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Antigenic Analyses of Sugi Basic Protein by Monoclonal Antibodies: I. Distribution and Characterization of B-Cell-Tropic Epitopes of *Cry j I* Molecules

Key Words

Cry j I
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mAb
Elisa
Gel filtration
Sugi basic protein

Abstract

Using 23 monoclonal antibodies raised against Sugi basic protein (SBP, major allergen of Japanese cedar pollen), composed of *Cry j I* and *Cry j II*, analyses of B-cell-tropic epitopes of *Cry j I* were performed. The following results were obtained. (1) As far as the mAbs were used, no major cross-reactive determinants were detected between *Cry j I* and *Cry j II* molecules. (2) 21 of the 23 mAbs were specific for *Cry j I*, and the anti-*Cry j II* mAbs were classified into four groups by their fine specificities, suggesting that *Cry j I* bears at least four antigenic determinant regions. (3) *Cry j I* molecules were found to take a monomeric form in solution and to display no repeating antigenic epitopes on their surfaces. (4) Some of the determinants seemed to be located in the interior of a *Cry j I* molecule, and when the Ag is coated on a plastic plate, the determinants become exposed on its surface. (5) Binding of human IgE antibodies to *Cry j I* and *Cry j II* was blocked by some of the obtained mAbs, suggesting that these epitopes recognized by the mAbs might have an important role in human allergic response against the cedar pollen.

Introduction

Sugi basic protein (SBP) was first identified and purified by Yasueda et al. [1] as a major allergen of Japanese cedar (*Cryptomeria japonica*) pollen in 1983 [1]. They showed that SBP could be separated into four subfractions on the bases of molecular weight and isoelectric point, all of which displayed the same antigenicity and allergenicity to each other by immunodiffusion, and by intradermal

skin test and the radioallergosorbent test. According to the WHO nomenclature, SBP was designated *Cry j I* in 1988 [2]. Taniai et al. [3] reported that SBP was found to be composed of multibands of 45- to 50-kD proteins and a single band of a 38-kD protein on SDS-PAGE analysis. After further purification of SBP by a HPLC Mono S column, they isolated 5 subfractions from the 45- to 50-kD proteins, all of which were found to be identical with each other in N-terminal amino acid sequence of 20 residues.

Sakaguchi et al. [4] found that the 37-kD protein has an N-terminal amino acid sequence of 10 residues different from those of the 45- to 50-kD proteins, and that IgE antibodies in serums from a small part of the patients with cedar pollen allergy react only to the 37-kD protein, but not to the 45- to 50-kD protein, and vice versa. Then, they named the 37-kD protein *Cry j I*, a second allergen of Japanese cedar pollen. To date, however, no specific mAbs for *Cry j I* and *Cry j II* have been reported, and little information has been available for the antigenic or allergenic structures of the allergen. Analyses on the epitopes recognized by mAbs will provide useful and important information for understanding the mechanisms of immune responses against the allergens and for making strategies to control the immune responses [5-15]. In this study, we obtained 23 mAbs, 21 of which are specific for *Cry j I* and the remaining 2 for *Cry j II*. Using these mAbs, analyses of B-cell-tropic epitopes of *Cry j I* were performed. Details are presented and discussed.

Materials and Methods

Antigens. SBP was prepared from Japanese cedar pollen according to Yasueda et al. [1] with slight modification in that sodium bicarbonate, instead of ammonium bicarbonate, was used for extraction of the antigens [3]. *Cry j I* and *Cry j II* were purified from SBP according to Sakaguchi et al. [4] by HPLC on Mono S column (Pharmacia Fine Chemicals, Sweden). SBP was immobilized onto CNBr-activated Sepharose (Pharmacia) and used for purification of mAbs.

Antibodies. Monoclonal antibodies were obtained from hybridoma cell lines established by a cell fusion technique using SP2/0 mouse myeloma cells and spleen cells from BALB/c mice immunized intraperitoneally 3 times with 1 µg SBP adsorbed on 4 mg alum. The mAbs were purified from the ascitic fluid produced in BALB/c mice using the Affigel protein A Bio Rad or the immobilized SBP. Isotypes of the mAbs were determined using a kit (MONOAB-ID, Zymed, USA). The purified mAbs were coupled to CNBr-activated Sepharose. Pooled serums from mice immunized with SBP were used as polyclonal anti-SBP antibodies.

