

IgE reactivity and cross-reactivity of Japanese monkeys (*Macaca fuscata*) to Japanese cedar (*Cryptomeria japonica*) and cypress (*Chamaecyparis obtusa*) pollen allergens

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Summary

Background The natural occurrence of Japanese cedar (*Cryptomeria japonica*, CJ) pollinosis has been reported in Japanese monkeys (*Macaca fuscata*). However, the reactivity to Japanese cypress (*Chamaecyparis obtusa*, CO) pollen allergens in these monkeys has not yet been reported.

Objectives The present study was designed to investigate the reactivity to CO pollen allergens in monkeys sensitized to CJ pollen allergens.

Methods Serum samples from 40 monkeys naturally sensitized to CJ pollen allergens were collected from four troops. We measured the specific IgE to CO pollen allergens and examined the reactivity to the allergens by intradermal test. Cross-reactivity between CJ and CO pollen allergens was examined by ELISA inhibition method. Furthermore, we examined the sensitivity to the allergens by histamine release assay from leucocytes.

Results All 40 monkeys had specific IgE to crude and purified major allergens (Cha o 1) of CO pollen. The monkeys showed a positive reaction to CO pollen allergens in the intradermal test. Allergenic cross-reactivity between Cha o 1 and Cry j 1 (a major allergen in CJ pollen) was also observed. Specific histamine release to both the major allergens was noted in two monkeys with CJ pollinosis.

Conclusion Japanese monkeys sensitized to Japanese cedar pollen allergens also demonstrate reactivity to Japanese cypress pollen allergens.

Keywords: cedar, cypress, IgE, monkey, pollen

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Introduction

Japanese cedar (*Cryptomeria japonica*, CJ) pollinosis is one of the most common allergic diseases in Japan [1,2], with an increasing number of the patients [3]. It is suggested that the prevalence of CJ pollinosis is $\approx 10\%$ in Japanese people [1,4].

In humans, Japanese cypress (*Chamaecyparis obtusa*, CO) pollen plays an important role in the pollinosis during the spring season. Many patients with CJ pollinosis demonstrated reactivity to CO pollen allergen based on skin tests and the CAP method [5,6]. Furthermore, cross-allergenicity has been reported between CJ and CO pollen allergens [7,8].

Two major allergens have been isolated from CJ pollen and characterized as Cry j 1 and Cry j 2 [9,10]. Humans possess specific IgE to Cry j 1 and Cry j 2 [10,11]. A major allergen has recently been isolated from CO pollen, and has

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been designated Cha o 1 according to WHO nomenclature [12]. The primary structure of Cha o 1 was determined by cDNA sequencing [13] and the amino acid sequence of Cha o 1 demonstrated 80% homology with that of Cry j 1 [13].

The natural occurrence of CJ pollinosis has also been reported in Japanese monkeys (*Macaca fuscata*) [14,15], in which the symptoms resemble those of human patients. The monkeys with CJ pollinosis had specific IgE to CJ major allergens (Cry j 1, Cry j 2) [16,17]. Furthermore, leucocytes from monkeys with pollinosis demonstrate pollen allergen-specific histamine release, as well as a significant proliferation of peripheral blood mononuclear cells in response to CJ pollen allergens [18]. Japanese monkeys have been well established as a suitable animal model for studying CJ pollinosis. The monkeys are exposed to both CJ and CO pollen. However, the reactivity to CO pollen allergens in monkeys has not yet been reported.

The present study investigates the reactivity to CJ pollen allergens in monkeys sensitized to CO pollen allergens. We measured specific IgE to CO pollen allergens and examined the reactivity to the allergens by the intradermal test. Furthermore, we analysed the cross-reactivity between CJ and CO allergens by ELISA inhibition methods. Finally, we examined the sensitivity to Cha o 1 by the histamine release assay.

