j 2

No.	Position	Amino acid sequence	
1	1–20	RKVEHSRHDA	INIFNVEKYG
2	11–30	INIFNVEKYG	AVGDGKHDCT
3	21–40	AVGDGKHDCT	EAFSTAWQAA
4	31–50	EAFSTAWQAA	CKKPSAMLLV
5	41–60	CKKPSAMLLV	PGNKKFVNN
6	51–71	PGNKKFVNN	LFENGPCQPHF
7	61–80	LFENGPCQPH	FTFKVDGIIA
8	71–90	FTFKVDGIIA	AYQNPASWKN
9	81–100	AYQNPASWKN	NRIWLQFAKL
10	91–110	NRIWLQFAKL	TGFTLMGKGV
11	101–120	TGFTLMGKGV	IDGQKQWWA
12	111–130	IDGQKQWWA	GQCKWVNGRE
13	121–140	GQCKWVNGRE	ICNDRDRPTA
14	131–150	ICNDRDRPTA	IKFDFSTGLI
15	141–161	IKFDFSTGLI	IQGLKLMNSPE
16	151–170	IQGLKLMNSP	EFHLVFGNCE
17	161–180	EFHLVFGNCE	GVKIIGISIT
18	171–190	GVKIIGISIT	APRDSPTDGD
19	181–200	APRDSPTDGD	IDIFASKNFH
20	191–210	IDIFASKNFH	LQKNTIGTGD
21	201–220	LQKNTIGTGD	DCVAIGTGSS
22	213–232	VAIGTGSS	NIVIEDLICGPG
23	221–240	NIVIEDLICG	PGHGISIGSL
24	231–250	PGHGISIGSL	GRENSRAEVS
25	241–260	GRENSRAEVS	YVHVNGAKFI
26	251–270	YVHVNGAKFI	DTQNGLRIKT
27	261–280	DTQNGLRIKT	WQGGSGMASH
28	271–290	WQGGSGMASH	IYENVEMIN
29	279–303	SHIYENVEMIN	SENPILINQFYCT
30	291–310	SENPILINQF	YCTSASACQN
31	301–320	YCTSASACQN	QRSVAQIQDV
32	310–330	NQRSVAQIQDV	TYKNIRGYSA
33	321–340	TYKNIRGYSA	TAAAIQLKCS
34	331–350	TAAAIQLKCS	DSMPCKDIKL
35	341–360	DSMPCKDIKL	SDISLKLTS
36	351–370	SDISLKLTS	KIASCLNDNA
37	361–380	KIASCLNDNA	NGYFSGHVIP
38	371–388	NGYFSGHVIP	ACKNLPS

Fluorometric ELISA for specific IgE against overlapping synthetic peptides in human patients and monkeys

The titre of specific IgE for Cry j 2 peptides in the sera of the human patients and monkeys with pollinosis was measured by a fluorometric ELISA as previously described [9, 10]. Briefly, the Cry j 2 protein (1 µg/mL) or Cry j 2 synthetic peptides (10 µg/mL) diluted with 0.05 M carbonate buffer (pH 9.5) were immobilized in the wells of a microtitre plate (Immulon 2; Dynex Technologies, Chantilly, VA, USA) overnight at 4 °C. After being washed, serum samples diluted with 1% bovine serum albumin–phosphate-buffered saline–Tween-20 (1:20) were added to the wells and incubated at room temperature for 3 h. After β-D-galactosidase-conjugated anti-human IgE (Pharmacia) and 0.2 mM 4-methylumbelliferyl-β-D-galactoside (Sigma Chemicals, St Louis, MO, USA) were reacted in turns, the reaction was stopped with 0.1 M glycine–NaOH (pH 10.3). The fluorescence unit (FU) of the reaction product, 4-methylumbelliferone, was measured using an automatic fluorometric

