

IgE-reactivity to major Japanese cedar (*Cryptomeria japonica*) pollen allergens (Cry j 1 and Cry j 2) by ELISA in dogs with atopic dermatitis

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Abstract

The present study investigated IgE-reactivity to two major Japanese cedar (*Cryptomeria japonica*, *C. japonica*) pollen allergens (Cry j 1 and Cry j 2) in dogs with atopic dermatitis by use of a fluorometric ELISA. The serum samples from 27 dogs that showed IgE-sensitivity to crude *C. japonica* pollen allergen by ELISA were tested for specific IgE to the two major allergens. All 27 dogs had anti-Cry j 1 IgE, and 10 (37%) had anti-Cry j 2 IgE. Inhibition of binding of dog specific IgE to crude *C. japonica* pollen allergen was carried out by addition of Cry j 1. When serum samples containing anti-Cry j 1 IgE but no anti-Cry j 2 IgE were incubated with Cry j 1, specific IgE

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binding to crude *C. japonica* pollen allergen was almost abolished. These findings suggest that Cry j 1 is a major allergen in dogs. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Canine atopic dermatitis is a common disease in veterinary dermatology (Halliwell and Schwartzmann, 1971; Nesbitt et al., 1984; Willemse, 1986). Various clinical signs have been reported, including pruritus, otitis externa, bilateral conjunctivitis, and recurrent and chronic dermatitis (Willemse, 1986).

Japanese cedar (*Cryptomeria japonica*, *C. japonica*) pollinosis has been reported in dogs of Japan (Sasaki et al., 1995). These dogs sensitive to *C. japonica* pollen showed clinical symptoms of atopic dermatitis and positive reaction to *C. japonica* pollen allergen in Prausnitz–Küstner test. Our recent epidemiological study reported that $\approx 10\%$ of atopic dogs had specific IgE to crude *C. japonica* pollen allergen (Hasegawa et al., 1996). These findings suggested that *C. japonica* pollen allergen might be associated with pathogenesis of atopic dermatitis in dogs. In humans, *C. japonica* pollinosis is one of the most common allergic diseases in Japan (Ishizaki et al., 1987). In a study of a periodic health examination for a large number of students in a university, the frequencies of specific IgE to crude *C. japonica* pollen allergen and clinical *C. japonica* pollinosis were 27 and 12%, respectively (Nakamura, 1990).

Two major allergens have been isolated from *C. japonica* pollen and characterized as Cry j 1 (Yasueda et al., 1983) and Cry j 2 (Sakaguchi et al., 1990). We previously showed that humans (Sakaguchi et al., 1990; Hashimoto et al., 1995) as well as Japanese monkeys (*Macaca fuscata*) (Sakaguchi et al., 1992; Hashimoto et al., 1994) had specific IgE to Cry j 1 and Cry j 2. However, specific IgE to these major allergens have not been examined in dogs.

The present study was carried out to identify IgE-reactivity to Cry j 1 and Cry j 2 by the ELISA in atopic dogs sensitive to crude *C. japonica* pollen allergen.

2. Materials and methods

2.1. Crude and major *C. japonica* pollen allergens

Crude *C. japonica* pollen allergen was prepared as described previously (Yasueda et al., 1983). Cry j 1 and Cry j 2 as major allergens were purified as described previously (Sakaguchi et al., 1990).

2.2. Dogs

Based on Willemse's criteria for clinical diagnosis of canine atopic dermatitis (Willemse, 1986), atopic dermatitis was diagnosed. Twenty seven dogs showed IgE-

sensitivity to crude *C. japonica* pollen allergen by a fluorometric ELISA. Of serum samples from 27 dogs, nine were supplied from the Veterinary Medical Center, the University of Tokyo and 18 from veterinary practitioners in Japan. These dogs consisted of 18 cases of female and nine cases of male, and were 1–15 years old (age $\pm$ S.D., 6 years 9 months $\pm$ 3 years 4 months). All the dogs showed typical clinical signs of atopic dermatitis, including pruritus, recurrent and chronic dermatitis, otitis externa, and surface staphylococcal pyoderma. Cases of dermatomycosis and ectoparasite infection were excluded.

The sera used as a negative control in the pollen-specific IgE assay were obtained from 10 dogs in the USA where there is no growth of *C. japonica*.