Materials and methods

Antigen

Pollen crude extract was prepared as previously described [9,10]. Cry j 1 was purified from Japanese cedar pollen as previously described [9,10]. Cha o 1 was purified from Japanese cypress according to the procedure of Ide *et al.* [12]. Briefly, 40 g of pollen was extracted with 1000 mL of 0.1 mol/L TRIS-HCl (pH 8.0) by shaking the sample gently at 4 °C for 16 h. The sample was centrifuged at 20 000 *g* for 15 min and the supernatant was collected. Next, the extracts were applied to a QAE-Toyopearl 550C column (Tosoh, Tokyo, Japan) equilibrated with 0.1 mol/L TRIS-HCl (pH 8.0) and the pass-through fraction was collected. The pass-through fraction was dialysed against 0.1 mol/L phosphate (pH 5.0) and applied to a SP-Toyopearl 550C column (Tosoh) equilibrated with 0.1 mol/L acetate buffer (pH 5.0), and the proteins were eluted with 0.2 mol/L phosphate buffer (pH 7.0). The sample was applied to a Sephacryl S-300 (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline. The allergenic activity of the fractions in the peak of the gel filtration was verified by fluorometric ELISA.

Monkeys

Sera from 40 Japanese monkeys (*Macaca fuscata*) that had specific IgE (more than 1 Ua/mL) to CJ pollen allergens were collected from four free-ranging troops (AW, Hyogo; GD, Nagano; KS, Miyazaki; TK, Miyazaki) [17]. All serum specimens were stored at –70 °C until use.

Of the 40 monkeys, we confirmed that nine demonstrated CJ pollinosis symptoms during the pollination season. The intradermal test and histamine release assay were performed. The monkeys were anaesthetized by intramuscular injection of the combination of ketamine hydrochloride of 5 mg/kg and xylazine hydrochloride of 1 mg/kg.

Measurement of specific IgE to crude pollen allergens

The CAP method (Pharmacia) was used according to the manufacturer's instructions to measure specific IgE to Japanese cedar and cypress pollen allergens.

Measurement of specific IgE to two major pollen allergens

Specific IgE to Cry j 1 and Cha o 1 was measured by fluorometric ELISA as previously described [17]. Briefly, purified protein (1 µg/mL) was used to coat the microplate wells. After incubation of monkey serum (diluted 1 : 8) with the immobilized antigens, antihuman IgE antibody conjugated with β -D-galactosidase (Pharmacia) diluted 1 : 25 was added to each well. As the enzyme reaction substrate, 0.2 mmol/L 4-methylumbelliferyl- β -D-galactoside (Sigma Chemicals, St Louis MO, USA) was added and the plates were incubated at 37 °C for 2 h. Then, 0.1 mol/L glycine-NaOH (pH 10.3) was added to stop the reaction. Fluorescence units (FU) were measured on a fluorometric microplate reader (Fluoroskan; Flow Laboratories, McLean VA, USA). The cut-off values were determined with sera from cynomolgus monkeys originating from Indonesia where there is no CJ vegetation as a negative control [17]. The specific IgE level was expressed as arbitrary units (U)/mL.

Intradermal test

CJ (40 µg/mL) and CO (25 µg/mL) pollen crude extract (diluted 1 : 50) and the purified allergens (200 ng/mL) were used for the intradermal test. The injection volume of each allergen was 0.05 mL, and the reaction (weal and erythema) was read 15 min after injection. Saline served as a negative control. The skin test results were judged according to the criteria of the human skin test [19].

ELISA inhibition test

The allergenic cross-reactivity between CJ and CO pollen allergens was analysed by fluorometric ELISA inhibition as

previously described [20]. Briefly, Cry j 1 or Cha o 1 ($0.2 \mu\text{g/mL}$) was adsorbed to microplate wells. After an overnight incubation at 4°C , the serum was mixed with Cry j 1 or Cha o 1 ($20 \mu\text{g/mL}$) as an inhibitor and added to the wells. After 3 h of incubation at room temperature, antihuman IgE antibody conjugated with β -D-galactosidase was added. The following procedures were the same as those for the above-mentioned fluorometric ELISA.

Histamine release assay for major allergens

The histamine release assay with leucocytes was carried out as previously described [11,21].

Results

Specific IgE to CJ and CO pollen allergens

Figure 1(a) showed specific IgE to two crude pollen allergens in the 40 monkeys sensitized to CJ pollen allergens. The level of IgE to CJ pollen allergen (mean value $\pm$ SD, $7.8 \pm 8.6 \text{ Ua/mL}$) was significantly higher than that of IgE to CO pollen allergen ($4.4 \pm 4.2 \text{ Ua/mL}$; $P < 0.05$).