Table 2. Small peptides of Cry j 2 peptide no. 13

No.	Position	Amino acid sequence	
13	121–140	GQCKWVNGRE	ICNDRDRPTA
13–1	121–139	GQCKWVNGRE	ICNDRDRPT
13–2	121–138	GQCKWVNGRE	ICNDRDRP
13–3	121–137	GQCKWVNGRE	ICNDRDR
13–4	121–136	GQCKWVNGRE	ICNDRD
13–5	121–135	GQCKWVNGRE	ICNDR
13–6	121–134	GQCKWVNGRE	ICND
13–7	121–133	GQCKWVNGRE	ICN
13–8	121–132	GQCKWVNGRE	IC
13–9	121–131	GQCKWVNGRE	I
13–10	121–130	GQCKWVNGRE	
13–11	122–140	QCKWVNGRE	ICNDRDRPTA
13–12	123–140	CKWVNGRE	ICNDRDRPTA
13–13	124–140	KWVNGRE	ICNDRDRPTA
13–14	125–140	WVNGRE	ICNDRDRPTA
13–15	126–140	VNGRE	ICNDRDRPTA
13–16	127–140	NGRE	ICNDRDRPTA
13–17	128–140	GRE	ICNDRDRPTA
13–18	129–140	RE	ICNDRDRPTA
13–19	130–140	E	ICNDRDRPTA
13–20	131–140		ICNDRDRPTA

microplate reader (Fluoroskan; Flow Laboratories Inc., McLean, VA, USA).

Fluorometric ELISA for specific IgE against overlapping synthetic peptides in immunized mice

The titre of specific IgE for Cry j 2 peptides in the sera of immunized mice was measured by a fluorometric ELISA as previously described [14]. The procedures were similar to those of the fluorometric ELISA described above.

Fluorometric ELISA for specific IgE against the peptide covalently linked via their carboxyl group to microplates

To confirm the results using Immulon 2 microplate, we measured the titre of specific IgE for the peptides covalently linked via their carboxyl group to the microplates [17].

Colourimetric ELISA for monoclonal IgG antibodies against overlapping synthetic peptides

The reaction of monoclonal IgG antibodies for Cry j 2 peptides was measured by a colourimetric ELISA as previously described [14].

Histamine release assay

The histamine release assay using leucocytes was carried out as previously described [9]. Peptide no. 13 was conjugated to human serum albumin (HSA) by the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce) coupling method [18]. Basophils from human patient no. 9 were incubated with various concentrations of the allergens, Cry j 2, peptide no. 13-conjugated HSA and HSA (negative control), at 37 °C for 40 min. The amount of released histamine was measured using a histamine-ELISA kit (IBL, Hamburg, Germany). The amount of specific histamine released was expressed as a percentage with respect to the total histamine content.

Interdermal skin test

Cry j 2 (200 ng/mL) and peptide no.13-conjugated HSA (200 ng/mL) were used for the interdermal skin test in monkeys (nos 1–4) with CJ pollinosis. Other monkeys were in poor physical condition, so a skin test was not conducted. The injection volume of each allergen was 0.05 mL, and the reaction was read 15 min after injection. Saline as a negative control and histamine phosphate (0.0275 mg/mL) as a positive control were served.

Identification of the minimum epitope in human patients, monkeys and monoclonal IgG antibodies

The minimum epitope in the peptide was identified by an IgE–ELISA inhibition test. Briefly, in the cases of the humans and monkeys with pollinosis, Cry j 2 peptide no. 13 (3 µg/mL) diluted with 0.05 M carbonate buffer was immobilized. Then, serum samples pre-incubated with peptide no. 13, nos 13-1-13-20 (4 µg/mL; Table 2) or Cry j 2 (4 µg/mL) as an inhibitor were reacted. Subsequent procedures were the same as those of the fluorometric ELISA described above. The percentage inhibition was calculated as follows:

$$\left(1 - \frac{\text{FU in the presence of inhibitor}}{\text{FU in the absence of inhibitor}}\right) \times 100$$

In the case of monoclonal IgG antibodies, the procedures using the colourimetric ELISA were essentially similar to the IgE–ELISA inhibition test described above.

