

M. Hashimoto^a
 T. Kobayashi^a
 H. Nigi^a
 S. Saito^b
 I. Nakayama^c
 T. Narita^c
 M. Iwata^c
 H. Yasueda^d
 Y. Taniguchi^e
 M. Kurimoto^e
 S. Inouye^f
 M. Sakaguchi^f

Responses of Monkeys with Pollinosis to Two Major Allergens of Japanese Cedar Pollen

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^a Division of Wild Animal Medicine,
 Nippon Veterinary and Animal Science
 University, Tokyo.

^b Institute of Medical Science,
 Jikei University School of Medicine, Tokyo.

^c Biological Research Laboratories,
 Sankyo Co., Tokyo.

^d Clinical Research Center for Allergy and
 Rheumatology, National Sagami
 Hospital, Kanagawa.

^e Hayashibara Biochemical Laboratories,
 Okayama, and

^f Department of Epidemiology,
 National Institute of Health, Tokyo, Japan

Key Words

Allergen • Pollen • Histamine • T cell • IgE

Abstract

We examined the responses of two Japanese monkeys with pollinosis to two major allergens (Cry j 1 and Cry j 2) of Japanese cedar pollen. The two monkeys (A and B) had specific IgE antibodies to the allergens and showed a strong positive reaction to both of them in the intradermal test. In the histamine release test with peripheral blood mononuclear cells (PBMC), monkey A showed a typical pattern similar to that seen in human patients, while monkey B released a low level of histamine. The proliferative response of PBMC to both allergens in monkey A was weak, but was typical in monkey B. From clinical as well as immunological points of view, these monkeys may be a suitable animal model for Japanese cedar pollinosis in humans.

Introduction

Japanese cedar (*Cryptomeria japonica*, CJ) pollinosis is one of the most common immediate-type allergic diseases in Japan [1–3]. The number of patients with the pollinosis has recently increased [4]. Our seroepidemiological study demonstrated that about 30% of Tokyo residents between 20 and 39 years of age had anti-CJ IgE antibodies [5]. In another periodical health examination of 892 university students, the percentage of the subjects with CJ-specific IgE antibody was found to be 27% and the frequency of CJ pollinosis sufferers was 12% [6].

The occurrence of CJ pollinosis has also been reported in Japanese monkeys (*Macaca fuscata*) [7]. These monkeys

showed symptoms similar to those seen in humans such as tear production, eye redness, sneezing, and rhinorrhea. The skin test by injection of the CJ extract produced wheals in the monkeys. The monkey serum produced a skin-allergic reaction to the extract with the Prausnitz-Küstner test using a normal monkey. Furthermore, levels of anti-CJ IgE assayed by the radioallergosorbent test in the monkeys with CJ pollinosis were significantly high. We established an ELISA to measure the human anti-CJ IgE [8] and applied it to monkey anti-CJ IgE determination [9].

Two major allergens of CJ pollen have been isolated and characterized as Cry j 1 [1, 10] and Cry j 2 [11]. Human patients could be classified into three groups according to their IgE reactivity to the major allergens: patients reacting to

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Correspondence to: Dr. Masahiro Sakaguchi
 Department of Epidemiology
 National Institute of Health
 1-23-1 Toyama, Shinjuku-ku
 Tokyo 162 (Japan)

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both allergens or only to Cry j 1 or Cry j 2 [11, 12]. In our preliminary study, we also classified monkeys into three IgE-reactive patterns [9]. Furthermore, we studied the prevalence of CJ-specific IgE in the sera of 276 Japanese monkeys in nine troops, and found that 45 monkeys (16%) in eight of the nine troops had CJ-specific IgE [13].

In the present paper, we examined the in vivo and in vitro immunological responses of two pollinosis monkeys kept in an animal laboratory after being captured.