Specific IgE to the two major allergens (Cry j 1 and Cha o 1) was measured by fluorometric ELISA (Fig. 1b). All 40 monkeys demonstrated specific IgE to Cry j 1 and Cha o 1. Figure 2 showed the correlation between the levels of specific IgE to the two major allergens in monkeys. The level of specific IgE to Cry j 1 showed a slight correlation with that to Cha o 1 (correlation coefficient of 0.59). Furthermore, the level of IgE to Cry j 1 (mean value $\pm$ SD, $3070 \pm 4350 \text{ U/mL}$) was significantly higher than that to Cha o 1 ($1160 \pm 1780 \text{ U/mL}$; $P < 0.001$).

Table 1. Level of specific IgE to CJ and CO pollen allergens in monkeys with pollinosis symptoms

Monkey number	Age (years)	Sex	Specific IgE			
			CAP (Ua/mL)		ELISA (U/mL)	
			CJ	CO	Cry j 1	Cha o 1
1	6	F	10.2	2.87	960	225
2	7	F	7.9	1.55	4350	415
3	11	F	21.5	0.72	1560	16
4	16	M	21.9	5.79	4530	1780
5	10	F	35.6	11.9	13 700	3100
6	14	F	21.0	0.92	3470	414
7	16	F	8.69	2.00	6800	354
8	18	F	7.83	0.49	6640	59
9	5	F	10.2	1.76	2640	371

Intradermal test for CJ and CO pollen allergens

Three monkeys (numbers 1–3 in Table 1) that demonstrated specific IgE to CJ and CO pollen allergens also showed allergic reactions to crude and major allergens of CJ and CO pollen in the skin test (Table 2). These antigens produced only weal in all monkeys. The negative control solution (saline) elicited a small weal ($< 5 \text{ mm}$) and no erythema.