Computer analysis

Sequence data of the epitope were analysed using GeneWorks Software (Intelligenetics, Geel, Belgium) and DNASIS-Software (Hitachi Software Engineering Co., Ltd, Ibaraki, Japan).

Results

Binding of IgE from humans, monkeys and immunized mice to Cry j 2 synthetic peptides

As shown in Fig. 1, 16 of the 20 patients had IgE that bound to Cry j 2 synthetic peptides. There are four IgE-binding epitopes on Cry j 2, namely, peptide nos 6, 13, 29 and 31. Eight patients showed IgE reactivity to peptide no. 13, and six showed IgE reactivity to peptide no. 31. Two patients showed IgE-reactivity to both peptide nos 13 and 31. As a negative control, 20 human patients with mite allergy and high titres of specific IgE for mite allergens had no anti-CJ IgE showed no IgE reactivity to these peptides (data not shown).

As shown in Fig. 2, an unexpected result was that five of the seven monkeys had IgE that bound to the same epitope recognized by human sera, Cry j 2 peptide no. 13. As a negative control, monkeys without pollinosis and anti-CJ IgE showed no IgE reactivity to these peptides (data not shown).

To examine the specificity of the sera antibodies, we conducted a binding inhibition assay using the sera from human patients (nos 1–10 except no. 4) and monkey (nos 1–5), and the results are shown in Fig. 3. In these human patients and in the monkey, the binding of IgE to peptide no. 13 was completely blocked by pre-incubation of the sera with peptide no. 13 or

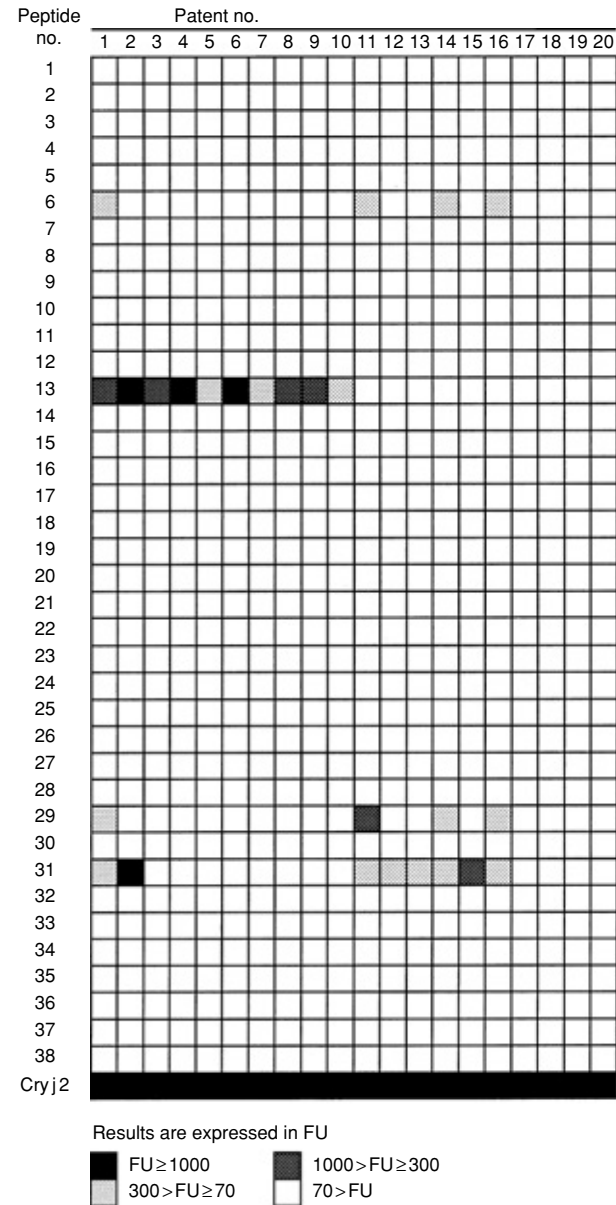


Fig. 1. Binding of human IgE to Cry j 2 synthetic peptides. Serum samples were collected from 20 pollinosis patients (abscissa) and analysed for specific IgE to Cry j 2 synthetic peptides (ordinate) by fluorometric ELISA, as described in Materials and methods. The binding activity was expressed in fluorescence units of the reaction and scored as shown in the figure.