Biotinylation of mAbs. Biotin-amidocaproate N-succinimide ester (Sigma, USA) was coupled to mAbs according to Guesdon et al. [16].

Elisa. Binding of these mAbs to the solid-phase Ag were examined by the following procedures. (1) Microtiter plates were coated with 50 µl of SBP (5 µg/ml), *Cry j I* (2 µg/ml) or *Cry j II* (2 µg/ml) by incubation at 37 °C for 1 h, followed by blocking with 1% BSA in PBS. 50 µl of culture supernatants of the hybridoma cells, purified mAbs (1-5 µg/ml) or biotinylated mAbs (1 µg/ml) were added to the wells. The reactions were visualized with horseradish peroxidase (HRPO)-labeled anti-mouse Ig (Dakopatts, Denmark) or HRPO-labeled avidin (Zymed) and substrate. (2) To assess the epitope specificities of the mAbs, two methods were used, one is a double-binding assay in which the ability of each biotin-labeled mAb was tested for binding to the *Cry j I* presented by the mAbs (5 µg/ml, 50 µl) coating solid phase. The other method is a cross-inhibition test based on the ability of the

Table 1. Monoclonal antibodies raised against SBP

Specificity	mAbs, n	Isotype	Hybridoma clones
<i>Cry j I</i>	21	IgG1 κ (17)	026, 027, 029, 052, 053, 028, 060, 065, 007, 013, 046, 070, 017, 016, 019, 073, 055
		IgG2a κ (2)	054, 057
		IgM κ (2)	004, 024
<i>Cry j II</i>	2	IgG1 κ (1)	N26
		IgG3 κ (1)	T27
<i>Cry j I+II</i>	0		

Culture supernatant of each hybridoma cell line was added to the wells previously coated with SBP (5 µg/ml), *Cry j I* (2 µg/ml) or *Cry j II* (2 µg/ml). Bindings of the mAbs were detected with HRPO-labeled anti-mouse Ig and substrate. Isotype of each mAb was determined by the MONO-ID.

mAbs to inhibit the binding of coexisting biotin-labeled mAbs to the solid-phase *Cry j I*.

Gel Filtration Analysis. 50 µl of a mAb (1 mg/ml) were mixed with 50 µl of *Cry j I* (1 mg/ml) or 50 µl of PBS. After incubation at room temperature for 1 h, 50 µl of another mAb (1 mg/ml) or PBS were added to the mixture. After a further 1-hour incubation, the resultant mixture was subjected to analysis on HPLC using a TSK-Gel G3000 SW column (Tohso, Japan) equilibrated with 0.3 M NaCl in 10 mM phosphate buffer, pH 7.2.

Blocking of Human IgE Antibody-Binding by mAbs. The ability of the mAbs to block the binding of human anti-*Cry j I* or -*Cry j II* IgE antibodies was determined by Elisa using the antigen-coated microplate. 50 µl of 10-100 µg/ml mAb or PBS was added to the Ag-coated wells and incubated at 37 °C for 1 h to saturate the antigenic determinants. Then, 50 µl of appropriately diluted human serum (1:2-4) was added to the wells. After overnight incubation at 4 °C, binding of human IgE antibodies to the coated antigen was detected with HRPO-labeled goat anti-human ε-chain Ab (Cappel, USA) and substrate.

Results

Antigen Specificities of the mAbs Raised against SBP

In total, 23 mAbs were obtained. 21 of the 23 mAbs were specific for *Cry j I*, and the remaining 2 were for *Cry j II*. No mAbs reactive to both *Cry j I* and *Cry j II* were obtained so far, suggesting that *Cry j I* and *Cry j II* share no major cross-reactive determinants (table 1). The isotype of each mAb is shown in table 1.

Table 2. Classification of anti-*Cry j I* mAbs by the binding assay of biotin-labeled mAbs to the *Cry j I* presented by coated mAbs

Biotin-labeled mAbs	Binding against <i>Cry j I</i> presented by solid-phase mAbs										Control solid phase <i>Cry j I</i>
	046	013	065	060	052	029	053	026	004	027	
046*	-	-	+++	+++	+++	+++	+++	-	-	-	+++
013	-	-	++	++	++	++	+++	-	-	-	++
065	+++	+++	-	-	-	-	-	-	-	-	+++
060	+++	+++	-	-	-	-	-	-	-	-	+++
052	++	++	-	-	-	-	-	-	-	-	+++
029	+++	++	-	-	-	-	-	-	-	-	+++
053	+++	+++	-	-	-	-	-	-	-	-	+++
026	-	-	-	-	-	-	-	-	-	-	+
004	-	-	-	-	-	-	-	-	-	-	+
027	-	-	-	-	-	-	-	-	-	-	++
poly	++	++	++	+++	+++	+++	+++	-	-	-	+++
a-mIg	#	#	#	#	#	#	#	+++	++	+++	#

