

Parasite infection and Japanese cedar pollinosis in monkeys

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Abstract

To evaluate the relationship between helminthic parasite infection and sensitization to Japanese cedar (*Cryptomeria japonica*; CJ) pollen allergens in Japanese monkeys (*Macaca fuscata*), we examined the parasite infection, presence of anti-pollen allergen IgE and development of pollinosis. Serum samples and fecal specimens were taken from 169 monkeys in five troops, and the presence of IgE antibody for CJ pollen allergen and of helminthic parasite eggs in their feces was examined. Of the 169 monkeys, helminthic parasite eggs from 138 monkeys were detected. The frequency of anti-CJ IgE (19%) and the level of total IgE did not differ significantly between the monkeys with and without parasite eggs. We examined the presence of anti-CJ IgE and pollinosis symptoms in 31 monkeys of a troop; six of the monkeys demonstrated anti-CJ IgE and pollinosis symptoms. Five of these six monkeys had parasite eggs. We found that the monkeys that demonstrated anti-CJ IgE and pollinosis symptoms had helminthic parasite infections. These data suggest that helminthic parasite infection does not reduce the development of clinical signs of CJ pollinosis in Japanese monkeys. © 1999 Elsevier Science B.V. All rights reserved.

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Abbreviations: CJ, *Cryptomeria japonica*

1. Introduction

Japanese cedar (*Cryptomeria japonica*: CJ) pollinosis is one of the most common allergic diseases in Japan (Ishizaki et al., 1987). Our previous seroepidemiological study showed that approximately 30% of the general population between 20 and 39 years of age who resided in the Tokyo area had anti-CJ IgE antibody (Inouye et al., 1986). In another study of 892 university students, the percentage of carriers of CJ-specific IgE was found to be 27%, and the frequency of CJ pollinosis sufferers was 12% (Nakamura, 1990).

The natural occurrence of CJ pollinosis has also been observed in Japanese monkeys (*Macaca fuscata*) (Yokota et al., 1987). These monkeys with pollinosis show symptoms similar to those of human patients; tear production, eye redness, sneezing, and rhinorrhea. A CJ extract injection into the skin produces a wheal in these monkeys, and monkey serum induces a skin allergic reaction to the extract in the Prausnitz-Küstner test. Furthermore, monkeys with CJ pollinosis had anti-CJ IgE (Sakaguchi et al., 1992). Japanese monkeys with CJ pollinosis had reactivity to major CJ allergens (Sakaguchi et al., 1997). In addition, leukocytes from these monkeys showed CJ pollen allergen-specific histamine release, as well as significant proliferation in response to CJ pollen allergens (Hashimoto et al., 1997).

Godfrey (1975) reported a low prevalence of asthma in a rural population in Gambia despite elevated IgE levels indicative of parasite infection. Similar observations have been reported elsewhere (Carswell et al., 1976; Woolcock et al., 1989). In contrast, bronchial asthma tends to be more wide-spread in areas where parasite infection is endemic (Master and Barrett-Connor, 1985). Other reports have suggested that parasite infection enhances the IgE antibody response to common environmental allergens (Alshishtawy et al., 1991; Di Prisco et al., 1993). There are two conflicting hypotheses: parasite infection protects against subsequent allergic diseases, and parasite infection predisposes a host to allergic diseases (Master and Barrett-Connor, 1985; Bell, 1996).

In Japan, sophisticated water and sewage systems have prevented widespread helminthic infection in humans. However, in Japanese monkeys, the prevalence of parasite infections is still very high (Hayama and Nigi, 1963). We previously detected *Oesophagostomum* sp., *Streptopharagus* sp., *Tricocephalus* sp., and *Strongyloides* sp. in 317 Japanese monkeys of seven troops, and we determined that approximately 85% of the monkeys were infected with one or more species of intestinal parasite (Tanaka and Nigi, 1967; Nigi and Tanaka, 1973).

In the present study, to evaluate the relationship between parasite infection and the sensitization of pollen allergens in Japanese monkeys, we examined the presence of anti-pollen allergen IgE and parasite eggs, and the development of pollinosis symptoms in monkeys.

2. Material and methods

2.1. Monkeys

The study was carried out in February, March and April (Japanese pollen season) from 1984 to 1994. The animals examined were 169 Japanese monkeys (*Macaca fuscata*) from

five provisioned free-ranging troops (31 from Awajishima [Hyogo Prefecture], 19 from Gigokudani [Nagano Prefecture], 16 from Iwatayama [Kyoto Prefecture], 25 from Koshima [Miyazaki Prefecture], 78 from Takasakiyama [Ohita Prefecture]). They were captured individually for the collection of serum and fecal samples. A mixture of ketamine HCl (Kettaral, Sankyo, Tokyo, Japan) at about 5 mg/kg body weight and xylazine HCl (Celactar, Bayer, Tokyo, Japan) at about 1 mg/kg body weight was administered intramuscularly by blowgun to each monkey, based on a rough estimation of its body weight. After being examined, each monkey was administered 4 mg/kg of torazoline HCl (T-6886, Sigma Chemicals, St. Louis, MO) intravenously or intramuscularly as an antagonist to the xylazine HCl, and the awakened monkey was then released.