Cry j 2 (data not shown), a finding that suggests the specificity of IgE binding to the peptide. Moreover, pre-incubation of the sera from the same humans and monkey with peptide no. 13 resulted in a dose-dependent inhibition of the IgE binding to peptide no. 13 (data not shown). Likewise, the binding of IgE to Cry j 2 was significantly blocked by pre-incubation of the sera with Cry j 2 and, to a lesser extent, with peptide no. 13 (Figs 3a and b). In human patients (nos 11 and 12) and monkeys (nos 6 and 7) as a negative control, the binding of IgE to Cry j 2 was not blocked with peptide no. 13 (data not shown).

Furthermore, IgE in the sera from the immunized mice strongly reacted with peptide no. 13 (Fig. 2). Pre-incubation of the sera from the immunized mice with peptide no. 13 or

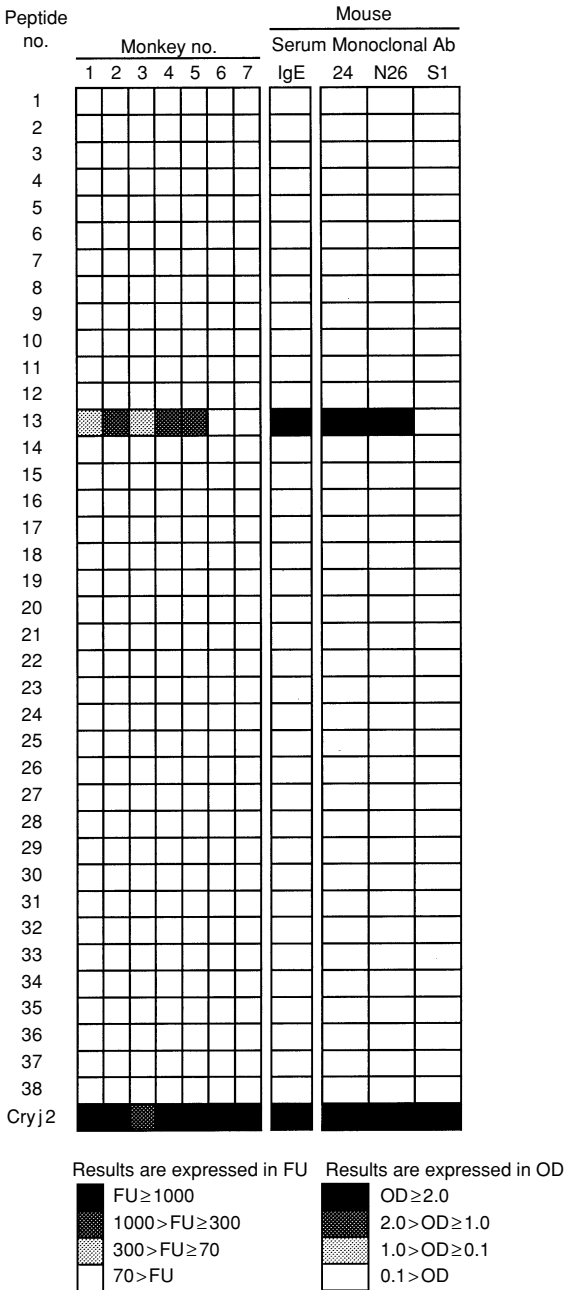


Fig. 2. Binding of monkey and mouse IgE, and Cry j 2-specific monoclonal IgG antibodies to Cry j 2 synthetic peptides. Serum samples were collected from seven pollinosis monkeys and immunized mice (abscissa) and analysed for specific IgE to Cry j 2 synthetic peptides (ordinate) by fluorometric ELISA, as described in Materials and methods. Furthermore, the reactivity of Cry j 2-specific mouse monoclonal antibodies (abscissa) to the peptides was investigated by colourimetric ELISA, as described in Materials and methods. The binding activity was expressed in fluorescence units and optical density of the reaction and scored as shown in the figure.