50 μ l of purified mAbs, 5 μ g/ml, were coated on microplates, then 50 μ l of 5 μ g/ml *Cry j I* were added to the wells. After a 1-hour incubation at 37°C and washing, biotinylated mAbs (2 μ g/ml) were added to the wells. As a control for the antibody activity of each biotin-labeled mAb, the biotin-labeled mAbs were reacted with solid-phase *Cry j I*. Results are presented by absorbance at 492nm. +++ = > 1; ++ = 0.5–1.0; + = 0.1–0.5; - = < 0.1; # = not done.

Epitope Specificities of Anti-*Cry j I* mAbs

The epitope specificity of each mAb was analyzed by the ability of each biotinylated mAb to bind to the *Cry j I* presented by the solid-phase mAbs. As shown in table 2, the results allowed the grouping of these mAbs into three families differing in their fine specificities: group 1, 046 and 013; group 2, 065, 060, 052, 029 and 053, and group 3, 026, 004 and 027. Group 1 mAbs and group 2 mAbs showed the reciprocal results as follows; biotinylated mAb in one group could bind to the *Cry j I* presented by the other group mAbs, but not to that presented by the mAbs within the same group. The results suggest that epitopes for group 1 mAbs are different from those for group 2 mAbs. Biotin-labeled group 3 mAbs gave no positive reactions against *Cry j I* presented by other mAbs, even by polyclonal Ab, and solid-phase-coating group 3 mAbs could not present the antigen to other mAbs. However, it is confirmed that biotin-labeled group 3 mAbs could bind to the solid-phase-coated *Cry j I*, and that group 3 mAbs were really coated on the plate (table 2, the last column and the last line). The epitopes for group 3 mAbs are considered to be located at the inner space of the molecule, and they would be exposed as cryptantigens to its surface when conformationally changed. The epitope specificity of each group 3 mAb could not be defined in this experiment. All of these findings suggest that *Cry j I* molecules take a

monomeric form in water solution, and they have no repeating expression of the determinants on their surface.

The grouping of the mAbs mentioned above was validated by the cross-inhibition test. In this method, if two or more mAbs could inhibit each other in a reciprocal fashion, they are considered to be members of the same group, moreover, a mAb in one group would not compete with other group mAb to bind to the solid-phase antigen. Table 3 shows the results. The mAbs within groups 1 or within group 2 inhibited each other in a reciprocal fashion (surrounded with thick lines). However, group 1 mAbs competed with biotin-labeled group 2 mAbs except 053 to bind to the solid-phase *Cry j I* (surrounded with thin lines), whereas group 2 mAbs did not inhibit binding of biotin-labeled group 1 mAbs. This one-way inhibition will be further studied in the next experiment. The epitope for 053 which is considered to be a member of group 2 was shown to be closely related, but not identical with those for other mAbs in group 2. The group 3 mAbs defined by the above double-binding assay could be further divided into two groups by the cross-inhibition test (group 3: 026 and 004; group 4: 027) because the mAb 027 shows no inhibiting ability for other mAbs except itself. The inhibition pattern of mAbs within group 3 shows that the epitopes for the two mAbs are closely related, but not identical with each other.

Table 3. The inhibition of biotin-labeled mAb binding to solid-phase *Cry j I* with coexisting unlabeled mAb

Unlabeled mAb	Biotin-labeled mAb									
	046	013	165	060	052	029	053	026	004	027
046	+++	+++	+++	+++	+++	+++	-	-	-	-
013	+++	+++	+++	+++	+++	++	-	-	-	-
065	±	±	+++	+++	+++	+++	+	-	±	-
060	±	-	+++	+++	+++	+++	+++	±	-	-
052	-	-	+++	+++	+++	+++	++	-	-	-
029	-	-	+++	+++	+++	+++	+	-	-	-
053	-	-	+++	+++	+++	+++	+++	-	±	-
026	-	-	±	±	-	-	-	+++	+++	-
004	-	-	±	±	-	±	-	+	+++	-
027	-	-	-	-	-	-	-	-	-	+++

50 µl of 2 µg/ml *Cry j I* were coated on microplates. Biotin-labeled mAbs (2 µg/ml) were reacted to the solid-phase antigen in the presence or absence of liquid-phase unlabeled mAbs (10, 30 and 100 µg/ml). +++ = More than 50% inhibition of the binding of biotin-labeled mAb; ++ = 30-49%; + = 15-29%; ± = 6-14%; - = <5% inhibition.

