

EPITOPES ON *Cry j* I AND *Cry j* II FOR THE HUMAN IgE ANTIBODIES CROSS-REACTIVE BETWEEN *CUPRESSUS SEMPERVIRENS* AND *CRYPTOMERIA JAPONICA* POLLEN

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Abstract—Forty sera from French patients allergic to *Cupressus sempervirens* pollen were tested for cross-reactivities against *Cry j* I, *Cry j* II (major allergens of *Cryptomeria japonica* pollen) and other pollen allergens from botanically related plants. Seventy-three per cent of the sera reacted with either *Cry j* I or *Cry j* II, or with both of them. These IgE cross-reactions were blocked effectively by mAb 046 (anti-*Cry j* I) or N26, T27 (anti-*Cry j* II), and weakly by mAbs 052, 027 and 026 (anti-*Cry j* I). Furthermore, the IgE antibodies in two sera, #40 and #11, bound to peptide fractions obtained from enzyme-digested *Cry j* I, and mAb 027 could also bind to the fractions. Analyses of the amino acid sequences of the peptides revealed that reactive peptides contained "NGNAT-PQLTKNAGVLTCSLSKR" sequence and the third residue N³ was glycosylated, however, when the N³ was not glycosylated, the IgE antibodies did not react, but mAb 027 could. The glycosylation of the N³ might be required for IgE-binding to the peptides. Sugar component on the N³ residue was found to be 0.4 mol galactose, 1.3 mol mannose, 0.8 mol fucose and 2.0 mol *N*-acetylglucosamine. Cross-reactivities against other pollen allergens from botanically related plants were found in most of the sera. However, many of these reactivities were detected by sandwich ELISA but not by an ELISA using allergen-coated plates, indicating that it is important to select an appropriate ELISA procedure in order to detect an allergen or an IgE antibody to an allergen.

INTRODUCTION

Cross-reactivity between the pollen of Taxodiaceae and Cupressaceae families has been shown by Panzani *et al.* (1986) in a study of the French and Japanese patients allergic to *Cupressus sempervirens* (Common cypress) and *Cryptomeria japonica* (Japanese cedar, Sugi) pollen respectively. *C. japonica* is located only in Japan and *C. sempervirens* is distributed in France, Italy and Israel (Panzani *et al.*, 1991), and both pollens independently caused the increasing seasonal allergic disease in respective countries. Thus analysis of the cross-reactivity between the two pollen allergens is of great interest for understanding immunological mechanisms of these allergic responses in human.

Cry j I, a major allergen of *C. japonica* pollen was first isolated and characterized by Yasueda *et al.* (1983) as Sugi Basic Protein (SBP), Taniai *et al.* (1988) and Sakaguchi *et al.* (1990) found in their SBP preparations an allergen different from *Cry j* I in molecular weight, in antigenicity and in *N*-terminal amino acid sequence. The new allergen was named *Cry j* II. In SDS-PAGE analysis, *Cry j* I showed multiband of 45–50 kDa, and *Cry j* II a single band of 37 kDa. However primary

structures of these allergens have not been available yet. Recently we obtained mAbs specific for *Cry j* I and *Cry j* II, and the primary structures of B cell epitopes on *Cry j* I were analyzed. We reported that four mAb-binding sites on the allergens were related to binding sites for serum IgE from patients allergic to *C. japonica* pollen, two of the four epitopes seemed to be conformational and the remaining two were of sequential structure (Kawashima *et al.*, 1992a, b). In this study, using the sera from French patients allergic to *C. sempervirens* pollen, many of which showed a positive reaction against *Cry j* I and/or *Cry j* II, we tried to know whether or not the epitopes for these cross-reactive human IgE Abs were related to the binding sites for the mAbs.

IgE-binding sites on pollen allergens are generally reported to be conformational (Duffort *et al.*, 1991; Polo *et al.*, 1991), and the importance of carbohydrate moiety for IgE-binding has been pointed out by Sward-Nordmo *et al.* (1988), Polo *et al.* (1991), Nilsen and Paulsen (1986) and Duffort *et al.* (1991). Here we show that IgE antibodies in two of the sera bound to a glycosylated, but not to unglycosylated, peptide of 22 residues derived from enzyme-digested *Cry j* I, and the C-terminal half region of the peptide was identical with the sequence of a peptide reactive to mAb 027.

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MATERIALS AND METHODS

Allergens

Cry j I and *Cry j II* were prepared as described before (Taniai *et al.*, 1988; Sakaguchi *et al.*, 1990). False cypress pollen extract was a kind gift from Dr Sakaguchi (The Institute of Public Health, Tokyo). Japanese cedar pollen was obtained from Akita Pref., Japan. Dry pollens of Mountain cedar, Red cedar, Bald cypress, Incense cedar, Monterey cypress and Arizona cypress were purchased from International Biologicals, Inc., U.S.A. The pollen extracts were prepared as described before (Taniai *et al.*, 1988).