Cry j 2 resulted in the inhibition of the IgE binding to peptide no. 13 (data not shown). These results suggest that IgE elicited by immunization with Cry j 2 reacts with a major epitope, peptide no. 13, across species from humans to mice.

Each peptide may be influenced by buffers of different pHs and ionic strengths for optimal binding to the solid phase on a conventional ELISA plate. Thus, we compared the IgE

reactivity to the peptides on conventional ELISA plates (Immulon 2) with the reactivity to those covalently linked to the microplates. The results for patient no. 6 in the experiment using Covalink plates are similar to those of the fluorometric ELISA using Immulon 2 microplates (data not shown). The results for the other human patients or monkeys who exhibited IgE reactivity to some peptides in the experiment using Covalink plates were similar to those of the fluorometric ELISA using Immulon 2 microplates (data not shown).

Binding of Cry j 2 specific monoclonal IgG antibodies to the synthetic peptides

We found that mouse Cry j 2-specific monoclonal IgG antibodies (24 and N26) strongly reacted with peptide no. 13 (Fig. 2), suggesting that peptide no. 13 is the major epitope for anti-Cry j 2 antibodies in different subclasses. However, S1 did not react with any of the peptides.

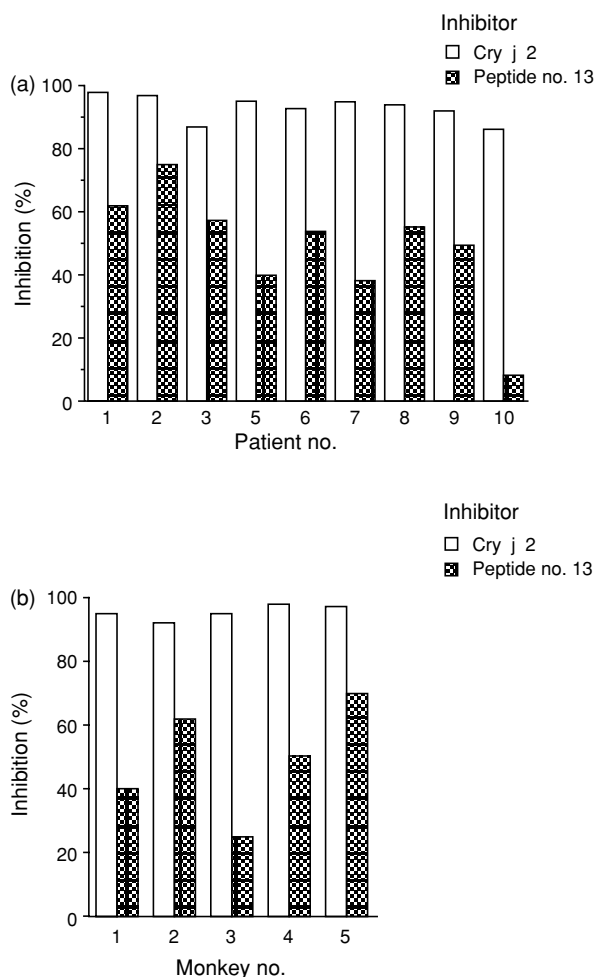


Fig. 3. Inhibition of binding of IgE to Cry j 2. Each serum sample from humans (a) and monkeys (b) was pre-incubated with Cry j 2 (25 µg/mL, □) or peptide no. 13 (25 µg/mL, ▨) for 1 h at room temperature and added to wells coated with Cry j 2. The subsequent procedures were the same as those of the IgE-ELISA inhibition test described in Materials and methods. The percentage inhibition was calculated as described in Materials and methods.