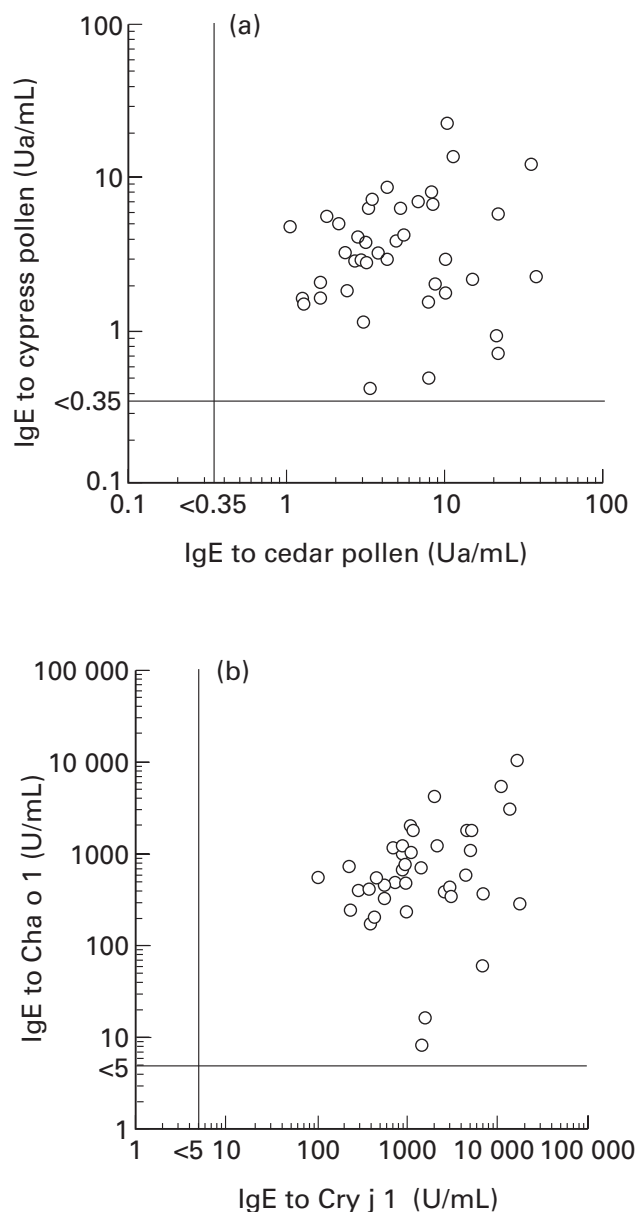


Fig. 1. Comparison of specific IgE to CJ and CO allergens in 40 Japanese monkeys. (a) Specific IgE to the crude pollen allergens were measured by CAP system. A value of more than 0.34 Ua/mL was regarded as positive. (b) Specific IgE to Cry j 1 and Cha o 1 were measured by ELISA method. A value of more than 5 U/mL was regarded as positive.

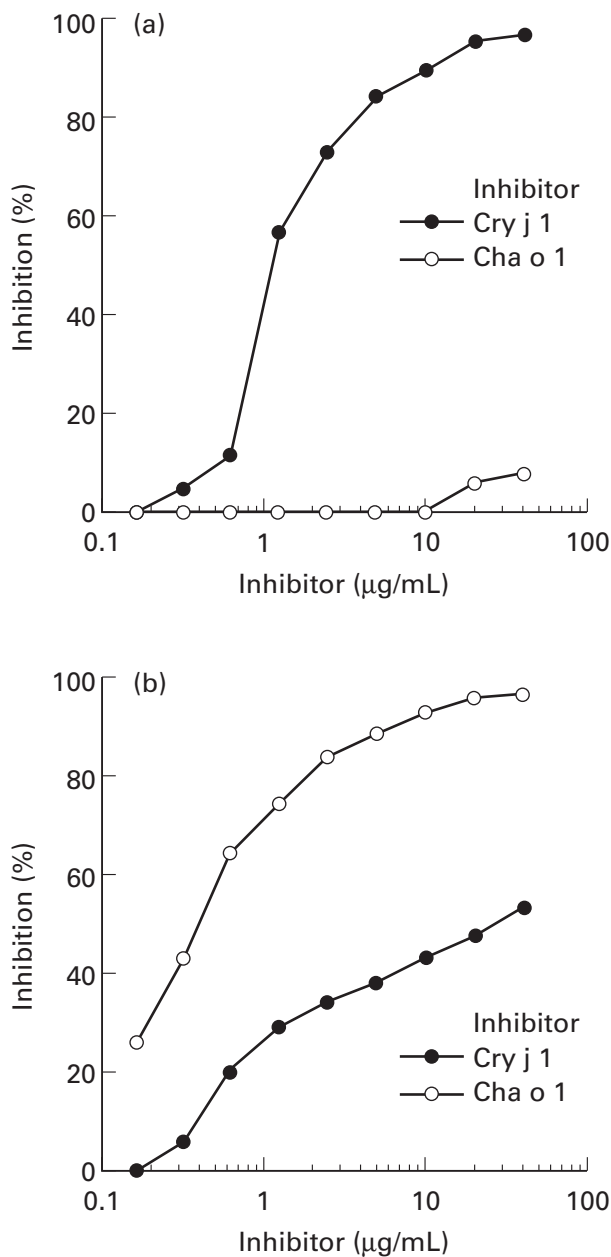


Fig. 2. Dose-dependent ELISA inhibition curves in a monkeys. Cry j 1 and Cha o 1 as inhibitors. (a) Immobilized antigen: Cry j 1 (b) Immobilized antigen: Cha o 1.

Allergenic cross-reactivity between CJ and CO pollen

Figure 2 showed dose-dependent ELISA inhibition curves in a monkey (number 4). In three monkeys (numbers 4–6), allergenic cross-reactivity between the major allergens of CJ and CO pollen was investigated by the ELISA inhibition test with 20 μg/mL of Cry j 1 or Cha o 1 as an inhibitor