Sera were also obtained from 20 wild cynomolgus monkeys (*M. fascicularis*) originating from Indonesia. It was considered that most of these monkeys had suffered from helminthic parasite infection. Sera were also obtained from 20 cynomolgus monkeys that were born and had bred at the Tsukuba Primate Center for Medical Science, National Institute of Infectious Diseases, Tsukuba, Japan. Few of these monkeys had helminthic parasite infections.

2.2. *Observation of CJ pollinosis in monkeys*

The 31 monkeys of the Awajishima troop were examined for the presence of anti-CJ IgE and CJ pollinosis. The troop has been fed artificially since 1967. When monkeys came feeding place, we checked them for the presence of CJ pollinosis symptoms (tear production, eye redness, sneezing, and rhinorrhea) throughout CJ pollen season.

2.3. *Detection of parasite egg*

Fecal specimens were collected from the recta of captured monkeys and fixed with 10% formalin solution. Egg examinations for helminthic parasites were conducted by medical general laboratory (MGL) method (Ritchie, 1948; Faust et al., 1970). The species of helminths were determined by microscopic examination.

2.4. *Measurement of anti-CJ IgE*

Specific IgE antibodies for Japanese cedar pollen were analyzed by a capsulated hydrophilic carrier polymer (CAP) assay (Pharmacia, Uppsala, Sweden) (Hashimoto et al., 1994). After collection, all serum specimens were stored at -70°C until use.

2.5. *Measurement of total IgE*

Total IgE antibodies in sera of monkeys were measured by CAP assay (Pharmacia). Established monkey IgE antibody as a reference is not available, and the levels of total IgE in monkeys are thus expressed as human IgE equivalent international unit (IU)/ml.

Table 1
Parasite infection in five troops of Japanese monkeys

Troop	Prefecture	No. of monkeys	Sex		Age (years)	Parasite infection (%)
			Female	Male	Mean $\pm$ SD	
Awajishima	Hyogo	31	17	14	9.5 $\pm$ 4.2	23 (74%)
Gigokudani	Nagano	19	18	1	11.9 $\pm$ 5.9	14 (74%)
Iwatayama	Kyoto	16	16	0	15.0 $\pm$ 7.2	11 (69%)
Koshima	Miyazaki	25	12	13	11.6 $\pm$ 5.6	23 (92%)
Takasakiyama	Ohita	78	54	24	— ^a	67 (86%)
Total		169	117	52	11.0 $\pm$ 5.6 ^b	138 (82%)

^aData not available.
^bData excluding monkeys in the Takasakiyama troop.

3. Results

3.1. Relationship between the presence of parasite eggs and anti-CJ IgE

Of the 169 monkeys, parasite eggs from 138 monkeys (82%) were detected (Table 1). The rates of the parasite infection among the five troops varied from 69 to 92%. Four species of parasite eggs were detected in the feces: *Tricocephalus* sp., *Strongyloides* sp., *Oesophagostomum* sp., and *Streptopharagus* sp., in 82, 42, 33 and 28% of the 138 monkeys, respectively. There was no correlation between the parasite infection and the age or sex of the monkeys (data not shown).

The CAP assay was performed on 169 serum specimens from the monkeys to detect anti-CJ IgE. Of the 138 monkeys with parasite eggs, 26 demonstrated anti-CJ IgE (19%) (Table 2). The same anti-CJ IgE rate (19%) was observed in the 31 monkeys without parasite eggs. There were thus no significant difference in the positive rate of anti-CJ IgE between the groups with and without parasite eggs.

3.2. Relationship between the presence of parasite eggs and development of pollinosis symptoms

We examined the presence of anti-CJ IgE and symptoms in the monkeys of the Awajishima troop. Of the 31 monkeys, nine demonstrated anti-CJ IgE (Table 3). Six (no.1–6) of the nine monkeys showed pollinosis symptoms during the pollination season.

Table 2
Positive rates of anti-CJ IgE in the monkeys with and without parasite eggs

	No. of monkeys	Anti-CJ IgE	
		Positive (%)	Negative (%)
Parasite egg (+)	138	26 (19%)	112 (81%)
Parasite egg (–)	31	6 (19%)	25 (81%)
Total	169	32 (19%)	137 (81%)

Table 3

Presence of CJ pollinosis and parasite egg in nine monkeys with anti-CJ IgE

Monkey	Sex	Age (Years)	Pollinosis symptom	Parasite egg	Anti-CJ IgE (Ua/ml)
1	M	9	+	+	15.0
2	M	15	+	+	14.0
3	F	6	+	+	3.8
4	F	7	+	+	3.2
5	F	9	+	+	0.8
6	M	12	+	–	6.3
7	M	7	–	+	1.0
8	F	15	–	+	1.1
9	M	12	–	–	1.6

Of these six monkeys, five (no. 1–5) had parasite eggs. Of the three other anti-CJ IgE-positive monkeys (no. 7–9) without pollinosis, two (no. 7, 8) had parasite eggs.