Materials and Methods

Monkeys

Two Japanese monkeys (*M. fuscata*) living on the Awaji Island, Hyogo Prefecture, Japan and observed to have pollinosis symptoms during the pollination season were captured and then kept in an animal laboratory of Sankyo, Tokyo. They showed symptoms similar to those seen in human patients such as eye redness, sneezing and rhinorrhea. In this study, they were anesthetized with ketamine hydrochloride.

Antigen

A crude extract of CJ pollen was prepared as described [1]. Two major allergens, Cry j 1 and Cry j 2, were purified as described previously [10, 11].

Assay of Specific IgE

The specific IgE antibody to major allergens was assayed by fluorometric ELISA as described previously [13]. Also, the CAP system (Pharmacia, Uppsala, Sweden) [14] was used according to the manufacturer's instructions.

Intradermal Test

CJ crude extract (1:1,000) and purified allergens (200 ng/ml) were used for the intradermal test. The injection volume of each allergen was 0.05 ml, and the reaction (wheal and erythema) was read 15 min after injection. Saline and 0.01% histamine hydrochloride solution were used as the negative and positive control, respectively. The skin test results were judged by the criteria of the human skin test [15].

Histamine Release Assay

The histamine release assay with peripheral blood mononuclear cells (PBMC) was carried out as previously described [12, 16]. Fresh heparinized venous blood was incubated with 1% dextran (T 2000; Pharmacia) solution at room temperature for 1 h and the supernatants were centrifuged. Then, the pellet was resuspended and these cells were counted as PBMC and used for the histamine release test. The PBMC were incubated with various concentrations of the allergen preparations at 37°C for 45 min. The released histamine was measured by a radioimmunoassay kit with high sensitivity (Immunotech, France) [17]. This kit was used because Japanese monkeys have a smaller number of circulating basophils than humans. The specific histamine release was expressed as percentage of the total histamine content.

Table 1. Serum IgE antibody activity to CJ allergens

Monkey	Sex	Age	Specific IgE	fluorometric ELISA ^a	
				Cry j 1	Cry j 2
A	m	19	13.6 (3)	2,450	2,440
B	f	15	9.3 (3)	4,340	140

Figures in parentheses represent score.

^a U/ml.

^b The specific IgE were expressed as arbitrary units/ml.

Proliferative Response to Allergens

PBMC (5×10^5) suspended in RPMI 1640 complete medium containing 5% human serum were cultured in 96-well plates in the presence of Cry j 1 or Cry j 2 (2.5 µg/ml), then incubated for 48 h at 37°C, 5% CO₂, humidified atmosphere. After pulsing for 16 h with 1 µCi/well of [³H]TdR, the cells were harvested and the incorporated radioactivity was measured on a scintillation counter.

Results

IgE Antibody to CJ Pollen

Specific IgE to CJ allergens was examined by both the CAP and the fluorometric ELISA (table 1). The two monkeys had IgE to crude CJ antigen and to both Cry j 1 and Cry j 2 (table 1).

Intradermal Test with CJ Allergens

Two monkeys had allergic reactions to both Cry j 1 and Cry j 2 in the skin test. The negative control solution (saline) elicited small wheals (<5 mm) but no erythema. These antigens produced only wheals (10×11 mm to Cry j 1; 13×14 mm to Cry j 2; 11×11 mm to crude extract) in monkey A, but caused both wheals (13×15 mm to Cry j 1; 11×12 mm to Cry j 2; 14×15 mm to crude extract) and erythema in monkey B (fig. 1). Similarly, the positive control (histamine) produced only wheals in monkey A and both wheals and erythema in monkey B.

