

Prevalence of IgE antibody to crude and purified allergens of Japanese cedar pollen among different troops of Japanese monkeys (*Macaca fuscata*)

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We measured specific IgE antibodies to the crude allergen as well as two purified allergens (*Cry j* I and *Cry j* II) of Japanese cedar (*Cryptomeria japonica*—CJ) pollen in the serum of 276 Japanese monkeys in nine troops. Of 45 monkeys with CJ specific IgE in eight of nine troops, 23 (51%) were found to have IgE to both *Cry j* I and *Cry j* II, 21 (47%) only to *Cry j* I, and one (2.2%) only to *Cry j* II. The positive rate of specific IgE antibody to each allergen varied among the troops.

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Introduction

Japanese cedar (*Cryptomeria japonica*—CJ) pollinosis is one of the most common immediate-type allergic diseases in Japan [15], and the number of patients with it are increasing [5]. In our previous study, about 30% of Tokyo residents between 20 and 39 years of age had the anti-CJ IgE antibody and more than 10% of the residents might be suffering from the pollinosis [6].

The occurrence of CJ pollinosis also in Japanese monkeys has been reported [9,16]. These monkeys showed symptoms similar to those shown by human

patients such as tear production, eye redness, sneezing, and rhinorrhea. The skin test by injection of the CJ extract produced a wheal in the monkeys. The monkey serum induced a skin allergic reaction to the extract on Prausnitz-Küstner test using a normal monkey. During the pollen season, significant nasal eosinophilia was observed in the monkeys. Furthermore, levels of anti-CJ IgE assayed by the radioallergosorbent test (RAST) in the monkeys with CJ pollinosis were significantly high.

Measurement of specific IgE is a useful method

for the diagnosis of allergic diseases. Pharmacia's RAST and its more recent version, the CAP system, have been used as the standard methods to measure the specific IgE antibody [1,2]. Recently, we established an ELISA method to measure human anti-CJ IgE [10] and applied it to monkey anti-CJ IgE determination [11].

Two major allergens of CJ pollen have been isolated and characterized as *Cry j* I [14,15] and *Cry j* II [12]. Human patients could be classified into three groups according to their IgE reactivity to the major allergens: patients reacting to both allergens or only to *Cry j* I or *Cry j* II [12]. In our preliminary study, we found that monkeys with pollinosis were also classified into three IgE-reactive patterns [11].

In this study we used serum specimens taken from 276 Japanese monkeys aged five years and over at nine locations without regard to their pollinosis symptoms and compared the two assay methods: fluorometric indirect ELISA and the CAP for determination of specific IgE to CJ crude antigens. Next, we measured specific IgE to the two major allergens in the serum of the monkeys, which showed positive reactions to the crude allergens.

Materials and methods

Serum

Serum specimens were taken from 276 Japanese monkeys (*Macaca fuscata*) aged five years and over in nine troops (three open enclosure troops in zoos and six provisioned free-ranging troops; see Fig. 1) between September 1989 and March 1993. Of 276 monkeys, 182 were female, and 94 were male. The average age of the 276 monkeys was 11.1 years (S.D. = 5.7) (Table 1). Negative control sera were obtained from 40 cynomolgus monkeys (*Macaca fascicularis*) that originated from Indonesia where there is no vegetation of CJ. Fluorescence units (FU) values in sera of the control monkeys were: for *Cry j* I, mean value + 3 S.D. = 36.0, maximal value = 130; for *Cry j* II, mean value + 3 S.D. = 36.5, maximal value = 58.0. Thus, cutoff values for *Cry j* I and *Cry j* II was determined to be 131 FU and 59 FU, respectively. All serum specimens were stored at -70°C until use.

Antigen

Pollen crude extract was prepared as described in reference [15]. *Cry j* I and *Cry j* II were purified as described previously [12].