Table 4. Retention time of the peaks eluted from a G3000 SW column and percentage of the peak s area

Samples	Retention time		
Experiment 1			
<i>Cry j I</i>		21.511 (43.1)	22.568 (48.9)
029	17.801 (91.9)		
046	17.385 (99.2)		
052	17.465 (97.9)		
029 + <i>Cry j I</i>	15.265 (83.7)	21.635 (5.8)	22.681 (5.6)
0.46 + <i>Cry j I</i>	15.318 (98.2)		
029 + <i>Cry j I</i> + 046	14.121 (96.8)		
029 + <i>Cry j I</i> + 052	15.335 (58.5)	17.455 (40.2)	
046 + <i>Cry j I</i> + 029	12.311 (7.5)		
	14.121 (63.4)	15.365 (26.0)	
Experiment 2			
<i>Cry j I</i>		21.511 (43.1)	22.568 (48.9)
026	17.068 (99.5)		
026 + <i>Cry j I</i>	17.078 (56.4)	21.578 (18.3)	22.638 (21.8)
<i>Cry j I</i>		21.048 (41.0)	21.868 (50.0)
027	17.801 (90.1)		
027 + <i>Cry j I</i>	17.815 (41.2)	21.071 (19.8)	21.895 (24.7)

Percentages are given in parentheses.

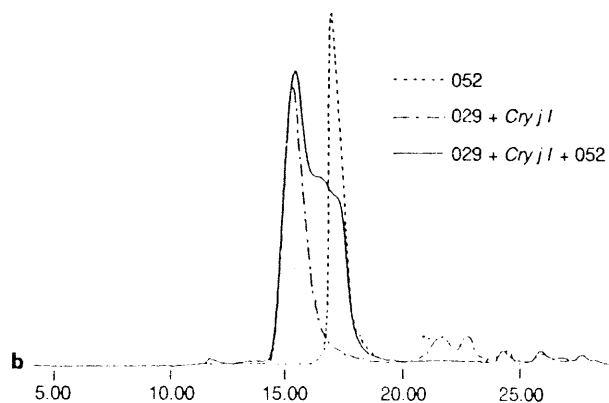
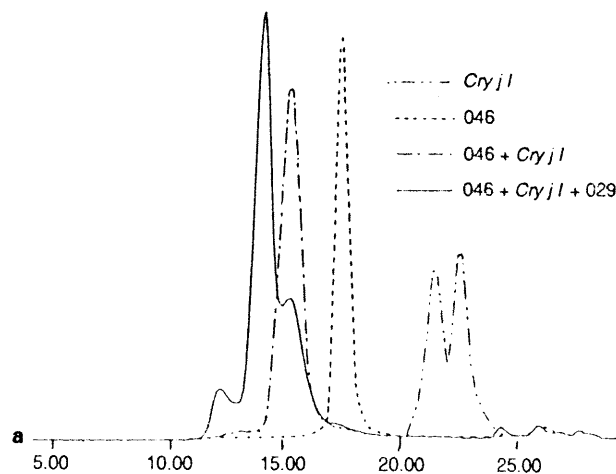


Fig. 1. Elution profiles of complexes consisting of *Cry j I* and mAbs in gel filtration analyses. 50 μ l of a purified mAb (1 mg/ml) was mixed with 50 μ l of *Cry j I* (1 mg/ml) or PBS. After a 1-hour incubation at room temperature, another mAb or PBS was added to the mixture with further incubation for 1 h. The resultant mixture was subjected to HPLC gel filtration on a TSK G3000 SW column.