Antibodies

Monoclonal antibodies specific for *Cry j I* or *Cry j II* were obtained and characterized as described before (Kawashima *et al.*, 1992a). Rabbit polyclonal antibodies containing anti-*Cry j I* and anti-*Cry j II* (named anti-SBP) was obtained in our laboratory (Taniguchi *et al.*, 1989) and an IgG fraction of the antiserum was prepared using Protein A Sepharose 4B (Pharmacia).

Human sera

Serum samples were obtained from 40 French individuals whose allergy to *Cupressus sempervirens* pollen had been diagnosed on the basis of case history, results of skin test and RAST. The patients were considered as never having been exposed to *Cryptomeria japonica* pollen (Panzani *et al.*, 1986). These sera were lyophilized and kept at 4°C until use. Sera from Japanese patients sensitive to *C. japonica* pollen were used as controls.

ELISA for IgE-binding to pollen allergens

Two methods were used. One is a sandwich method in which an allergen extract is captured by SBP-specific

rabbit IgG previously coated on microplates after which serum samples were added to the wells. The other method is to coat an allergen extract directly to microplates followed by addition of serum samples. Serum samples were used in 1:3 dilution. Biotin-labeled goat anti-human ϵ chain (Kirkegaard & Perry Laboratories Inc., U.S.A.) was used to detect the bound IgE-allergen complex. Reactions were visualized by HRPO-labeled avidin (Zymed, U.S.A.) and substrate.

Peptide fractions obtained from enzyme-digested *Cry j I*

Cry j I was digested with V8 protease (from *Staphylococcus*, Sigma), pepsin (from porcine stomach mucosa, Sigma) or lysylendopeptidase (from *Acromobactor*, Seikagaku kogyo, Japan) according to the procedures shown in Fig. 1, and peptide fractions were prepared by reversed-phase HPLC as described before (Kawashima *et al.*, 1992b). Test for binding of sera and mAbs with the peptide fractions was performed after covalently fixing peptides to microplates as described before (Kawashima *et al.*, 1992b). Determination of amino acid sequences was performed using a 473A Protein Sequencer (Applied Biosystems, U.S.A.) The existence of any sugar component in a peptide was determined according to Takemoto *et al.* (1985).

RESULTS

Cross-reactivities against *Cry j I* or *Cry j II* of the sera from French patients sensitized with *Cupressus sempervirens* pollen

Thirty-eight of the 40 French patients sensitized with *C. sempervirens* pollen reacted positively to *C. sempervirens* pollen extract in skin test (data not shown). In

Procedures for enzymatic digestion of *Cry j I* used in this study

V8 protease-digestion	Pepsin-digestion	Lysylendopeptidase-digestion
1.5 mg <i>Cry j I</i> in 1 ml H ₂ O	1.67 mg <i>Cry j I</i> in 1 ml H ₂ O	1.5 mg <i>Cry j I</i> in
↓	185 μ l 0.1 M HCl	0.01 M PB + 0.3 M NaCl
boil for 5 min	480 μ l H ₂ O	1 ml of 0.2 M Tris-HCl
↓	3.2 μ l 1 mg/ml pepsin	buffer pH 8.5
add 1 ml of 0.2 M	↓	↓
ammoniumacetate in 10 mM EDTA	mix together	boil for 5 min
pH 5.0	↓	↓
↓	incubate at room temperature	add 15 μ l of 1 mg/ml
add 50 μ l of 1 mg/ml	for 20 hrs	lysylendopeptidase
V8 protease	↓	↓
↓	add 1 drop of pyridine to	incubate at 37°C for
incubate at 37°C for 48 hrs	stop the enzymatic reaction	18 hrs
during the incubation, the		↓
same amount of the enzyme		boil for 10 min to stop
was added twice.		the enzymatic reaction
↓		
boil for 5 min to stop		
the enzymatic reaction		

Fig. 1.

Table 1. Reactivities of serum IgE from French patients sensitized with *Cupressus sempervirens* pollen against *Cry j* I and *Cry j* II

Reactivity to <i>Cry j</i> I	<i>Cry j</i> II	Number of cases	
		Allergen-coated plates ELISA	Sandwich ELISA
+	+	10	13
+	-	17	19
-	+	1	0
-	-	12	8

Fifty microliters of 2 µg/ml *Cry j* I or *Cry j* II were coated on 96-well microplates. After blocking with 1% BSA, 50 µl of 1:3 diluted serum were added to the wells (allergen-coated plates ELISA). *Cry j* I or *Cry j* II were added to the microplate wells previously coated with 5 µg/ml rabbit IgG fraction containing both anti-*Cry j* I and anti-*Cry j* II, followed by addition of a diluted serum sample (Sandwich ELISA). After overnight incubation at 4°C, binding of the human IgE was detected by biotin-labeled anti-human epsilon chain, HRP-labeled avidin and substrate.