Fluorometric indirect ELISA for specific IgE

The specific IgE antibody to CJ crude antigen was assayed in all serum specimens. Then, the sera with

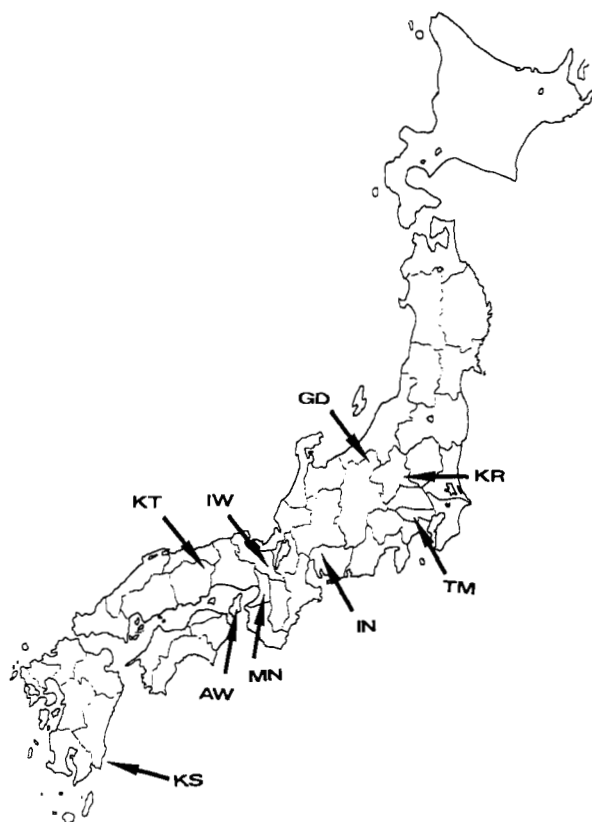


Fig. 1. Sampling location of Japanese monkey sera used in this study.

positive reactions were examined for specific IgE to *Cry j* I and *Cry j* II by fluorometric ELISA as described previously [11]. Briefly, 0.1 ml of crude protein (10 µg/ml) or purified protein (1 µg/ml) was used for coating of microplate wells. After incubation of monkey serum (diluted 1:8) with the immobilized antigens, anti-human IgE antibody conjugated with β-D-galactosidase (Phadezym kit; Pharmacia, Uppsala, Sweden) diluted 1:25 was added to each well. As enzyme reaction substrate, 0.2 mM 4-methylumbelliferyl -β-D-galactoside (Sigma Chemicals, St. Louis, MO) was added and the plates were incubated at 37°C for two hours. Then 0.1 M glycine-NaOH (pH 10.3) was added to stop the reaction. FU were measured on a fluorometric microplate reader (Fluoroskan; Flow Laboratories, McLean, VA).

The CAP system

The CAP system (Pharmacia) [1] was used according to the manufacturer's instructions. Relationship between CAP class and UA/ml was as follows: CAP class 0, <0.34 (UA/ml); class 1, 0.35–0.69; class 2, 0.7–3.49; class 3, 3.5–17.4; class 4, 17.5–49.9; class 5, 50–99.9; class 6, >100.

Table 1. Positive rates of CJ specific IgE in various troops of Japanese monkeys (>5 years old)

Troop (prefecture)	Total number	Age mean $\pm$ S.D.	IgE positives (%)	Positives (number)		
				<i>Cry j</i> I, II	<i>Cry j</i> I	<i>Cry j</i> II
KR (Gunma)	29	9.9 $\pm$ 4.2	8 (27.6)	5	2	1
AW (Hyogo)	51	9.1 $\pm$ 3.6	13 (25.5)	10	3	0
IN (Aichi)	31	11.8 $\pm$ 7.3	5 (16.1)	2	3	0
IW (Kyoto)	16	5.0 $\pm$ 7.2	5 (31.3)	1	4	0
GD (Nagano)	22	12.5 $\pm$ 5.6	2 (9.1)	0	2	0
MN (Osaka)	16	11.5 $\pm$ 5.8	1 (6.3)	0	1	0
TM (Tokyo)	20	10.6 $\pm$ 5.8	1 (5.0)	1	0	0
KS (Miyazaki)	66	11.6 $\pm$ 5.6	10 (15.1)	4	6	0
KT (Okayama)	25	11.5 $\pm$ 6.4	0 (0)	0	0	0
Total (%)	276	11.1 $\pm$ 5.7	45 (16.3)	23 (51.1)	21 (46.7)	1 (2.2)

Results

Positive rate of specific IgE antibody to CJ crude antigen

The cutoff value for specific IgE antibody to CJ crude antigen was determined to be 200 FU from the values obtained on the 40 cynomolgus monkeys serving as negative control [11]. Of 276 monkeys, 45 had anti-crude CJ IgE (16%). Table 1 shows the positive rate of anti-CJ IgE in each troop. It varied from 0% to 31%. The CAP system was performed on 31 serum specimens which were antibody positive with more than 200 FU by the indirect ELISA. Figure 2 shows the correlation between FU and CAP unit (UA). The correlation coefficient was 0.91.