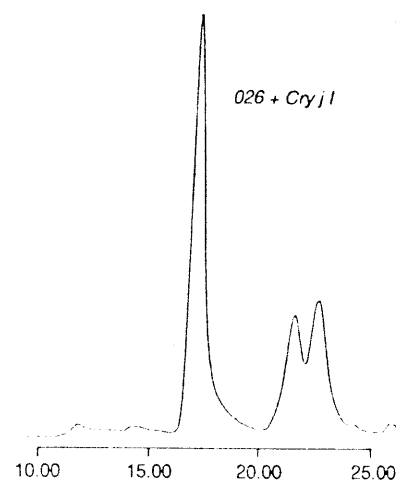
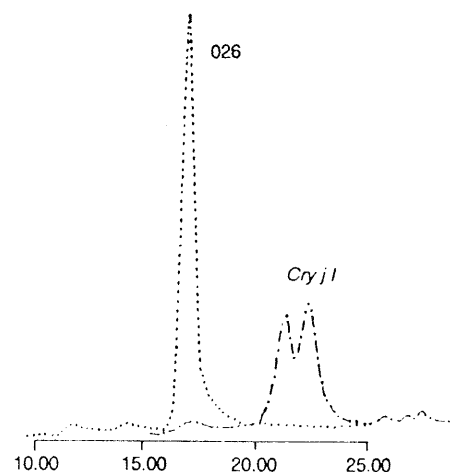


Fig. 2. Elution profiles from a HPLC G3000 SW column of mAb 026, *Cry j I* and of the mixture of *Cry j I* and 026. 50 μ l of *Cry j I* (1 mg/ml) and 50 μ l of mAb 026 (1 mg/ml) were mixed together. After a 1-hour incubation at room temperature, the mixture was subjected to HPLC gel filtration using a G3000 SW column.

Analyses on the Binding of the mAbs to Liquid-Phase Cry j I by HPLC Gel Filtration

Since the one-way inhibitions were observed between biotin-labeled group 2 mAbs and unlabeled group 1 mAbs in the cross-inhibition test, HPLC gel filtration analyses were performed to determine whether group 1 mAb 046 and group 2 mAbs 029 and 052 are capable of cobinding to *Cry j I* to form the complex Ab-Ag-Ab in the liquid phase. Table 4 summarizes the retention time and percent area of

each peak obtained in the series of gel filtration tests. Figure 1a shows the elution profiles from a G3000 SW column of *Cry j I*, mAb 046 (group 1) and of the complexes formed by 046 + *Cry j I* or 046 + *Cry j I* + 029 (group 2). The retention time of the mixture composed of 029 + *Cry j I* + 046 was clearly reduced compared with those of 046 + *Cry j I* and of 046 or *Cry j I*. On the other hand, as shown in figure 1b, the retention time of the mixture 029 + *Cry j I* + 052 (group 2) was the same as that of 029 + *Cry j I*, and the elu-

tion profile of the mixture is composed of the pattern of the Ag-Ab complex plus the mAb alone. The results indicate that group 1 mAb and group 2 mAb could bind simultaneously to *Cry j I* in the liquid phase to form an Ab-Ag-Ab complex larger than the complex of group 1 mAb + *Cry j I* or group 2 mAb + *Cry j I*. Similar results were obtained in the case of 013 (group 1) + *Cry j I* + 029 (data not shown). It becomes clear that on the surface of liquid-phase *Cry j I*, the binding sites for group 2 mAbs are located apart from those for group 1 mAbs, and a mAb in the group 1 does not compete with a group 2 mAb to get each epitope in the liquid phase.

Figure 2 shows that the group 3 mAb 026 and *Cry j I* do not form any complex when mixed together in the liquid phase, as indicated by the fact that the elution profile and the retention time of the mixture consist of three peaks corresponding to that of the mAb alone and those of the Ag alone. Similar results were obtained in the case of 027 (elution profiles are not shown). In groups 3 and 4, the mAbs were confirmed to be incapable of binding to *Cry j I* in the liquid phase.

Blocking of the Binding of Human IgE Antibody to Cry j I and Cry j II by the Mouse mAbs

It is very important to determine whether the epitopes recognized by the mouse mAbs are related to the allergenic determinants for human allergic reactions. Human serums from cedar pollen allergic individuals were added to the solid-phase *Cry j I* or *Cry j II* in the presence or absence of 10–100 µg/ml of the representative mouse mAbs from the four groups. As shown in table 5, the most effective mAb on the blocking of human IgE binding to *Cry j I* is the mAb 046 (group 1), which inhibits the IgE antibody binding of almost all cases, and the degree of the inhibition was 40–80%. In the cases of the mAbs 052 (group 2), 026 (group 3) and 027 (group 4), inhibition exceeding 50% could not be observed. However, human IgE binding to *Cry j II* could be well inhibited by anti-*Cry j II* mAbs N26 or T27 (27–78%, table 6). Since human IgE antibodies against *Cry j I* or *Cry j II* might be polyclonal, inhibition more than 40% could be considered to be significant. The epitopes recognized by the group 1 mAbs and those for N26 and T27 might be important determinants on *Cry j I* and *Cry j II*, respectively, for allergic responses in human beings.