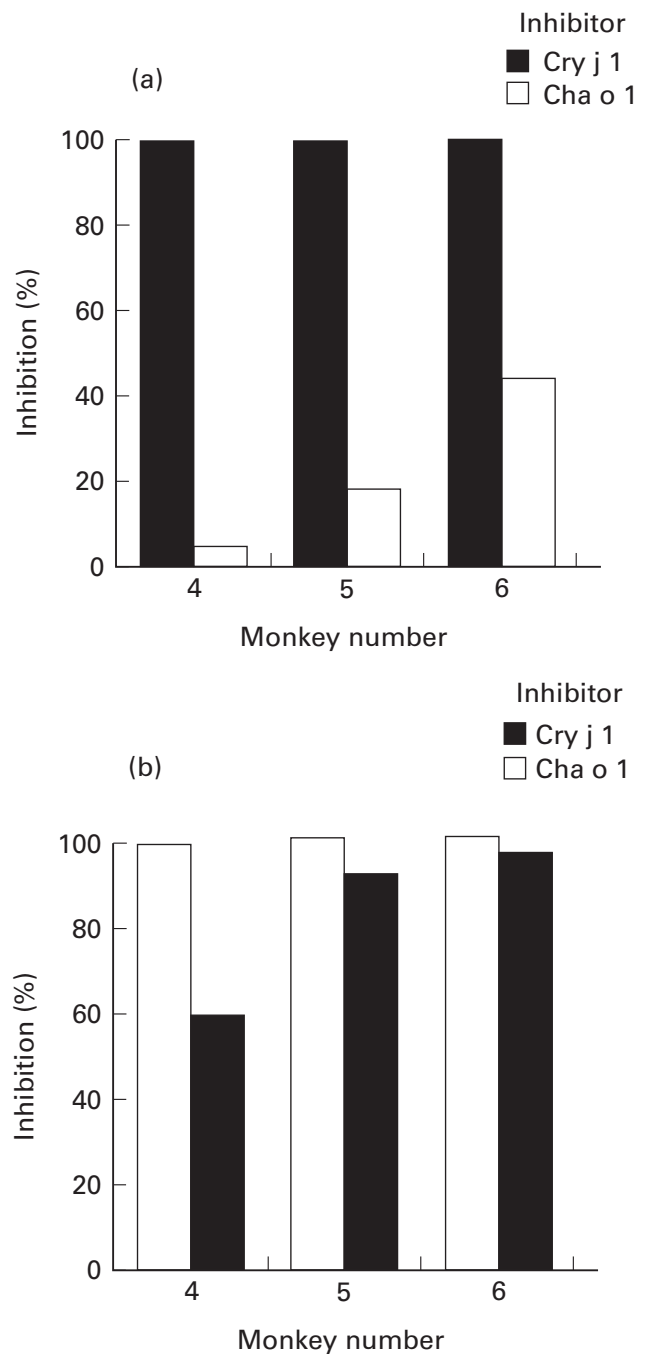
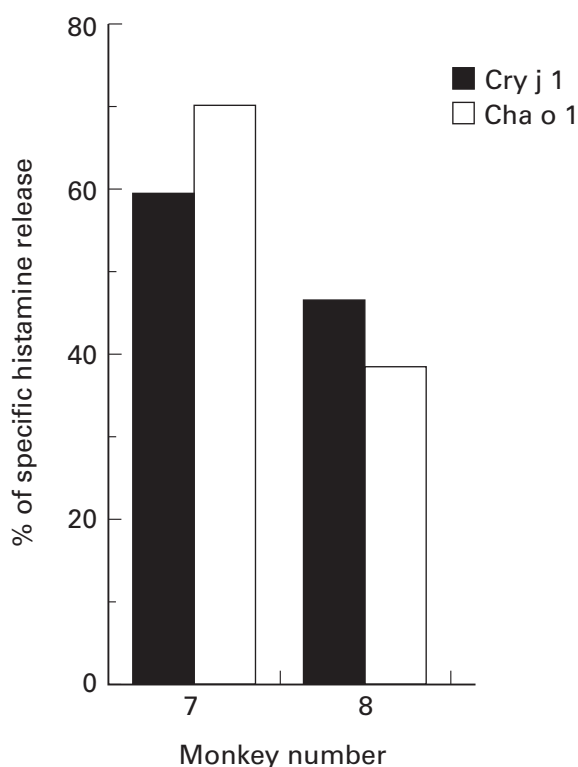


Fig. 3. Allergenic cross-reactivity between CJ and CO pollen allergens according to ELISA inhibition test. Cry j 1 and Cha o 1 as inhibitors. (a) Immobilized antigen: Cry j 1. (b) Immobilized antigen: Cha o 1.