ELISA using allergen-coated plates, 28 of the 40 sera (73%) reacted with allergens of *C. japonica*. Ten sera showed positive reactions against both *Cry j* I and *Cry j* II, 17 sera were positive to *Cry j* I but negative to *Cry j* II, and one serum was positive only to *Cry j* II. The remaining 12 sera were negative to both *Cry j* I and *Cry j* II (Table 1). The binding of these IgE antibodies with the solid-phase *Cry j* I was completely inhibited by *Cry j* I solution (data not shown). In analysis with a sandwich ELISA, similar results were obtained although the sensitivity was slightly increased, 80% of the sera showed positive reaction to the one or two allergens (Table 1).

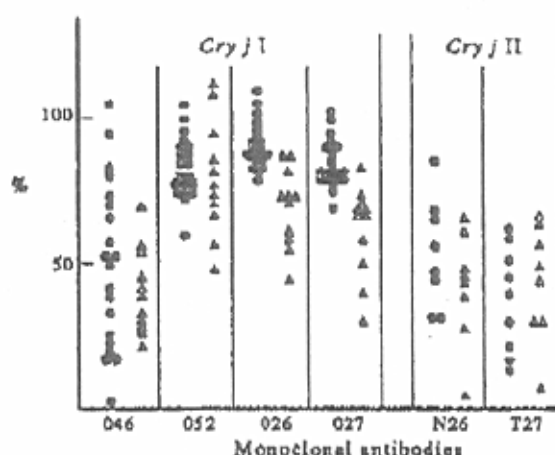


Fig. 2. Percentage binding of serum IgE to *Cry j* I and *Cry j* II in the presence of mAbs specific for *Cry j* I or *Cry j* II. Fifty microliters of 2 µg/ml *Cry j* I or *Cry j* II were coated on microplate wells. After blocking with 1% BSA, 40 µl of 25 µg/ml mAbs or PBS were added to the wells. After incubation at 37°C for 2 hr, 10 µl of each serum were added to the wells without removing the mAbs or PBS. After overnight incubation at 4°C, binding of human IgE to the antigens was detected by biotin-labeled anti-human epsilon chain, HRP-labeled avidin and substrate. A closed circle represents per cent binding of IgE in each French individual, and an open triangle represents that in each Japanese individual.

Blocking of the IgE-binding to *Cry j* I or *Cry j* II by the mAbs specific to *Cry j* I or *Cry j* II

In a previous paper (Kawashima *et al.*, 1992), we reported that at least four antibody-binding sites were present on *Cry j* I molecules. In order to determine whether or not these binding sites were related to the ones that cross-reacted with the IgE, four anti-*Cry j* I mAbs, 046, 052, 026 and 027, showing an epitope specificity different from each other, and anti-*Cry j* II mAbs N26 and T27 were selected. The inhibitory effect of these mAbs on binding between the cross-reactive IgE antibodies and *Cry j* I or *Cry j* II was tested in 20 sera positive to *Cry j* I and in eight sera positive to *Cry j* II. Figure 2 shows the results. In the presence of mAb 046, the percentage of binding of serum IgE among the 20 French patients ranged from 100% to 3%. Ten of the 20 sera showed 50% inhibition or more, whereas, in the presence of mAbs 052, 026 or 027, the inhibition of serum IgE-binding to *Cry j* I in the 20 French patients was less than 50%. This indicates that mAb 046 was the most effective of the four anti-*Cry j* I mAbs in blocking the binding of the cross-reactive IgE to *Cry j* I. Serum IgE-binding to *Cry j* II was considerably inhibited by both mAb N26 and T27. As expected, inhibition of serum IgE-binding in Japanese patients by these mAbs was slightly higher than that in French patients. The results suggest that the epitopes recognized by these mAbs are closely related to the cross-reactive determinants for IgE antibodies between the pollen from *Cryptomeria japonica* and *Cupressus sempervirens*.

Detection of the IgE-reactive fragments of enzyme-digested *Cry j* I

Sera #40 and #11 showing a strong IgE-binding activity to *Cry j* I were tested in order to see whether or not the IgE could bind to the fragments obtained from enzyme-digested *Cry j* I. Since mAb 046, the most effective mAb for the blocking of IgE-binding, was found to be incapable of binding to the enzyme-cleaved *Cry j* I (Kawashima *et al.*, 1992), a greater part of the IgE in the sera cross-reactive to *Cry j* I is considered to be also incapable of binding to the digested *Cry j* I. However, as Fig. 3 shows, fraction nos 31 and 32 (V8-31, V8-32) from V8 protease-digested *Cry j* I bound to the IgE in #40, and also in #11 (data not shown), although the binding strength was markedly reduced. Fractions 27, 31, 32 and 38 bound well to mAb 027. Determination of the amino acid sequences of these four fractions revealed that the sequences of the three fractions 31, 32 and 38 were identical to each other as seen in Table 2. However, the third residue of fractions 31 and 32 could not be identified, whereas that of fraction 38 was identified as N, suggesting that the third residue Asn (N³)s of fractions 31 and 32 might be glycosylated but not that of fraction 38. Furthermore, the sequence "N¹AGVL-TCSLSK²¹" was previously reported to be the sequence reactive to mAb 027 (Kawashima *et al.*, 1992b). The amino acid sequence of fraction 27 was not determined.