3.3. Relation between total IgE and the presence of anti-CJ IgE

The geometric mean levels of total IgE equivalent in the Japanese monkeys with and without parasite eggs were 735 and 604 IU/ml, respectively (Fig. 1). There was no significant difference between these two groups. The geometric mean levels of total IgE equivalent in the wild cynomolgus monkeys from Indonesia and those bred in the laboratory were 560 and 153 IU/ml, respectively. There were significant differences between the cynomolgus monkeys bred in the laboratory and other three wild groups ($p < 0.001$).

4. Discussion

Helminthic parasites have been believed to modulate the development of allergic diseases (Bell, 1996). Turton (1976) reported that a self-infection of *Necator americanus* cured his seasonal hay fever. This was attributed to the ability of hookworms to increase specific and total IgE levels, thus preventing mast cell sensitization by allergen-specific IgE (Ogilvie et al., 1978). This blocking theory is supported by the observation that parasite infections appear to raise total IgE levels disproportionately to parasite-specific IgE (Jarrett and Bazin, 1974; Turner et al., 1979). However, due to the very low incidence of helminthic infection in Japanese people, it is difficult to study the relationship between parasite infection and the development of CJ pollinosis in Japanese patients.

In Japanese monkeys, naturally occurring CJ pollinosis has been detected (Yokota et al., 1987; Sakaguchi et al., 1992). These monkeys demonstrate anti-CJ IgE as well as symptoms similar to those of human patients. We reported the prevalence of anti-CJ IgE among nine troops of Japanese monkeys (Hashimoto et al., 1994). Of the 276 monkeys, 45 (16%) had anti-CJ IgE. In Japanese monkeys, the prevalence of the parasite infection is very high (Nigi and Tanaka, 1973). These monkeys thus appear to be a suitable animal

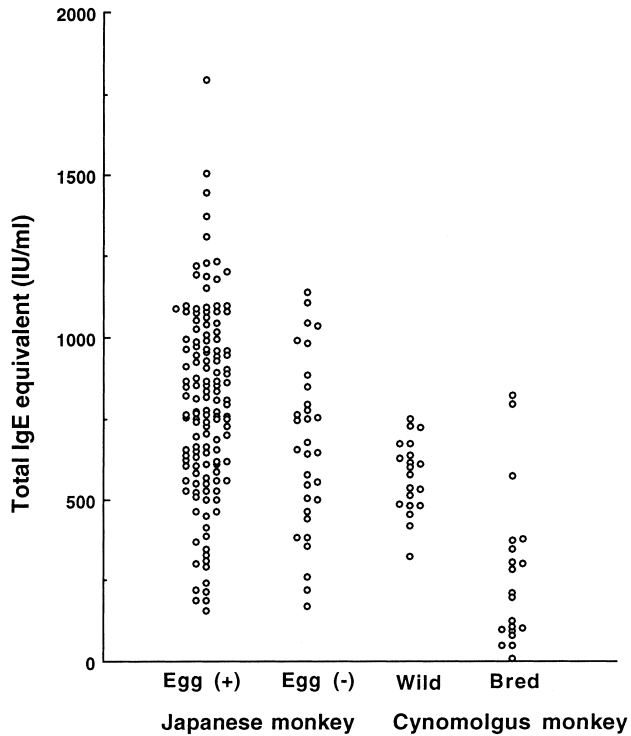


Fig. 1. The levels of total IgE equivalent in sera of monkeys.

model for evaluating the relationship between parasite infection and the development of allergic disease.

In the present study, 138 of 169 monkeys in five troops were infected with parasites. The rate of parasite infection (82%) is similar to those reported earlier (Nigi and Tanaka, 1973). We measured anti-CJ IgE in sera of the 169 monkeys. The positive rate of anti-CJ IgE (19%) did not differ between the monkeys with and without parasite eggs. Parasite infection thus does not appear to have an effect on the sensitization of CJ allergen in the monkeys.

There was no significant difference between the levels of total IgE equivalent in the Japanese monkeys with or without parasite eggs. Cynomolgus monkeys that were born and had bred in our institute and suffered little from helminthic parasite infection showed low levels of total IgE. These findings suggest that the Japanese monkeys without parasite eggs may have been infected with helminthic parasite infection before the egg examination. The high level of total IgE in the Japanese monkeys also reconfirmed that the prevalence of helminthic parasite infections is very high among Japanese monkeys.

Of the 31 monkeys from one troop examined for the presence of CJ pollinosis during the CJ pollen season, six demonstrated anti-CJ IgE and pollinosis symptoms. Of these six monkeys, five monkeys were infected by parasites, which indicates that parasite infection might not suppress the development of CJ pollinosis symptoms in Japanese monkeys.

Based on the present results, we think that parasite infections does not prevent the development of CJ pollinosis in Japanese monkeys.

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