Histamine release assay

To examine whether peptide no. 13 is a biological epitope of allergy from Cry j 2, we performed a histamine release assay using basophils isolated from the sera of patient no. 9. The results showed that a dose-dependent histamine release was induced by Cry j 2 and peptide no. 13-conjugated HSA (data not shown), and that the maximum histamine release was obtained using 10 ng/mL of peptide no. 13-conjugated HSA as well as Cry j 2. Figure 4 shows the percentage of maximum histamine release obtained in the assay with respect to the total histamine content. As a negative control, an experiment was carried out in which histamine release was not induced by HSA.

Interdermal skin test for peptide no. 13

Four monkeys (nos 1–4) that demonstrated specific IgE to peptide no. 13 also showed allergic reactions to this peptide in skin test (Table 3). Cry j 2, this peptide and positive control produced only weal in all monkeys. The negative solution (saline) elicited a small weal (≤ 5 mm) and no erythema.

Determination of minimum IgE epitope in peptide no. 13

To determine the minimum IgE epitope in Cry j 2 peptide no. 13 ($^{121}\text{GQCKWVNGREICNDRDRPTA}^{140}$), we conducted the IgE-ELISA inhibition experiment using synthetic peptides (Table 4). It was difficult to immobilize short peptides on the wells of the microplate, so we conducted the IgE-ELISA inhibition experiment. In the pooled sera from human patients (nos 1–3, 6, 8 and 9), IgE binding to peptide no. 13 was inhibited by peptide nos 13-1-13-9 and 13-11-13-13, but it was not inhibited by the other peptides (Table 4). We found that the core of the epitope required for human IgE binding to Cry j 2 was $^{124}\text{KWVNGREI}^{131}$. In the pooled monkeys' sera (nos 2, 4 and 5), pooled mice sera and monoclonal antibody 24, IgE binding to peptide no. 13 was inhibited by peptide nos 13-1-13-9 and 13-11-13-13, but was not inhibited by the other peptides (Table 4).

Discussion

The present study demonstrated that at least four IgE-binding epitopes of Cry j 2, namely, peptide nos 6, 13, 29 and 31, might

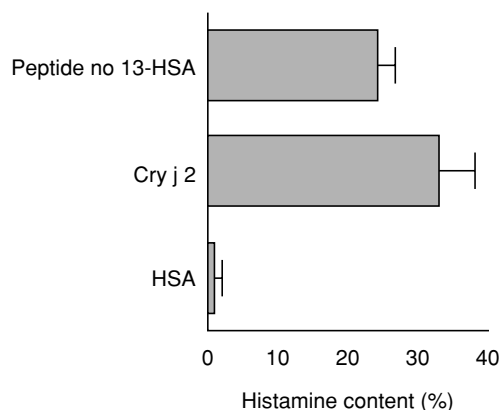


Fig. 4. Percentage of maximum histamine released (abscissa) with respect to the total histamine content using basophils from the human sera of patient no. 9. The ordinate shows the allergens incubated with basophils. The maximum release was obtained using 10 ng/mL Cry j 2 and peptide no. 13-conjugated HSA. As a negative control, an experiment was carried out in which histamine release was not induced by HSA.

Table 3. Interdermal skin test for the monkeys

Monkey no.	Saline	Cry j 2	Peptide	Histamine
1	—	++	++	+++
2	—	+++	+++	+++
3	—	++	++	+++
4	—	++	++	+++

+++ : equal to or greater than the diameter of the positive control. ++ : equal to or greater than mean diameter of the positive and negative controls. + : larger than the diameter of the negative control but small than the mean diameter of the positive and negative control. — : equal to or smaller than the diameter of negative control.

Table 4. Inhibition of IgE binding to Cry j 2 peptide no. 13

Peptide		Inhibition			
No.	Residue	Humans*	Monkey†	Mouse‡	mAb (24)
13	121–140	+§	+	+	+
13–1	121–139	+	+	+	+
13–2	121–138	+	+	+	+
13–3	121–137	+	+	+	+
13–4	121–136	+	+	+	+
13–5	121–135	+	+	+	+
13–6	121–134	+	+	+	+
13–7	121–133	+	+	+	+
13–8	121–132	+	+	+	+
13–9	121–131	+	+	+	+
13–10	121–130	—	—	—	—
13–11	122–140	+	+	+	+
13–12	123–140	+	+	+	+
13–13	124–140	+	+	+	+
13–14	125–140	—	—	—	—
13–15	126–140	—	—	—	—
13–16	127–140	—	—	—	—
13–17	128–140	—	—	—	—
13–18	129–140	—	—	—	—
13–19	130–140	—	—	—	—
13–20	131–140	—	—	—	—