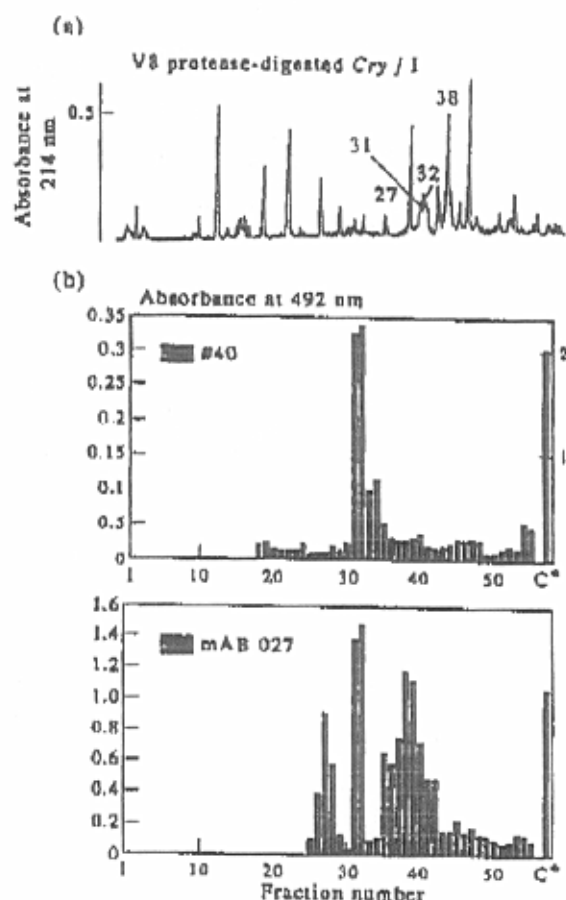


Fig. 3. Peptide map of the V8 protease-digested *Cry j I* and the binding profiles of #40 IgE and mAb 027 against the fractions. V8 protease-digested *Cry j I* was fractionated by reversed-phase HPLC using RP C18 column (A). Each fraction was covalently fixed on the microplate wells previously treated with Bis(sulfosuccinimidyl)suberate. After blocking with 1% BSA, serum #40 or mAb 027 was reacted with the fixed fractions. Binding of human IgE was detected by biotinylated anti-human ϵ chain, HRPO-labeled avidin and substrate. Binding of mAb was detected by HRPO-labeled anti-mouse Ig and substrate. C means positive control, native *Cry j I* (2 μ g/ml, 50 μ l).

Similar experiments were performed using pepsin- or lysylendopeptidase-digested *Cry j I*. Results are summarized in Table 2. mAb 027 reacted to both P-39 and P-45 (fractions from pepsin-digested *Cry j I* containing the sequence mentioned above), while IgE in serum #40 reacted only to P-39, which is probably glycosylated at the N³, and not to the unglycosylated P-45 fraction. Among the fractions from lysylendopeptidase-digested *Cry j I* (Fig. 4), no fractions could bind to IgE in serum #40 and #11, whereas mAb 027 bound to fractions L-9 and L-26, of which amino acid sequences were found to be identical with "N¹¹AGVLTCSLSK²¹" (Table 2). Fractions L-7 and L-11 did not bind to IgE of #40, #11 nor to mAb 027. However, the fractions L-7 and L-11 were found to share the sequence N¹GNATPQLTK¹⁰ with the V-31, -32, -38, P-39 and P-45, whereas both L-7 and L-11 lack the sequence N¹¹AGVLTCSLSK²¹. In addition, L-7 is probably glycosylated at the N³, while L-11 is not. The fact that the IgE in serum #40 and #11 failed to bind to the fractions L-7, L-9 and L-11 indicates that neither the sugar chain alone, nor the peptide L-7 alone (even if the N³ is glycosylated), nor the N¹¹AGVLTCSLSK²¹ sequence alone is sufficient for IgE-binding. These results suggest that IgE in sera #40 and #11 reacts with a conformational epitope formed by the glycosylated peptide NGNATPQLTKNAGVLTCSLSKR, alternatively, the IgE might recognize the C-terminal half region of the peptide, N¹¹ ~ K²¹, as mAb 027 does, and the glycosylation of the N³ located in the N-terminal half region of the peptide, might play an important role for the human IgE-binding to the peptide.

