NOTE *Internal Medicine*

Experimental Sensitization with Japanese Cedar Pollen in Dogs

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ABSTRACT. Japanese cedar pollinosis is a type I allergic disease mediated by immunoglobulin E (IgE) antibodies to Japanese cedar (*Cryptomeria japonica*) pollen antigen (CPAg). By using 22 dogs consisting of 20 dogs aged 3 months and 2 dogs aged 3 years, immunization was performed by subcutaneous injections of CPAg with aluminum hydroxide gel. Variable levels of CPAg-specific IgE antibody response were detected in 21 of the 22 immunized dogs two weeks after the second immunization. This study provided an experimental sensitization system with CPAg in dogs, which will be useful for further immunological studies on Japanese cedar pollinosis.
KEY WORDS: canine, IgE antibody, Japanese cedar.

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In recent years, the number of patients with allergic diseases has been increasing in dogs as well as in humans [2, 4, 5, 8, 15]. Immunoglobulin E (IgE) antibodies play an important role in the development of immediate hypersensitivity (type I allergic disease) [5, 8, 15]. Binding of multivalent allergens to specific IgE antibody on mast cells or basophils leads to the release of histamine and arachidonic acid metabolites. These substances act as inflammatory mediators inducing various symptoms of allergic diseases such as dermatitis, urticaria, asthma, rhinitis and conjunctivitis. Japanese cedar pollinosis, originally described in humans, is a type I allergic disease caused by sensitization to Japanese cedar (*Cryptomeria japonica*) pollen antigen (CPAg) which is one of the important allergens indigenous to Japan. It has been reported that about 10% of dogs suffering from atopic dermatitis have specific IgE antibodies against CPAg in Japan [5, 9, 10]. A proportion of atopic dogs with high levels of serum CPAg-specific IgE antibody shows a seasonality from February to April in the aggravation of the dermatologic conditions, however, the clinical significance of CPAg for the development of atopic dermatitis should be further investigated. To understand the pathogenesis and to develop a novel therapeutic procedure for Japanese cedar pollinosis, a canine model experimentally sensitized to CPAg was tried to be established in this study.

CPAg was extracted from a crop of Japanese cedar pollen in 1997 with a method reported by Yasueda *et al.* [16]. First, stock of the pollen was defatted with ether and then extracted in 0.125 M ammonium bicarbonate overnight at room temperature. After the extraction, ammonium sulfate was added to the extract with stirring to 80% saturation. After stirring overnight at 4°C, the precipitate was collected and dissolved in phosphate buffer saline (PBS). The dissolved solution was dialyzed with PBS overnight at 4°C. Aluminium hydroxide gel (alum) was prepared as adjuvant by the following method: 1 N NaOH was added to 5.5% aluminium potassium sulfate solution with stirring until pH of the solution reached 7.0. After stirring for 30 min at room temperature, the precipitate was collected by centrifugation and suspended in physiological saline.

We used 22 dogs with no clinical sign of allergic disease which had been kept for experimental purposes. They had no detectable levels of specific IgE antibody to CPAg prior to the immunization. The dogs consisted of 18 female beagles aged 3 months, 2 female beagles aged 3 years and 2 French bulldogs (one female and one male) aged 3 months. These dogs were subcutaneously injected with 100 μg of CPAg in 3 m*l* of saline-emulsified alum. The same injection was repeated 2 weeks after the first injection. Blood samples were collected 2 weeks after the second immunization. The levels of CPAg-specific IgE antibody in the serum samples were determined by a fluorometric enzyme-linked immunosolvent assay (ELISA) by a method reported previously [14]. Briefly, each well of a microtiter plate was coated with 1 μg of CPAg overnight at 4°C. Serum samples were added to the wells and incubated for 3 hr at room temperature. The plates were then incubated with a mouse monoclonal anti-dog IgE antibody (0.5 μg/m*l*) [1] overnight at 4°C. After incubation, the plates were incubated with biotinylated anti-mouse IgG1 antibody (Zymed Laboratories, CA, U.S.A.) for 1 hr at room temperature, and then incubated with β-D-galactoside conjugated streptavidin (Zymed) for 1 hr at room temperature. Finally, 4-methylumbelliferyl-β-D-galactoside (Sigma, MO, U.S.A.) was added to the wells for development of the fluorescence. After the enzyme reaction was stopped with 0.1 M glycine-NaOH (pH 10.2), the fluorescence intensity was measured with a microplate fluorescence reader (Fluoroscan, Flow Laboratories, VA, U.S.A.). The concentration of