Reactivity to *Cry j* I and *Cry j* II

Specific IgE to two major allergens were examined by the fluorometric ELISA. Forty-five sera with IgE antibodies to the crude antigen were used in this examination. Of these samples, 23 (51%) reacted to both *Cry j* I and *Cry j* II, 21 (47%) only to *Cry j* I, and one (2.2%) only to *Cry j* II (Table 1). There are monkeys with specific IgE antibodies to both two allergens in six troops. Three monkeys in two troops, GD and MN, reacted only to *Cry j* I.

Discussion

In this study, we found the variations of reactivity to each allergen among different troops. The positive rate of anti-crude CJ IgE ranged from 0% to

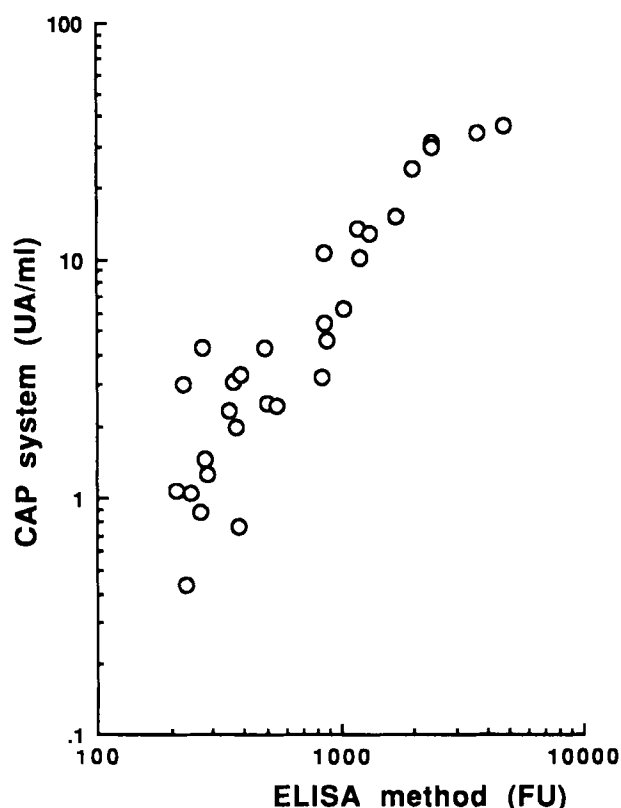


Fig. 2. Correlation between ELISA method and CAP system. Relationship between ELISA FU and CAP class was as follows. CAP class 1, 87–174 FU; class 2, 175–692 FU; class 3, 693–2,570 FU; class 4, 2,571–6,761 FU; class 5, 6,762–12,023 FU; class 6, > 12,023 FU.

31%. The habitat of the monkey troops is restricted throughout their lifetime, and their life is influenced by local environmental factors, e.g., amount of airborne CJ pollens, air pollution, food, parasite infection [3,6,9]. In human subjects, we found that positive rates of IgE to CJ pollen in young adult populations in 13 regions of Japan varied from 13% to 37% [6] and that there seemed to be a correlation between these positive rates and pollen counts in these regions. The incidence of pollinosis was suggested to be related to both airborne pollen numbers and pollution from automobile exhausts [7]. Moreover, it was reported that diesel-exhaust particulates have an enhancing activity on the anti-CJ IgE production in mouse experiments [13]. Also, nonresponsiveness to CJ pollen antigen in some human subjects with certain HLA (human leukocyte antigen) types have been reported [8]. In the future, the relationship between environmental factors and production of specific IgE in monkey troops should be investigated.

In our previous study on monkeys with pollinosis, seven monkeys with anti-crude CJ IgE had specific IgE to at least one of *Cry j* I and *Cry j* II allergens [11]. In this study, all 45 monkeys with anti-crude CJ IgE had also specific IgE to at least one of the two allergens: 23 reacted to both allergens, 21 to only *Cry j* I, and one to only *Cry j* II.

Reactivity to the two major allergens was different in each troop. Nonresponsiveness to *Cry j* II was remarkable in some troops. Distribution of their major histocompatibility complex (MHC) genes may vary in each troop, thus their immune responses to different allergens may be different in each troop [9].

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