(Fig. 3). When Cry j 1 was used as the solid-phase antigen, prior incubation of the sera with homologous Cry j 1 as an inhibitor resulted in complete inhibition (Fig. 3a). However, IgE binding to the solid-phase Cry 1 was not almost inhibited by Cha o 1. When Cha o 1 was used as the

Table 2. Intradermal test for monkeys sensitized to CJ and CO pollen allergens

Monkey no.	CJ allergen		CO allergen		
	Extract	Cry j 1	Extract	Cha o 1	Ct
1	15×12*	15×12	20×10	15×10	<5×5
2	13×12	15×12	12×12	10×10	<5×5
3	10×10	13×10	10×10	10×10	<5×5

**Fig. 4.** Specific histamine release in response to major allergens. Leucocytes obtained from two monkeys with CJ pollinosis. Cry j 1 and Cha o 1 as challenge allergens. Results are expressed as percentage histamine release of the total histamine content.

solid-phase antigen, prior incubation of the sera with homologous Cha o 1 resulted in greater than 96% inhibition (Fig. 3b). IgE binding to the solid-phase Cha o 1 was inhibited by Cry j 1 to various extents. These results indicate that allergenic cross-reactivity appears to occur between CJ and CO major allergens.

Allergen-specific histamine release

The histamine release assay was carried out using leucocytes from two monkeys (numbers 7, 8). The dose-

dependent histamine release curves for the two major allergens were constructed (data not shown). Figure 4 shows the histamine release induced by both Cry j 1 and Cha o 1 in these monkeys.

Discussion

In the present study, Japanese monkeys sensitized to CJ pollen demonstrated specific IgE to CO pollen allergen. The level of IgE to CJ pollen allergen was higher than that to CO pollen allergen. These findings are similar to those reported in human patients [5,8,12,13]. We found that monkeys sensitized to CJ pollen allergens also demonstrated reactivity to CO pollen allergens.

Most human patients with anti-Cry j 1 IgE had specific IgE to Cha o 1 [12,13]. Similarly, all the monkeys in the present study that demonstrated specific IgE to Cry j 1 also demonstrated specific IgE to Cha o 1. These findings suggest that, as in human patients, Cha o 1 is an important allergen in monkeys.

In the present study, leucocytes for monkeys showed allergen-specific histamine release. In our previous study, Cry j 1-specific histamine release from leucocytes of the pollinosis monkeys was also observed. The maximum release was obtained with about 10 ng/mL of Cry j 1 [18]. This amount of Cry j 1 and Cha o 1 also induced the maximum histamine release in the present study. Furthermore, three monkeys that demonstrated IgE to CJ allergens showed positive reactions in the skin test after injection of CO pollen allergens. In the skin test, all the monkeys showed only weal. Most monkeys with CJ pollinosis showed only weal [18]. These results indicate that Cha o 1 has allergenic potency in monkeys.

As mentioned above, the level of IgE to CJ pollen was higher than that of IgE to CO pollen. Similarly, the level of IgE to Cry j 1 was higher than that to Cha o 1. In ELISA inhibition, we found that allergenic cross-reactivity appears to occur between CJ and CO major allergens and Cry j 1 showed a greater inhibition than Cha o 1. From these results, sensitization to CJ pollen allergen might be predominant in monkeys. However, we think that it is difficult to find the primary sensitizing allergen, because the pollen season for CJ and CO are overlapping.

In conclusion, the monkeys demonstrated specific IgE to the crude and purified major allergens (Cha o 1) of CO pollen. Furthermore, monkeys with pollinosis showed specific histamine release in response to CO major allergens. Based on the present findings, monkeys sensitized to Japanese cedar pollen allergens appear to demonstrate reactivity to Japanese cypress pollen allergens. There have been some reports about experimental sensitization on monkeys and other animals with CJ allergens [22–25]. In this study, Japanese monkey with CJ pollinosis symptoms similar to

those showed by human patients is a unique animal with naturally occurring pollinosis. Thus, the Japanese monkey presents a suitable animal model for the study of pollinosis associated with cedar and cypress.

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