Table 1. The levels of IgE antibody specific to CPAg

Dog	Breeds	Age (months)	Sex	Specific IgE (U/m*l*)
1	Beagle	3	F	1,240
2	Beagle	3	F	1,850
3	Beagle	3	F	2,000
4	Beagle	3	F	550
5	Beagle	3	F	140
6	Beagle	3	F	4,520
7	Beagle	3	F	1,300
8	Beagle	3	F	2,160
9	Beagle	3	F	2,080
10	Beagle	3	F	338
11	Beagle	3	F	2,480
12	Beagle	3	F	6,620
13	Beagle	3	F	92,600
14	Beagle	3	F	439
15	Beagle	3	F	7,310
16	Beagle	3	F	11
17	Beagle	3	F	12,300
18	Beagle	3	F	1,220
19	French bulldog	3	F	4,720
20	French bulldog	3	M	<3
21	Beagle	36	F	4,250
22	Beagle	36	F	28,600

CPAg-specific IgE antibody in each serum sample was calculated from a standard titration curve of a reference standard (pooled serum) and expressed in arbitrary units (U/m*l*). Repeated immunizations induced appreciable CPAg-specific IgE antibody responses in 21 of the 22 dogs two weeks after the second immunization, whereas one French bulldog (Dog No. 20) did not produce any detectable amount of CPAg-specific IgE antibody (<3 U/m*l*) (Table 1). The responses of CPAg-specific IgE antibody markedly differed among the 21 positive dogs. The levels of CPAg-specific IgE antibody ranged from 11 U/m*l* to 92,600 U/m*l*.

Further, the intradermal skin test (IDST) was performed in 4 of the 22 dogs according to the method by Masuda *et al.* [11]. Briefly, CPAg was diluted to a rate of 1:20 or 1:50 with a diluent containing 0.9% sodium chloride and 0.4% phenol. The diluent without the CPAg was also used as a negative control. Histamine solution (27.5 μg/m*l*) was used as a positive control. These solutions were injected into the clipped skin of ventro-lateral thorax at a volume of 0.05 m*l* by using a test syringe with a 26-gauge needle. The sizes of wheal produced 15–20 min after injection were measured with slide calipers. All of the 4 dogs consisting of two beagles aged 3 years (Dog Nos. 21 and 22) and two French bulldogs aged 3 years (Dog Nos. 19 and 20) showed strong positive reaction for CPAg in the IDST as judged from the size of wheal produced by the injection with CPAg larger than that of positive control. Of these 4 dogs, 3 dogs (Dog Nos. 19, 21 and 22) were positive for serum CPAg-specific IgE antibodies, however, 1 dog (Dog Nos. 20) was negative for the IgE antibodies.

No clinical symptoms indicating Japanese cedar pollinosis such as rhinitis, conjunctivitis or atopic dermatitis were observed in the 22 dogs used in this study during the study period.

It has been shown that immunization of newborn dogs within 20 hr after the birth easily induced serum antigen-specific IgE antibody responses to the immunized antigen, however immunization of adult dogs frequently failed to induce the serum IgE antibody responses [7, 13]. On the other hand, Frick and Brooks reported that inbred dogs predisposed to atopic dermatitis readily developed IgE antibody specific to grass pollen antigen after immunization with its extract, whereas dogs without such predisposition did not produce the IgE antibodies after the immunization with the same protocol [3]. In our preliminary study, 3 female beagles aged 2 years appeared to produce detectable levels of CPAg-specific IgE antibody 2 weeks after the first immunization with CPAg in alum. From the literatures on experimental sensitization in dogs [7, 13], it was conceivable that young animals were easily sensitized with antigens. Although female beagles and French bulldogs aged 3 months were mainly used in this study, further efforts are required to choose most appropriate age, breed and sex of the dogs for the experimental sensitization with antigen.

Despite the high titers of CPAg-specific IgE antibody, no clinical signs were observed in the dogs experimentally sensitized to CPAg during the study period. This suggests that an inhalant exposure of the antigen and/ or some other factor may be required to develop some clinical symptoms of allergic disease. In fact, our preliminary experiments demonstrated that intrabronchial challenge of two sensitized dogs (Dog Nos. 21 and 22) with CPAg induced significant bronchial constriction (data not shown). Further experiments are needed whether these experimentally sensitized dogs show rhinitis, conjunctivitis or atopic dermatitis after inhalation challenge with CPAg, which are common symptoms of Japanese cedar pollinosis in humans.

In humans, the predisposition of atopic dermatitis and the levels of IgE antibody response are shown to be genetically controlled at least in part [6, 12]. It is well known that there exist breed and family predisposition to atopic dermatitis in dogs. Experimental sensitization system as employed in this study will provide useful finding for the investigation on the predisposition to allergic diseases in conjunction with the study on natural cases with allergic diseases.

In conclusion, sensitization of dogs with CPAg could be experimentally induced by immunization of CPAg with alum. It is expected that the dogs experimentally sensitized with CPAg will provide useful information on the involvement of the antigen specific IgE antibodies in allergic diseases. Moreover, the experimental sensitization system with CPAg in dogs can be considered as an animal model for the development of novel therapeutic procedure for allergic diseases.

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