2.3. Fluorometric ELISA for specific IgE to crude and major *C. japonica* pollen allergens

Specific IgE to crude and major *C. japonica* pollen allergens were assayed by a fluorometric ELISA with a slight modification as previously described (Sakaguchi et al., 1997). A microplate (Immulon 2, Dynatech, Chantilly, VA) was coated with crude *C. japonica* pollen allergen (10 μ g/ml) or purified allergens, Cry j 1 or Cry j 2 allergens (1 μ g/ml), at 4°C overnight. After washing, the plate was incubated with several concentrations of serum samples at room temperature for 3 h. The plate was then washed, and incubated with a mouse monoclonal anti-dog IgE antibody (De Boer et al., 1993) (0.5 μ g/ml) was then added. The plate was incubated at 4°C overnight. After washing, the plate was then incubated with biotinylated rat monoclonal anti-mouse IgG₁ (Zymed Laboratories, San Francisco, CA) at room temperature for 1 h. After washing, the plate was incubated with β -D-galactosidase conjugated streptavidin (Zymed) at room temperature for 1 h. After final washing, the plate was incubated with 0.1 mM 4-methylumbelliferyl- β -D-galactoside (Sigma, St. Louis, MO, USA) at 37°C for 2 h. The enzyme reaction was stopped with 0.1 M glycine–NaOH (pH 10.2), and the fluorescence intensity was read as fluorescence units (FU) on a microplate fluorescence reader (Fluoroskan, Flow Laboratories, McLean, VA). The level of specific IgE to each *C. japonica* pollen allergen was calculated from a standard titration curve of a reference standard (pooled serum) and was expressed in arbitrary units (U/ml).

2.4. Inhibition of dog specific IgE to crude *C. japonica* pollen allergen by addition with Cry j 1

To examine what proportion of anti-Cry j 1 IgE occupies whole anti-*C. japonica* pollen allergen IgE in dogs, ELISA inhibition for specific IgE to crude *C. japonica* pollen allergen was carried out by addition with Cry j 1. Crude *C. japonica* pollen allergen (2 μ g/ml) were coated to microplate wells. The dog serum samples were incubated with crude *C. japonica* pollen allergen (100 μ g/ml), Cry j 1 allergen (10 μ g/ml), or crude *Dematophagoies farinae* mite allergen (100 μ g/ml) (Yasueda et al., 1994). After 3-h-incubation at room temperature, the plate was incubated with the anti-dog IgE antibody (0.5 μ g/ml). The following procedures were the same as those of the above-described fluorometric ELISA. The percentage of inhibition was calculated

as follows:

$$\left(1 - \frac{\text{FU in presence of allergen}}{\text{FU in absence of allergen}}\right) \times 100 = \text{percentage of inhibition}$$

2.5. Cross-reactivity between Cry j 1 and Cry j 2

The allergenic cross-reactivity between Cry j 1 and Cry j 2 was analyzed by a fluorometric ELISA inhibition (Sakaguchi et al., 1990). Each Cry j 1 or Cry j 2 allergen (0.5 µg/ml) was coated to microplate wells at 4°C overnight. The serum samples mixed with Cry j 1 or Cry j 2 (10 µg/ml) as an inhibitor were added to the microplate wells coated with the Cry j 1 or Cry j 2. After 3-h-incubation at room temperature, the anti-dog IgE antibody (0.5 µg/ml) was added. The following procedures were the same as those in the above-described fluorometric ELISA.

3. Results

3.1. IgE-reactivity to crude *C. japonica* pollen allergen in atopic dogs

Specific IgE to crude *C. japonica* pollen allergen was measured in the sera of the 27 atopic dogs by the ELISA (Fig. 1). Of the 27 dogs, all had specific IgE to crude *C.*

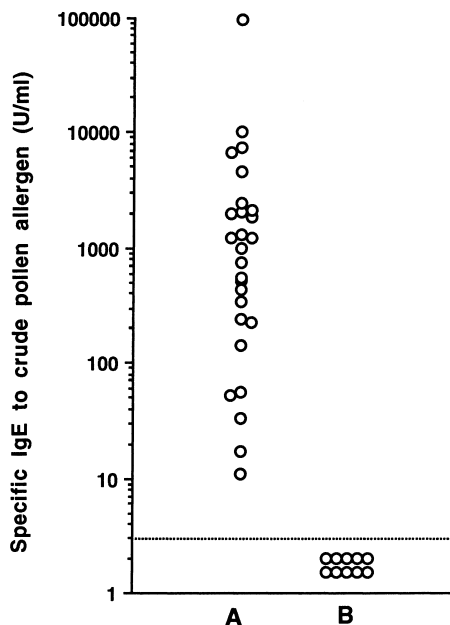
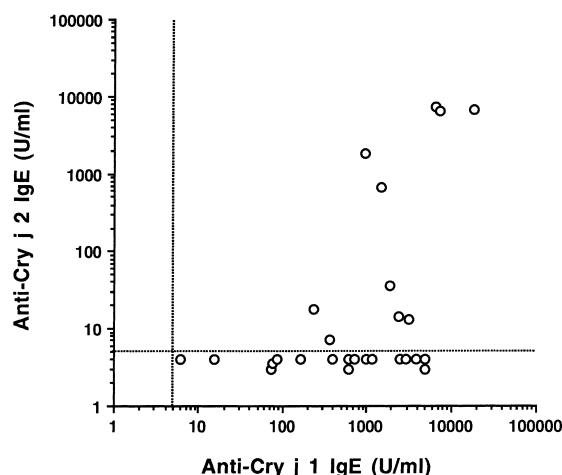


Fig. 1. IgE-reactivity to crude *C. japonica* pollen allergen in dogs. (A) Atopic dogs sensitive with crude *C. japonica* pollen allergen, (B) American dogs as a negative control.