Determination of sugar component on the N³ of the peptide V8-31

In order to confirm the presence of sugar on the N³ of V8-31 peptide, a sugar composition analysis was performed. The peptide V8-31 was found to contain 0.4 mol

Table 2. Binding profiles of enzyme-cleaved *Cry j I* fragments against human serum IgE (#40, #11) and mAb 027, and amino acid sequences of the fragments

Fragments	Binding to			Amino acid sequence ^a				
	#40	#11	027	1	5	10	15	20
V8-31	+	+	+	NG ^b ATPQLTKNAGVLTCS ^c ?....				
V8-32	+	+	+	NG ^b ATPQLTKNAGVLTCS ^c SL ^d ...				
V8-38	-	-	+	NGNATPQLTKNAGVLTCS ^c SLSKR				
P-39	+	-	+	NVENG ^b ATPQLTKNAGVL ^d				
P-45	-	-	+	NVENGNATPQLTKNAGVLTCS ^c SLSKR				
L-7	-	-	-	EAPNVENG ^b ATPQLTK				
L-11	-	-	-	EAPNVENGNATPQLTK				
L-9	-	-	+	NAGVLTCS ^c SLSK				
L-26	-	-	+	NAGVL ^d ?....				

^aThe primary structure of the parent protein, *Cry j I*, has not been available yet, therefore, we don't know where these peptides are located in the amino acid sequence of the protein. So the first amino acid of V8-31 peptide was assigned tentatively as 1.

^bNo residues were identified.

^cIdentified as *S*-pyridylethyl cysteine.

^dNot determined.

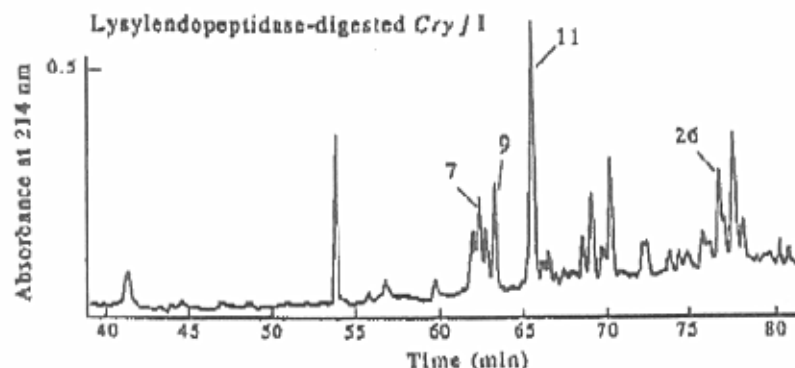


Fig. 4. Peptide map of lysylendopeptidase-digested *Cry j* I.

of galactose, 1.3 mol of mannose, 0.8 mol of fucose and 2.0 mol of *N*-acetylglucosamine as sugar components. The result indicates that the peptide V8-31 has an *N*-linked sugar chain on the N³ residue.

Cross-reactivities of the serum IgE against pollen allergens from botanically related plants

Pollen extracts from botanically related plants, shown in Table 3, were examined for binding to IgE antibodies in sera from three Japanese patients (Nos 1–3, sensitive to *C. japonica*) and four French patients (nos 4–7, sensitive to *C. sempervirens*) by the two ELISA methods described in the *Materials and Methods*. In the cases of False cypress, Bald cypress, Incense cedar and Monterey cypress, many sera tested showed positive reactions when the pollen antigens were presented by anti-SBP antibodies previously coated on the plates, whereas they showed little or no positive reactions with the same antigens when the antigens were directly coated on the plates (Fig. 5). These results suggest that some of the common antigenic determinants on these pollen allergens might be changed conformationally by an interaction with plastic plates.

DISCUSSION

Cross-reactions between *Cupressus sempervirens* and *Cryptomeria japonica* pollen allergens have been studied by Panzani *et al.* (1986) by skin test and RAST in French patients and in Japanese patients. In this study, we analyzed the cross-reactive determinants on *Cry j* I or *Cry j* II using sera from French patients sensitized with *Cupressus sempervirens* and monoclonal antibodies specific to *Cry j* I or *Cry j* II. We previously reported that at least four antibody binding sites are present on *Cry j* I

molecules and one or more sites on *Cry j* II molecules by these mAbs (Kawashima *et al.*, 1992a). In order to know whether these antibody-binding sites are related to those responsible for the IgE cross-reaction between *C. sempervirens* and *C. japonica* pollen allergens, the mAbs were examined for blocking activities against serum IgE from French patients cross-reactive to *Cry j* I or *Cry j* II. mAbs 046 (anti-*Cry j* I) and N26, T27 (anti-*Cry j* II) were found to inhibit effectively the binding of IgE antibodies to *Cry j* I and *Cry j* II respectively. These results are consistent with those reported previously by Kawashima *et al.* (1992a) using sera from Japanese patients. The epitopes recognized by these 3 mAbs are considered to be the most important sites on *C. japonica* pollen for specific or cross-reactive allergic responses. Using specific mAbs, Mao *et al.* (1992) showed that both human IgE antibodies and mAb can be directed against identical or closely related epitopes on an allergen *Der f* I.