Table 5. Inhibition of human IgE Abs from binding to *Cry j I* by mouse anti-*Cry j I* mAbs

Human serum	Binding to solid-phase <i>Cry j I</i>	Percent inhibition by anti- <i>Cry j I</i>			
		mAb 046 (group 1)	mAb 052 (group 2)	mAb 026 (group 3)	mAb 027 (group 4)
H.I.	522 ± 32	58.7	32.8	38.6	36.6
No. 120	901 ± 32	29.5	24.5	26.0	28.2
M.K.	201 ± 25	74.3	1.8	8.6	15.5
Y.M.	1,239 ± 36	56.5	16.6	16.4	14.6
A.N.	1,170	45.6	31.5	38.6	28.9
K.K.	126 ± 28	80.9	8.0	27.4	22.0
Y.Y.	355 ± 14	70.0	23.1	24.0	23.0
No. 199	298	68.2	42.0	11.8	24.9
No. 432	238	62.0	0	42.3	46.5
Means		59.9 ± 15.1	20.7 ± 13.2	25.8 ± 10.3	27.1 ± 9.7

50 µl of 100 µg/ml mAbs were reacted to the solid-phase *Cry j I*. After a 1-hour incubation at 37°C, 50 µl of human serums (1:2–4 diluted) from patients with cedar pollinosis were added to the wells. After overnight incubation at 4°C, the bindings of human IgE antibodies to the solid-phase antigens were detected with anti-human ε chain.

Table 6. Inhibition of human IgE Abs from binding to *Cry j II* by mouse anti-*Cry j II* mAbs

Human serum	Binding to solid-phase <i>Cry j II</i>	Percent inhibition by anti- <i>Cry j II</i>	
		mAb N26	mAb T27
H.I.	270 ± 8	27.0	27.4
Y.Y.	285 ± 6	31.1	37.6
Y.M.	355 ± 10	48.9	51.1
M.K.	151 ± 3	36.1	56.8
A.N.	164	45.2	42.8
K.K.	104 ± 2	31.2	34.4
No. 432	144	51.4	47.3
No. 430	109 ± 0	78.0	67.0
Means		50.3 ± 19.2	49.3 ± 14.6

50 µl of 100 µg/ml mAbs were reacted to the solid-phase *Cry j II*. After a 1-hour incubation at 37°C, 50 µl of human serums (1:2–4 diluted) from patients with cedar pollinosis were added to the wells. After overnight incubation at 4°C, the bindings of human IgE antibodies to the solid-phase antigens were detected with anti-human ε chain.

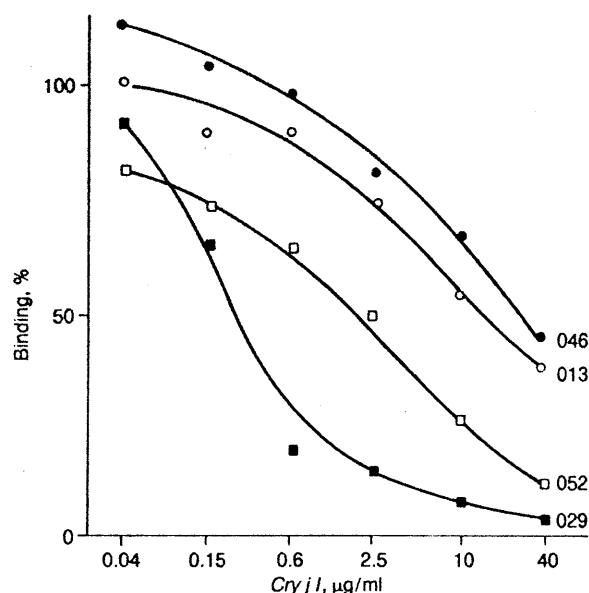


Fig. 3. Percent binding of the mAbs to solid-phase *Cry j I* in the presence of liquid-phase *Cry j I*. 50 µl of 2 µg/ml mAbs were reacted with solid-phase *Cry j I* in the presence or absence of various concentrations of liquid-phase *Cry j I*.