*Humans: the sera were pooled from human patients (nos 1–3, 6, 8 and 9). †Monkey: the sera were pooled from monkeys (nos 2, 4 and 5). ‡Mouse: the sera were pooled from mice immunized with Cry j 2 and aluminium hydroxide gel. §+, ≥80% inhibition.

exist. These peptides consist of 20–25 amino acids with different antigenicities. We observed that, of the 20 human patients, 10 had IgE reactivity to peptide no. 13. Furthermore, we found that IgE in five of the seven monkeys had the same epitope, peptide no. 13, and that IgE established in mice immunized with Cry j 2 reacted with peptide no. 13. Although we do not know why antibodies have limited epitope specificity across species, these findings indicate that peptide no. 13 could be a major epitope of IgE in some human patients, Japanese monkeys and mice. Approximately one-half of the patients did not exhibit a reaction against epitopes other than peptide no. 13. However, considering the polymorphism of the Ig gene present in human patients, this result may mean that the antibody repertoire against Cry j 2 is limited in humans. The reactivity to Cry j 2 peptides was probably influenced by unknown genetic backgrounds, or by an individual's history of clinical stages.

It may be worthwhile to analyse IgE-binding epitopes to understand the aetiology of this allergy.

In ELISA inhibition, pre-incubation of the sera with peptide no. 13 resulted in the inhibition of the IgE binding to Cry j 2 to some degree. The results in Fig. 3(b) imply that peptide no. 13 is the major epitope recognized by IgE from the sera of human patient nos 6 and 2, and monkey no. 2. We could not attain 100% inhibition because some other conformational epitopes of Cry j 2 may exist. Further analysis is needed to clarify this issue.

Peptide no. 13, identified as the epitope, was combined with a carrier protein. The allergens cross-link IgE receptors on basophils or mast cells, and these cells are activated and release chemical mediators, such as histamine. IgE binding to allergens must induce functional allergic reactions that induce the release of histamine [19]. The peptide no. 13-conjugated HSA showed the release of histamine from human basophils. Thus, we found that peptide no. 13 was the functional IgE-binding epitope.

Cry j 2 possesses a very high degree of homology at the amino acid sequence level with a major allergen of Japanese cypress (Cha o 2) [20]. However, none of the possible group of four epitopes of Cry j 2, from peptide no. 6 to no. 31, had homology with Cha o 2. Moreover, ¹²⁴KWVNGREI¹³¹ in Cry j 2 does not exist in other proteins, including Cha o 2.

Some T cell epitopes of Cry j 2 in humans and mice have been identified [21–25]. The main T cell epitopes of humans have been identified as peptides 66–80, 141–155, 186–200 and 346–365 [22]. The T cell epitopes of Cry j 2 in C57BL/6 mice immunized with Cry j 2 have been identified as peptides 78–91, 246–259, 318–331 and 370–383 [25]. We compared the B cell epitopes of Cry j 2 in this study with the T cell epitopes of Cry j 2. We found that all B cell epitopes in this study, including ¹²⁴KWVNGREI¹³¹, are located in regions different from those occupied by T cell epitopes of the Cry j 2 amino acid sequence. This information will be useful in performing immunotherapy using T cell epitopes [23].

In conclusion, we found that ¹²⁴KWVNGREI¹³¹ is an important major epitope recognized by IgE in humans, monkeys and mice. It is very interesting to note that IgE in humans, monkeys and mice, and mouse monoclonal IgG antibodies bind to the same epitope, i.e. peptide no. 13. These results indicate that it is appropriate to use monkeys or mice as suitable animal models to study Japanese cedar pollinosis in humans.

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