It is of great interest to know the primary structures of those epitopes in order to understand the mechanisms of human sensitization to the allergens, with a view to the development of immunotherapy against the disease. However, the epitope recognized by mAb 046 could not be determined by peptides obtained from enzyme-digested *Cry j* I, because the digested *Cry j* I completely lost the binding activity to the mAb, which is considered as recognizing a conformational epitope on *Cry j* I (Kawashima *et al.*, 1992b). The cross-reactive IgE antibodies blocked by mAb 046 are considered to bind to that conformational epitope. In order to know the structure of the conformational epitope, strategies different from those applied in this study are required, such as the use of recombinant peptides expressed from fragments of cDNA encoding *Cry j* I, as shown in the

Table 3. Plants used in this study (botanically related to *Cupressus sempervirens*)

Family	Genus	Species	English name
Taxodiaceae	<i>Cryptomeria</i>	<i>japonica</i>	Japanese cedar (Sugi)
	<i>Taxodium</i>	<i>distichum</i>	Bald cypress
Cupressaceae	<i>Chamaecyparis</i>	<i>obtusa</i>	Flame cypress (Hinoki)
	<i>Cupressus</i>	<i>arizonica</i>	Arizona cypress
	<i>Cupressus</i>	<i>macrocarpa</i>	Monterey cypress
	<i>Juniperus</i>	<i>virginiana</i>	Red cedar
	<i>Juniperus</i>	<i>sabinoidea</i>	Mountain cedar
	<i>Libocedrus</i>	<i>decurrens</i>	Incense cedar