Discussion

Distribution and characteristics of B-cell-tropic epitopes on *Cry j I* molecules were analyzed using mAbs obtained in our laboratories. In this study, no mAbs reactive to both *Cry j I* and *Cry j II* were found so far, suggesting that *Cry j I* and *Cry j II* share no major cross-reactive determinants. The result is consistent with the observation in the human IgE Elisa inhibition test reported by Sakaguchi et al. [4]. However, since we could have only two mAbs specific for *Cry j II*, the possibility remains that a total of 23 mAbs is insufficient for the detection of such determinants.

IgE antibodies from almost all patients with the cedar pollinosis have been reported to react with both *Cry j I* and *Cry j II*, while serums from a minor group of patients were found to be reactive only to *Cry j I* or *Cry j II* [4]. The N-terminal sequence of the 10 residues of *Cry j II* has been reported to be different from that of *Cry j I*, so *Cry j I* and *Cry j II* are considered to be different from each other. Since the complete primary structure of SBP has not been available yet, we could not define whether *Cry j II* is an artificial

breakdown product of *Cry j I* during the purification procedures.

Our anti-*Cry j I* mAbs tested could be classified into four groups by their fine specificities. Group 1 mAbs, 046 and 013, were confirmed by HPLC gel filtration to be capable of cobinding to liquid-phase *Cry j I* with group 2 mAb 029 (fig. 1a). In the double-binding assay, it is also indicated that epitopes for group 1 mAbs are different from those for group 2 mAbs. However, in the cross-inhibition assay, where the Ag is coated on a plate, group 1 mAbs, 046 and 013, did inhibit group 2 mAbs from binding to the solid-phase Ag, whereas group 2 mAbs did not inhibit binding of group 1 mAbs. This one-way inhibition might be explained as follows: the epitopes for groups 1 and 2 mAbs are different from each other, and they are located sufficiently apart from each other so that the two group mAbs do not compete in binding to the Ag in the liquid phase, in contrast, some conformational changes would occur in the Ag molecule to make the two epitopes get so closely that the two mAbs interact to each other in binding to the antigen coated on the solid phase. In addition, since group 1 mAbs 046 and 013 were shown to have a higher affinity than group 2 mAb 052 and 029 (as suggested by fig. 3), the one-way inhibition would be easily induced. Conformational changes in antigen molecules induced by the interaction between the antigen and plastic plate have been reported in several papers. Many of the changes are reported as changes in antigenicity, for example, Frieguet et al. [17] reported that mAbs directed to an epitope on the β_2 -subunit of *Escherichia coli* tryptophan synthetase bind slowly to the native Ag solution, while the mAbs react quite rapidly to the antigen adsorbed on a microplate, and the authors mentioned that the protein might undergo some denaturation when it was adsorbed on a microplate. The epitopes for our group 3 and 4 mAbs belong to such a category. Conversely, several mAbs had been reported which could bind to a corresponding antigen in solution, but not to the Ag adsorbed on a plate: some mAbs to ragweed AgE [8], tobacco mosaic virus protein [19], the β_2 -subunit of *E. coli* tryptophan synthetase [17] and interferon α [20]. Such cases, however, have not been found in our mAbs, because our mAbs were selected by using solid-phase antigens.

The mAb 046 could block the binding, at the degree of 40–80%, to *Cry j I* of human IgE antibodies in serums from patients with cedar pollinosis. Some degree of blocking of human IgE antibody binding by other mAbs was observed in some serums, but not in all. The binding to *Cry j II* was blocked by mAbs N26 and T27. Since human IgE antibodies against *Cry j I* or *Cry j II* are polyclonally produced, over

40% inhibition by a mAb may suggest that the epitope seen by the mAb is the main and important target of the IgE antibodies to the allergens. At present, since we have little information on the epitopes corresponding to the human blocking antibodies, the determination of the epitopes for these mAbs will give much benefits for under-

standing the mechanisms for immune responses to the allergens and for making strategies to control the responses as reported by other investigators [7-13, 15, 20]. Results obtained in the analysis of the structures of these epitopes will be presented in the following paper.