Allergen-Specific Histamine Release

The histamine release assay was carried out with PBMC from monkeys. Figure 2 shows the dose-response curve of the histamine release assay in monkey A. Dose-dependent histamine release curves were induced by the two major allergens and the maximum release was obtained with about 10 ng/ml of Cry j 1 and Cry j 2. With 100 ng/ml of Cry j 1 and Cry j 2, the allergen-specific histamine release was suppressed. In monkey B, the level of allergen-specific histamine

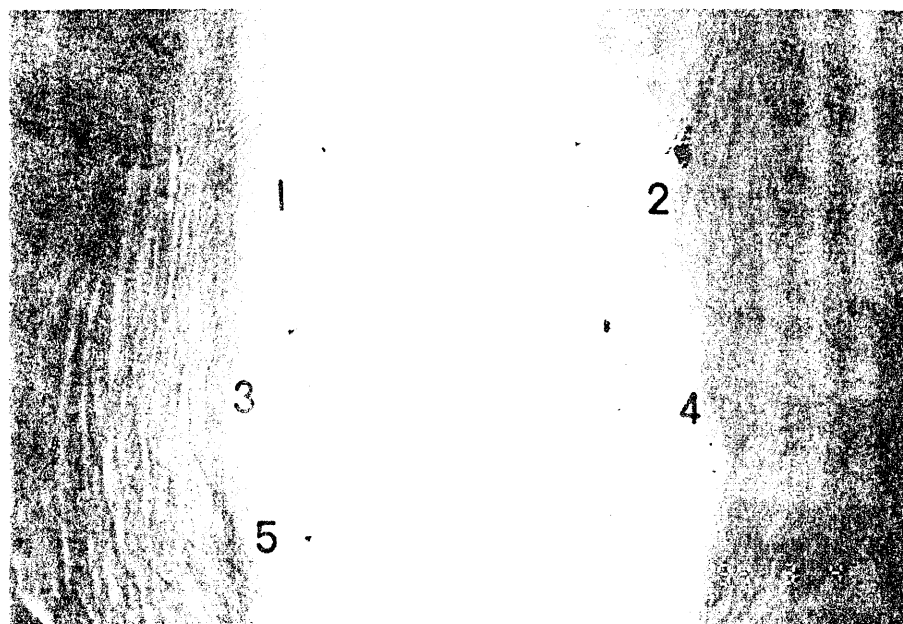


Fig. 1. Skin test reaction in monkey A. 1 - Saline; 2 - histamine; 3 - Cry j 1; 4 - Cry j 2; 5 - crude CJ extract.

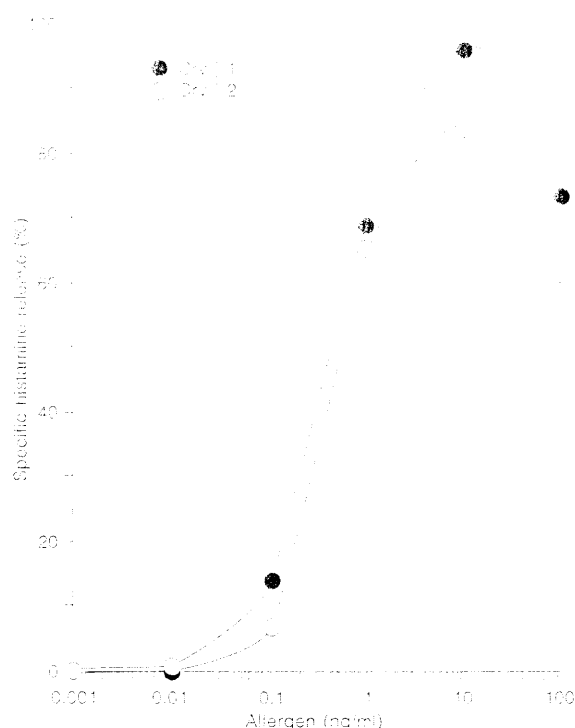


Fig. 2. Allergen-specific histamine release from PBMC of monkey A.

mine release was low [data not shown]. As a negative control, two monkeys without anti-CJ IgE antibody showed no allergen-specific histamine release [data not shown].

Proliferative Response to the Two Allergens

A proliferative response to both allergens was detected in the PBMC from the two monkeys (fig. 3). Monkey B showed a strong positive response to both allergens, but monkey A showed a weak positive response. As a negative control, two monkeys without anti-CJ IgE antibody showed no response to these allergens [data not shown].