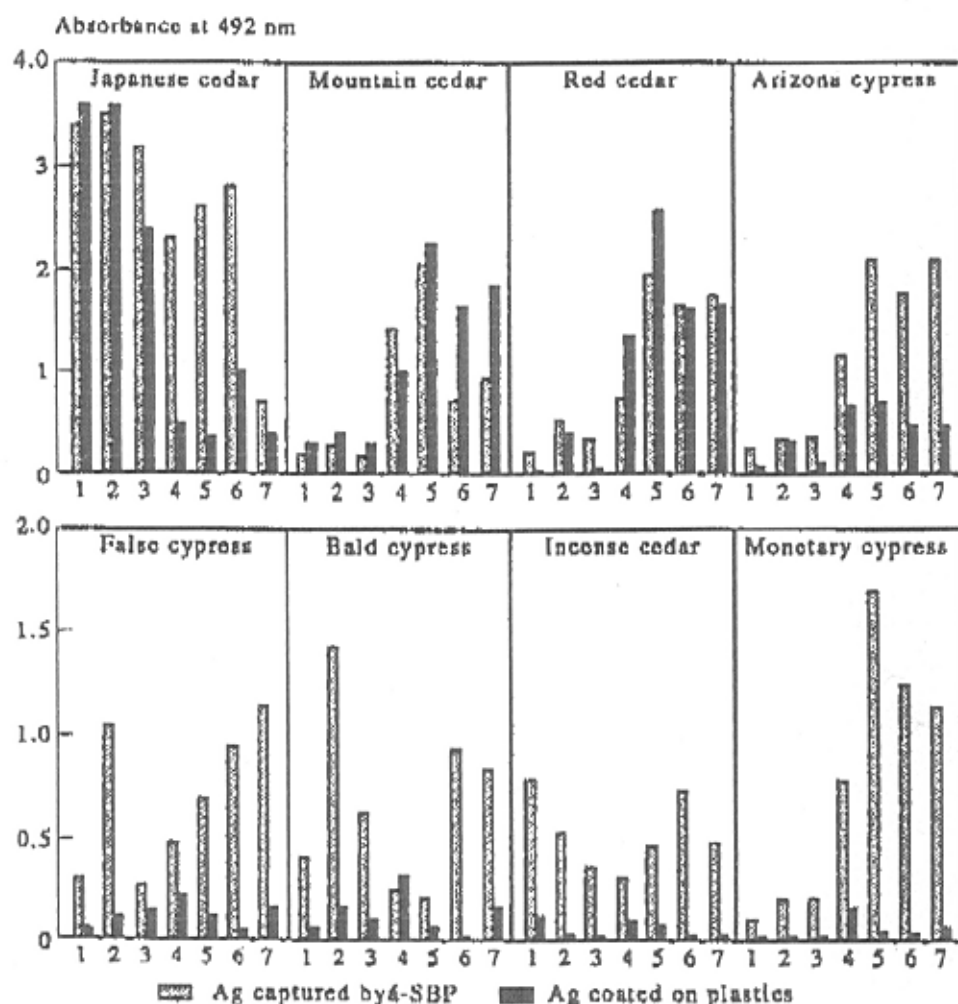


Fig. 5. Binding profiles of human cross-reactive IgE antibodies against pollen extracts from Taxodiaceae and Cupressaceae plants. Fifty microliters of 10 μ g/ml of each pollen extract were coated on microplates or captured by rabbit anti-SBP IgG previously coated on microplates. Then 50 μ l of human serum diluted in 1:3 or 1:5 were reacted with the antigens. Binding of IgE antibodies was detected by biotin-labeled anti-human ϵ chain, HRP-labeled avidin and substrate. Numbers 1–3 sera were from Japanese patients sensitive to *C. japonica* and Nos 4–7 were from French patients allergic to *C. sempervirens*.

analysis on mite allergen *Der p 1* by Greene *et al.* (1991, 1992), or the use of peptides obtained by the limited proteolysis of an mAb-bound protein antigen (Jemmerman *et al.*, 1986; Sheshberadaran *et al.*, 1988), or the use of an approach described by Burnens *et al.* (1987).

However, we found that IgE antibodies in some sera from French patients (#40 and #11) could bind to peptide fractions obtained from digested *Cry j 1*, although binding strength to the peptide was markedly reduced. The mAb 027 also well reacted with those peptide fractions. The amino acid sequences of the reactive fractions were found to contain the sequence "NGNATPQLTKNAGVLTCSLSKR", and the N³ is glycosylated. However, when the N³ of the sequence is not glycosylated, the IgE could not react with the peptide, whereas mAb 027 could (Table 2). The reduction of strength of IgE-binding to the peptide fractions suggests that some conformational structure might be involved in the effective binding between the IgE and the native glycoprotein. However, here we show that some IgE antibodies bound to a glycosylated peptide, indicating the importance of carbohydrate moiety of the

peptide for IgE binding. Whether the sugar moiety is directly involved in the epitope for the IgE is not known. Pole *et al.* (1991) showed that the binding sites for human IgE on *Par j 1* do not contain carbohydrates although these seemed to be important for the conformation of the glycoprotein and influenced the affinity of IgE antibody binding. When recombinant peptides of an allergen are used for epitope analysis of the allergen, we think that a possible role of the glycosylation of the peptides for IgE-binding should be considered.

IgE antibodies from French patients were tested for cross-reactivities against pollen extracts from Taxodiaceae and Cupressaceae families other than *C. sempervirens* pollen. Many sera showed cross-reactions against one or more of the pollen extracts tested. However, in some pollen extracts, such as False cypress, Bald cypress, Incense cedar and Monterey cypress, IgE cross-reactions in many sera were observed only when the sandwich ELISA was used. The possible mechanism of the discrepant results between the two methods, sandwich and allergen-coated plates ELISA, may be as follows: some of the common antigenic determinants on these pollen

allergens might be changed conformationally by interaction with plastic as reported previously (Al Moudallal *et al.*, 1984; Friguet *et al.*, 1984; Norvick *et al.*, 1982; Oslon and Klapper, 1986; Spangler, 1991), which results in non-reactivity of antibodies specific for only the native antigen. Or IgE antibodies induced by a pollen allergen might differ in affinity and in fine specificity from pollen to pollen. These observations show that the selection of an appropriate ELISA method is important for the detection and characterization of allergens and IgE antibodies.

Finally, the question as to whether the structure of the glycosylated peptide mentioned in this study is contained in the allergic sites of pollen allergens showing cross-reaction with *Cry j* I is of great interest for the understanding of the mechanisms of human sensitization to those pollens as well as for devising therapeutic strategies.

