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Short communication

IgE reactivity to a Cry j 3, an allergen of Japanese cedar (*Cryptomeria japonica*) pollen in dogs with canine atopic dermatitis

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ABSTRACT

The present study investigated IgE reactivity to a new *Cryptomeria japonica* pollen allergen (Cry j 3) in dogs with atopic dermatitis by using a fluorometric ELISA. Serum samples from 15 dogs that showed IgE sensitivity to crude *C. japonica* pollen allergen by ELISA were tested for specific IgE to each allergen, individually. All 15 dogs had anti-Cry j 1 IgE, 6 (40%) had anti-Cry j 2 IgE, and 11 (73%) had anti-Cry j 3 IgE. Further, we found that these anti-Cry j 3 IgE reacted to Cry j 3 with immunoblotting analysis. These findings indicate that Cry j 3 may be a major allergen in dogs.

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1. Introduction

Japanese cedar (*Cryptomeria japonica*) pollinosis is one of the most common immediate-type allergic diseases in Japan (Yasueda et al., 1983), which has been also reported in dogs (Sasaki et al., 1995) as well as in humans. The dogs sensitive to *C. japonica* pollen showed clinical symptoms of canine atopic dermatitis (CAD) and positive immune reactions to the pollen allergen in Prausnitz–Küstner test (Rupa et al., 2008). CAD is a common dermatitis in dogs and defined as an inflammatory and pruritic skin disease with IgE antibodies, most commonly directed against environmental allergens (Halliwell, 2006). Our epidemiological study in Japan reported that approximately 20% of atopic dogs had specific IgE to crude

C. japonica pollen allergen (Masuda et al., 2000a). These findings suggested that *C. japonica* pollen allergen might be associated with the pathogenesis of CAD.

Two major allergens have been previously isolated from *C. japonica* pollen and characterized as Cry j 1 (Yasueda et al., 1983) and Cry j 2 (Sakaguchi et al., 1990). Cry j 1 was identified as a 41–46 kDa allergen with pectate lyase enzyme activity (Yasueda et al., 1983; Taniguchi et al., 1995), while Cry j 2 was identified as a 45 kDa allergen with polymethylgalacturonase enzyme activity (Sakaguchi et al., 1990; Ohtsuki et al., 1995). We previously showed that humans (Sakaguchi et al., 1990; Hashimoto et al., 1995), Japanese monkeys (*Macaca fuscata*) (Sakaguchi et al., 1992; Hashimoto et al., 1994), and dogs (Masuda et al., 2000b) had specific IgE to Cry j 1 and Cry j 2.

Of the 100 human patients with *C. japonica* pollinosis, 27 serum samples contained IgE against the recently isolated Cry j 3, suggesting that Cry j 3 is an important allergen in some patients (Fujimura et al., 2007). Further, we

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found that Cry j 3 is 27 kDa protein and has relatively high homology with thaumatin-like protein categorized in the pathogenesis-related-5 (PR-5) family protein which may act as causative allergens of oral allergy syndrome (OAS) in human patients (Fujimura et al., 2007). OAS is a condition characterized by IgE-mediated immediate allergic symptoms restricted to the oral mucosa, which may involve itching, and vascular edema of the lips, tongue, and pharynx with a sudden onset (Kondo and Urisu, 2009). OAS symptoms occur in association with IgE cross-reactivity arising from structural homologies between pollen allergens and food proteins. A typical manifestation of OAS would be the oral mucosal symptoms that appear when a patient with birch pollen allergy has eaten some foods associated with the family *Rosaceae* such as an apple, cherry, or peach. However, to date, there have been no studies to investigate the allergic responses to Cry j 3 in dogs.

In the present study, we investigated IgE reactivity to Cry j 3 in atopic dogs sensitive to crude *C. japonica* pollen allergen by a fluorometric ELISA.

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). Cry j 3 was purified as described previously (Fujimura et al., 2007).

2.2. Sera

CAD was diagnosed based on Willemse's criteria (Willemse, 1986) and Prélud's major criteria (Prélud et al., 1998). The sera were obtained from 100 dogs with AD visiting to Fujimura Animal Hospital owned by one of the authors (M.F.) (Osaka, Japan). The sera used as negative controls in the pollen-specific IgE assay were obtained from 19 dogs that had been kept indoors as experimental laboratory animals and had never been exposed to *C. japonica* pollen. They had shown no signs of AD and demonstrated no specific reaction to crude *C. japonica* allergen by intradermal skin testing (IDST).