Discussion

In our previous study, all 145 human pollinosis patients with anti-crude CJ IgE had specific IgE to at least one of the two purified allergens and about 90% of the patients had specific IgE to both allergens [12]. Also, in another study, we measured the allergen-specific IgE in serum specimens taken from 276 Japanese monkeys 5 years of age and older at nine locations without regard to their pollinosis symptoms, and we found that all 45 monkeys with anti-CJ IgE also had specific IgE to at least one of the two allergens and 23 reacted to both allergens [13]. These findings suggested that, like humans, Cry j 1 and Cry j 2 are important allergens in monkeys. In this study, we further examined the immunological responses of two monkeys with pollinosis that were transferred to an animal laboratory.

The monkeys showed positive reactions in the skin test after injection of CJ allergens. In the skin test, monkey A showed only wheals. Most monkeys with CJ pollinosis showed only wheals [data not shown]. Monkey B showed erythema, which was a relatively rare case. The intradermal test is more difficult to perform in monkeys than in human beings because monkeys must be subjected to anesthesia.

In this study, PBMC from monkeys showed an allergen-specific histamine release pattern similar to that shown by PBMC of human patients [12, 16]. The same amount of Cry j 1 and Cry j 2 induced the maximum histamine release, indicating the similarity of the allergenic potency of the two allergens. Furthermore, the histamine release was suppressed by excessive amounts of the allergens, the same finding as we obtained with human patients [12].

Monkeys showed a significant proliferation of PBMC in response to the allergens. One monkey showed a strong positive response to both allergens. This monkey may be useful for the study of T cell response to CJ allergens (e.g. T cell epitopes, T cell-derived cytokines). To our knowledge, this is the first report of a proliferative response of PBMC or T cell to an allergen in allergic monkeys, and is consistent with our recent study of human patients with CJ pollinosis [18].

In summary, these monkeys with CJ pollinosis had symptoms and immunological responses to the major allergens similar to those seen in human patients. CJ allergy in monkeys appears to be homologous to the human allergy. We believe that these monkeys with CJ pollinosis are a most suitable animal model for developing antiallergy therapies, such as desensitization treatment.

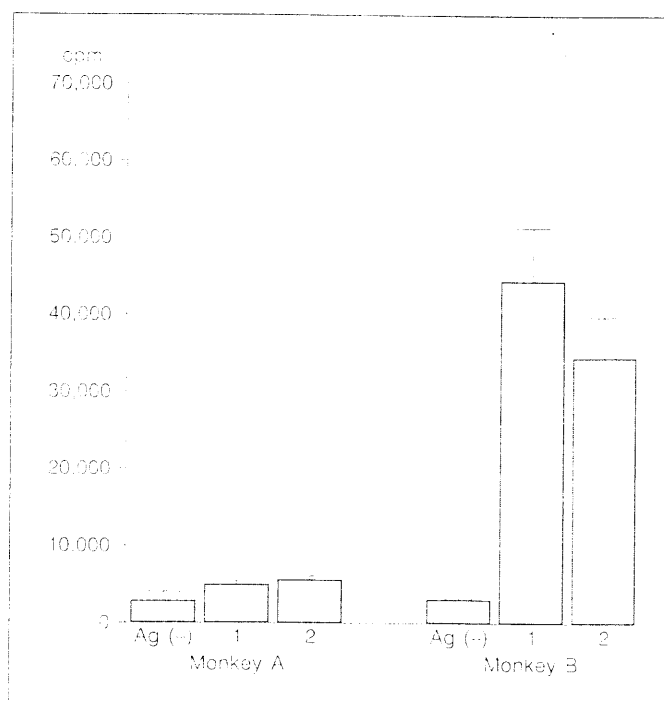


Fig. 3. Proliferative response of PBMC to Cry j 1 (1) and Cry j 2 (2).

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