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REFERENCES

- Al Moudallal Z., Altschuh D., Briand J. P. and van Regenmortel M. H. V. (1984) Comparative sensitivity of different ELISA procedures for detecting monoclonal antibodies. *J. Immun. Meth.* 68, 35–43.
- Burnens A., Demotz S., Corradin G., Binz H. and Bosshard H. R. (1987) Epitope mapping by chemical modification of free and antibody-bound protein antigen. *Science* 235, 780–783.
- Duffort O. A., Carreira J., Nitti G., Polo F. and Lombardero M. (1991) Studies on the biochemical structure of the major cat allergen *Felis domesticus* I. *Molec. Immun.* 28, 301–309.
- Friguet B., Djavadi-Ohanian L. and Goldberg M. E. (1984) Some monoclonal antibodies raised with a native protein bind preferentially to the denatured antigen. *Molec. Immun.* 21, 673–677.
- Greene W. K., Cyster J. G., Kaw-Yan C., O'brine R. M. and Thomas W. R. (1991) IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen *Der p* I. *J. Immun.* 147, 3768–3773.
- Greene W. K. and Thomas W. R. (1992) IgE binding structures of the major house dust mite allergen *Der p* I. *Molec. Immun.* 29, 257–262.
- Jemmerson R. and Paterson Y. (1986) Mapping peptides on a protein antigen by the proteolysis of antigen-antibody complex. *Science* 232, 1001–1004.
- Kawashima T., Taniai M., Taniguchi Y., Usui M., Ando S., Kurimoto M. and Matuhasi T. (1992) Antigenic analyses on Sugi Basic Protein by monoclonal antibodies. I. Distribution and characterization of B cell epitopes of *Cry j* I molecules. *Int. Arch. Allergy appl. Immun.* 98, 110–117.
- Kawashima T., Taniai M., Usui M., Ando S., Kurimoto M. and Matuhasi T. (1992) Antigenic analyses on Sugi Basic Protein by monoclonal antibodies II. Detection of immunoreactive fragments in enzyme-cleaved *Cry j* I. *Int. Arch. Allergy appl. Immun.* 98, 118–126.
- Mao J. L., Weyer A., Mazie J. C., Rouyre S., Marchand F., Gall A. L. and David B. (1992) Identification of allergenic epitopes on *Der f* I, a major allergen of *Dermatophagoides farinae*, using monoclonal antibodies *Molec. Immun.* 29, 203–211.
- Nilsen B. M. and Smestad-Paulsen B. (1986) Allergens in pollen from Mugwort (*Artemisia vulgaris* L.) I. Characterization of a crude and a partly purified extract of Mugwort pollen with particular emphasis on the glycoprotein allergen Ag7. *Int. Archs Allergy appl. Immun.* 80, 113–121.
- Norvick D., Eschbar Z. and Rubinstein M. (1982) Monoclonal antibodies to human alpha-interferon and their use for affinity chromatography. *J. Immun.* 129, 2244–2247.
- Osilon J. R. and Klapper D. G. (1986) Two major human allergenic sites on ragweed allergen E identified by using monoclonal antibodies. *J. Immun.* 136, 2109–2115.
- Panzani R., Yasueda H., Shimizu T. and Shida T. (1986) Cross-reactivity between the pollen of *Cupressus sempervirens* (Common cypress) and of *Cryptomeria japonica* (Japanese cedar). *Ann. Allergy* 57, 26–30.
- Panzani R., Zerboni R. and Ariano R. (1991) Allergenic significance of Cupressaceae pollen in some parts of the Mediterranean area. In *Allergenic pollen and pollinosis in Europe* (Edited by D'amato G., Spiekma F.Th.M. and Bonini S.), p. 81. Blackwell Scientific Publications, Oxford.
- Sakaguchi M., Inouye S., Taniai M., Ando S., Usui M. and Matuhasi T. (1990) Identification of the second major allergen of Japanese cedar pollen. *Allergy* 45, 309–312.
- Sheshberadaran H. and Payne L. G. (1988) Protein antigen-monomonal antibody contact sites investigated by limited proteolysis of monoclonal antibody-bound antigen: Protein "footprinting". *Proc. natn. Acad. Sci. U.S.A.* 85, 1–5.
- Spangler B. D. (1991) Binding to native proteins by antipeptide monoclonal antibodies. *J. Immun.* 146, 1591–1595.
- Sward-Nordmo M., Paulsen B. S. and Wold J. K. (1988) The glycoprotein Ag-54 (*Cl a* h II) from *Cladosporium herbarum*; Further biochemical characterization. *Int. Archs Allergy appl. Immun.* 85, 295–301.
- Takemoto H., Hase S. and Ikenaka T. (1985) Microquantitative analysis of neutral and amino sugars as fluorescent pyridylamino derivatives by high-performance liquid chromatography. *Analyt. Biochem.* 145, 245.
- Taniai M., Ando S., Usui M., Kurimoto M., Sakaguchi M., Inouye S. and Matuhasi T. (1988) N-terminal amino acid sequence of major allergen of Japanese cedar pollen (*Cry j* I). *FEBS Lett.* 239, 329–332.
- Taniguchi Y., Ikegami H., Usui M., Ando S., Kurimoto M., Sakaguchi M., Inouye S. and Matuhasi T. (1989) Biological and immunological properties of Sugi Basic Protein-pullulan conjugate. I. Suppressive effect on IgE antibody production and on IgE-mediated reactions. *Int. Arch. Allergy appl. Immun.* 89, 136–142.
- Yasueda H., Yui Y., Shmizu T. and Shida T. (1983) Isolation and characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J. Allergy Clin. Immunol.* 71, 77–86.