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 measured by a fluorometric ELISA. A microplate (NUNC-Immuno Plate Maxisorp F96; NalgeNunc International, Roskilde, Denmark) was coated with crude *C. japonica* pollen allergen (4 µg/ml) or the purified allergens, Cry j 1, Cry j 2, or Cry j 3 allergens (1 µg/ml), at 4 °C overnight. After washing, the plate was incubated with diluted sera (1:10) at room temperature for 3 h. The plate was then washed and incubated with a mouse monoclonal anti-dog IgE antibody (0.5 µg/ml) (DeBoer et al., 1993) 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, USA) at room temperature for 1 h. After washing, the plate was incubated with β-D-galactosidase-conjugated streptavidin (Zymed Laboratories) at room temperature for 1 h. After final washing, the plate was incubated with 0.1 mM 4-methylumbelliferyl-β-D-galactopyranoside (Sigma, St. Louis, MO, USA) at 37 °C for 2 h. The enzyme reaction was stopped with 0.1 M glycine-NaOH (pH10.2), and the fluorescence intensity was read as fluorescence units (FU) on a microplate fluorescence reader (Fluoroskan; Flow Laboratories, McLean, VA, USA). The fluorometric ELISA for specific IgE in each sample was repeated more than three times. The cut-off value was determined as the average + 3SD FU in serum samples of 19 dogs as negative control.

2.4. Detection of allergen-specific IgE by immunoblotting analysis

Cry j 1 and Cry j 3 were electrophoresed by SDS-PAGE and then electrotransferred to PVDF membrane (GE Healthcare, Piscataway, NJ, USA). The three sera showing higher FU values of anti-Cry j 1 or anti-Cry j 3 IgE had been respectively pooled for immunoblotting analysis. After blocking with PBS containing 5% skimmed milk, the membrane was incubated with pooled sera diluted 1:10 in PBS containing 0.1% Tween-20 and 5% non-fat dried milk for 2 h. After washing, horseradish peroxidase (HRP)-conjugated goat anti-dog IgE (0.1 µg/ml) (Bethyl Laboratories, Montgomery, TX, USA) was added on the membrane and incubated for 2 h. After washing, the membrane was incubated with ECL reagent (GE Healthcare) for 5 min and exposed to a radiograph film for 5–30 s.

3. Results and discussion

Specific IgE to crude *C. japonica* pollen allergen was measured in the sera of the 100 dogs with AD by ELISA (Fig. 1). The cut-off value calculated from negative control samples was 55 FU. Of the 100 dogs with AD, 15 had IgE sensitivity to crude *C. japonica* pollen allergen similar to the results of our previous epidemiological study (Masuda et al., 2000a). Next, IgE reactivity to Cry j 1 and Cry j 2 was examined in these 15 dogs sensitive with crude *C. japonica* pollen allergen by ELISA (Fig. 2A and B). The cut-off values of specific IgE levels to Cry j 1 and Cry j 2 calculated from the negative control samples were found to be 114 and 96 FU, respectively. Of the 15 serum samples which included specific IgE to crude *C. japonica* pollen allergen, all had specific IgE to Cry j 1, and 6 (40%) had specific IgE to Cry j 2. In humans, more than 90% of the patients with *C. japonica* pollinosis had specific IgE to Cry j 1 and Cry j 2 (Hashimoto et al., 1995). Further, in wild Japanese monkeys, we found that of the 45 monkeys sensitive to crude *C. japonica* pollen allergen, 23 (51%) had specific IgE to both Cry j 1 and Cry j 2, 21 (47%) had specific IgE only to Cry j 1, and 1 (2%) had specific IgE only to Cry j 2 (Hashimoto et al., 1994). In humans and monkeys, Cry j 1 and Cry j 2 are major pollen allergens. In the present study, however, 40% of the dog sera that had specific IgE to crude pollen had anti-Cry j 2 IgE. This result was similar to that of our previous study (Masuda