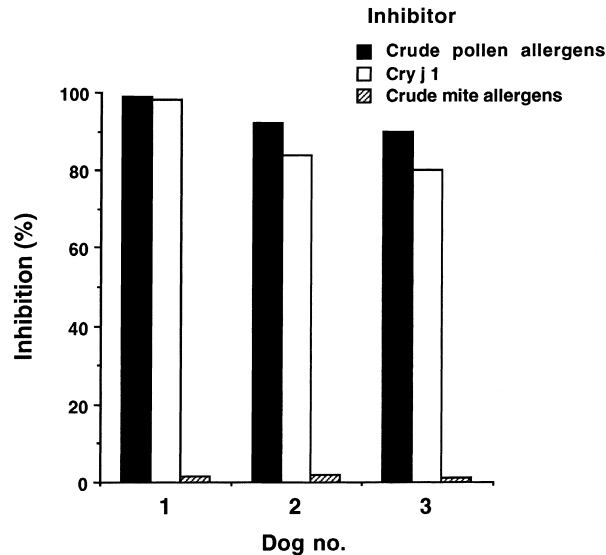


Fig. 3. Inhibition of dog specific IgE to crude *C. japonica* pollen allergen by addition with Cry j 1. Serum samples from three dogs (nos. 1–3) having anti-Cry j 1 IgE were mixed with crude *C. japonica* allergen, Cry j 1, or crude mite allergen. Three dogs (nos. 1–3) have anti-Cry j 1 IgE but no anti-Cry j 2 IgE.

Cry j 1, anti-Cry j 1 IgE was almost completely abolished, whereas anti-Cry j 2 IgE was not inhibited. Similarly when the pooled serum was incubated with Cry j 2, anti-Cry j 1 IgE was not inhibited.

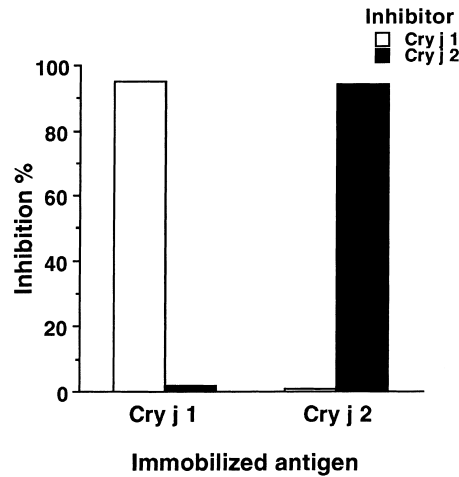


Fig. 4. Cross-reactivity between Cry j 1 and Cry j 2 by the ELISA inhibition using sera of atopic dogs. Pooled serum was collected from three dogs having specific IgE to both Cry j 1 and Cry j 2.

4. Discussion

In this study, 27 atopic dogs were shown to have specific IgE to crude *C. japonica* pollen allergen by the ELISA. Of these 27 dogs, all had anti-Cry j 1 IgE and only 10 (37%) had anti-Cry j 2 IgE, indicating that Cry j 1 is a major allergen in these dogs. We previously measured specific IgE to Cry j 1 and Cry j 2 in the sera of 145 human patients with *C. japonica* pollinosis and found that >90% of the patients had specific IgE to both allergens, while the remainder had specific IgE to either Cry j 1 or Cry j 2 (Hashimoto et al., 1995). We also examined specific IgE to Cry j 1 and Cry j 2 in Japanese monkeys with *C. japonica* pollinosis which showed symptoms similar to these of human patients (Hashimoto et al., 1994). Of the 45 Japanese monkeys sensitive to crude *C. japonica* pollen allergen, 23 (51%) were found to have specific IgE to both Cry j 1 and Cry j 2, 21 (47%) only to Cry j 1, and one (2.2%) only to Cry j 2. From these findings, Cry j 1 can be considered as a major allergen in three species including the human, Japanese monkey, and dog. However, the frequency of specific IgE to Cry j 2 seems to be variable among the three species. Anti-Cry j 2 IgE frequency in dogs is lower than other species. Some HLA class II molecules associated with purified allergens have been reported (Blumenthal, 1998). We think that a few dogs might have major histocompatibility complex (MHC) molecules associated with Cry j 2.

In this study, when dogs' sera with only anti-Cry j 1 IgE were incubated with Cry j 1, specific IgE-reactivity to crude *C. japonica* pollen allergen was markedly reduced by Cry j 1. Of course, when the sera was incubated with unrelated mite allergen as a negative control, the specific IgE-reactivity to crude *C. japonica* pollen allergen did not change. From these data, we found that most specific IgE to *C. japonica* pollen allergen in these dogs are anti-Cry j 1 IgE.

In this study, we confirmed that there is also no cross-reactivity between Cry j 1 and Cry j 2 in dogs. We found similar findings that there is no cross-reactivity between Cry j 1 and Cry j 2 in human patients (Sakaguchi et al., 1990; Hashimoto et al., 1995). We confirmed that Cry j 1 and Cry j 2 show different allergenicities in dogs.

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