References

- 1 Yasueda H, Yui Y, Shimizu T, Shida T: Isolation and partial characterization of the major allergen from Japanese cedar pollen. *J Allergy Clin Immunol* 1983;71:77-86.
- 2 Marsh DG, Goodfried L, King TP, Platts-Mills TEA: Allergen nomenclature. *Allergy* 1988;43:161-168.
- 3 Taniai M, Ando S, Usui M, Kurimoto M, Sakaguchi M, Inouye S, Matuhasi T: N-terminal amino acid sequence of major allergen of Japanese cedar pollen (*Cry j I*). *FEBS Lett* 1988;239:329-332.
- 4 Sakaguchi M, Inouye S, Taniai M, Ando S, Usui M, Matuhasi T: Identification of the second major allergen of Japanese cedar pollen. *Allergy* 1990;45:309-312.
- 5 Ekramaddoullah AKM, Kisil FT, Schon AH: Partial characterization of an antigenic site of high molecular weight basic antigen, a ryegrass pollen allergen, using a monoclonal antibody. *Mol Immunol* 1986;23:111-117.
- 6 Chapman MD, Heymann PW, Platts-Mills TAE: Epitope mapping of two major inhalant allergens, *Der p I* and *Der f I*, from mites of genus *Dermatophagoides*. *J Immunol* 1984;139:1479-1484.
- 7 Bose JR, Rector SE, Fischer J, Taronno R, Deslespesse G: Production and characterization of mouse monoclonal antibodies to allergenic epitopes on *Lol p I* (Rye I). *Immunology* 1986;59:309-315.
- 8 Oslon JR, Klapper DG: Two major human allergenic sites on ragweed allergen E identified by using monoclonal antibodies. *J Immunol* 1986;136:2109-2115.
- 9 Lind P, Hansen OS, Horn N: The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, *Der p I*, of *Dermatophagoides pteronyssinus*. Relative binding site location and species specificity studies by solid phase inhibition assays with radiolabeled antigen. *J Immunol* 1988;140:4256-4262.
- 10 Esch RE, Klapper DG: Identification and localization of allergenic determinants on grass group I antigens using monoclonal antibodies. *J Immunol* 1989;142:178-184.
- 11 Chapman MD, Sutherland WM, Platts-Mills TAE: Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P1 by using monoclonal antibodies: Binding to each epitope can be inhibited by serums from dust mite-allergic patients. *J Immunol* 1984;133:2488-2495.
- 12 Iwata M, Katamura K, Kubo RT, Ishizaka K: Relationship between T cell receptor and antigen-binding factors. I. Specificity of functional T cell receptors on mouse T cell hybridomas that produce antigen-binding factors. *J Immunol* 1989;143:3909-3916.
- 13 Iwata M, Katamura K, Kubo RT, Grey HM, Ishizaka K: Relationship between T cell receptors and antigen-binding factors. II. Common antigenic determinant and epitope recognition shared by T cell receptors and antigen-binding factors. *J Immunol* 1989;143:3917-3924.
- 14 Baur X, Ashaure H, Mazur G, Dewair M, Prelicz H, Steigemann G: Structure, antigenic determinants of some clinically important insect allergens: *Chironomid* hemoglobins. *Science* 1986;233:351-354.
- 15 Saint-Remy IMR, Lebrun PM, Lebecque SJ, Masson PL: The human immune response against major allergens from house dust mite, *Dermatophagoides pteronyssinus*. II. Idiotypic cross-reactions of allergen-specific antibodies. *Eur J Immunol* 1986;16:575-580.
- 16 Guesdon JL, Ternynck T, Averameas S: The use of avidin-biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 1979;27:1131-1139.
- 17 Frieguet B, Djavadi-Ohanian L, Goldberg ME: Some monoclonal antibodies raised with a native protein bind preferentially to the denatured antigen. *Mol Immunol* 1984;21:673-677.
- 18 Al Moudallal Z, Altschuh D, Briand JP, van Regenmortel MH: Comparative sensitivity of different ELISA procedures for detecting monoclonal antibodies. *J Immunol Methods* 1984;68:35-43.
- 19 Norvick D, Eschhar Z, Rubinstein M: Monoclonal antibodies to human alpha-interferon and their use for affinity chromatography. *J Immunol* 1982;129:2244-2247.
- 20 Bose R, Ekramaddoullah AKM, Kisil FT, Schon AH: Human and murine antibodies to rye grass pollen allergen *Lol p I* share a common idio type. *Immunology* 1988;63:579-584.
- 21 Heymann PW, Chapman MD, Platts-Mills TAE: Antigen *Der f I* from the dust mite *Dermatophagoides farinae*: Structural comparison with *Der p I* from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 1986;137:2841-2847.