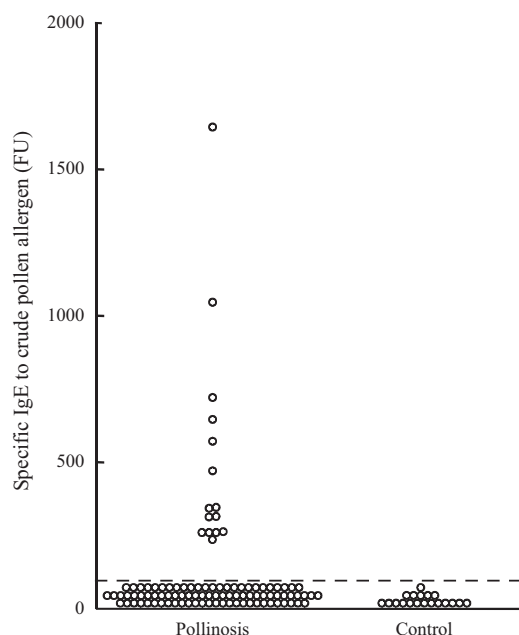


Fig. 1. IgE reactivity to crude *C. japonica* pollen allergen in dogs. The data were obtained by using diluted sera (1:10). The dashed line shows the cut-off value. The cut-off value calculated from negative control samples was 55 FU.

et al., 2000b). These findings indicate that Cry j 1 is a major allergen in dogs as well as humans and Japanese monkeys.

IgE reactivity to Cry j 3 was examined in these 15 dogs sensitive to crude pollen allergen (Fig. 2C). The cut-off values of specific IgE to Cry j 3 calculated from negative control samples were 126 FU. Of the 15 sera which contained specific IgE to crude *C. japonica* pollen allergen, 11 (73%) had specific IgE to Cry j 3. Of 11 sera samples, five also reacted with Cry j 1, and six with both Cry j 1 and 2. The IgE-binding frequency of Cry j 3 in the human patients with *C. japonica* pollinosis was estimated as 27% (Fujimura et al., 2007). More than 70% of CAD with specific IgE to crude *C. japonica* pollen allergen had anti-Cry j 3 IgE. These findings suggest that Cry j 3 may be a more important allergen in dogs than in humans.

To further evaluate anti-Cry j 3 IgE in dogs reactivity with Cry j 3, we conducted immunoblotting analysis. Cry j 1 and Cry j 3 were electrophoresed by SDS-PAGE and then electrotransferred to PVDF membrane. The pooled serum of 3 dogs with anti-Cry j 1 IgE was added to the membrane, and Cry j 1 was detected with anti-Cry j 1 IgE (Fig. 3, lane 1). Similarly, the pooled serum of 3 dogs with anti-Cry j 3 IgE was added to the membrane, and Cry j 3 was detected with anti-Cry j 3 IgE (Fig. 3, lane 3). In contrast, the pooled serum of 3 dogs as negative controls with no anti-Cry j 1 and Cry j 3 IgE was added to the membrane, and Cry j 1

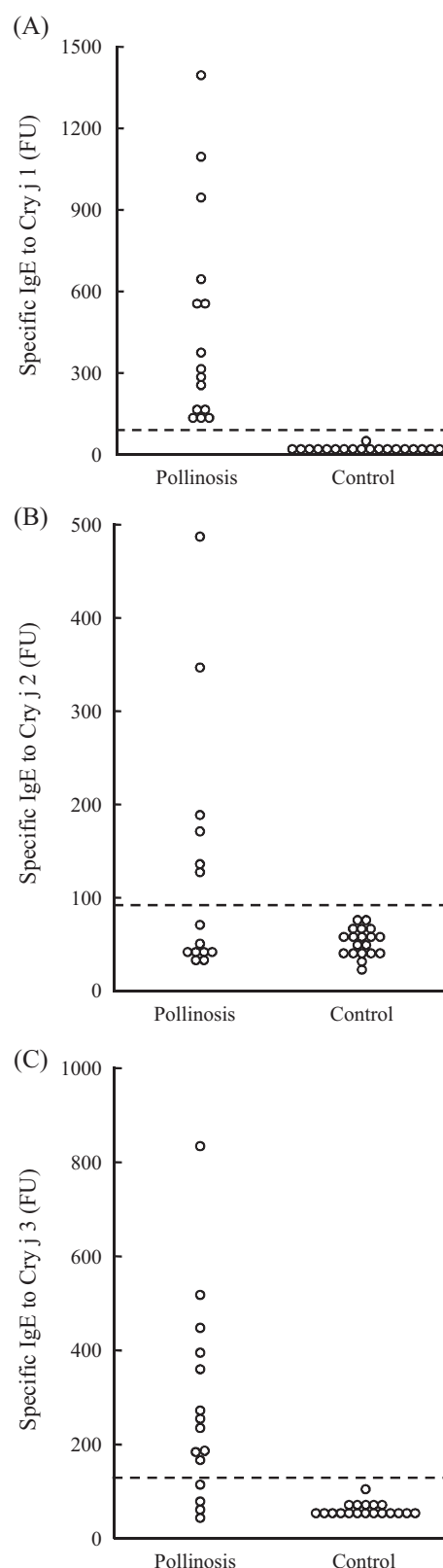


Fig. 2. IgE reactivity to (A) Cry j 1, (B) Cry j 2, and (C) Cry j 3 in atopic dogs sensitive to crude *C. japonica* pollen allergen. The data were obtained by using diluted sera (1:10). The dashed line shows the cut-off value. The cut-off values calculated from negative control samples were 114, 96 and 121 FU, respectively.

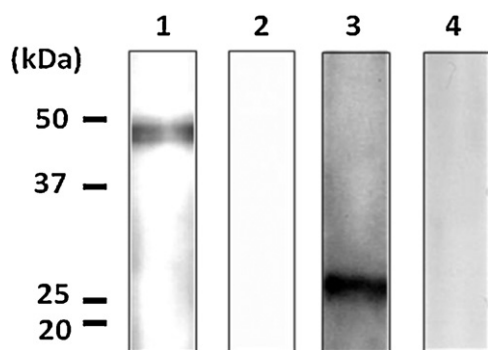


Fig. 3. Immunoblotting analysis of Cry j 1 and Cry j 3. Cry j 1 (lanes 1 and 2) or Cry j 3 (lanes 3 and 4) was electrophoresed by SDS-PAGE and then electrotransferred to PVDF membrane. Lane 1: pooled serum of 3 dogs with anti-Cry j 1 IgE (FU values: 1410, 1090, and 925), lanes 2 and 4: pooled serum of 3 dogs as negative controls with no anti-Cry j 1 and Cry j 3, lane 3: pooled serum of 3 dogs with anti-Cry j 3 IgE (FU values: 812, 507, and 412).

and Cry j 3 were not detected (Fig. 3, lanes 2 and 4). These results reconfirmed that sera of dogs with anti-Cry j 3 IgE reacted to Cry j 3 with immunoblotting analysis.

We found that Cry j 3 has significant homology with the thaumatin-like protein categorized in PR-5 family protein which might act as a causative allergen in OAS (Fujimura et al., 2007). Cross-reactivity between allergens from *C. japonica* pollen and tomato has been reported in dogs and humans (Fujimura et al., 2002; Kondo et al., 2002). PR-5 protein, which was considered as one of the important pollen allergens responsible for pollinosis and OAS, have been identified as Jun a 3 from mountain cedar (*Juniperus ashei*), Jun v 3 from eastern red cedar (*Juniperus virginiana*), and Cup a 3 from Arizona cypress (*Cupressus arizonica*) (Midoro-Horiuti et al., 2000, 2001; Cortegano et al., 2004). Fujimura et al. (2007) revealed that Cry j 3 has significant homology with the thaumatin-like protein and suggested that Cry j 3 might act as a causative allergen for OAS in human patients. Unfortunately, we could not evaluate the presence of OAS in the dogs with IgE to Cry j 3. The present results, however, suggest that Cry j 3 is an important allergen in dogs and might play an important role in the etiology of OAS in dogs.

In conclusion, we found that many dogs with specific IgE to crude *C. japonica* pollen had anti-Cry j 3 IgE. Our results indicate that Cry j 3 can be considered as one of the major allergens in CAD. Further studies are needed to reveal the role of cross-reactivity to other allergens in the etiology of OAS in CAD.

Conflicts of interest

The authors declare no conflicts of interest.

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