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Original Article

Specificity of an enzyme-linked immunosorbent assay for dog IgE antibody to Japanese cedar (*Cryptomeria japonica*) pollen

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Abstract

We developed a fluorometric enzyme-linked immunosorbent assay (ELISA) for allergen-specific IgE in dogs with the use of monoclonal anti-dog IgE; we assayed IgE antibody to Japanese cedar pollen in the sera of dogs with Japanese cedar pollinosis. To assess the specificity of this ELISA, a pooled serum sample from pollinosis dogs was subjected to gel chromatography. The peak of anti-pollen allergen IgE activity was different from the peaks of total IgA, IgG and IgM. When IgE antibody positive serum was heated at 56°C for 4 h, antibody activity was markedly reduced. Furthermore, polyclonal anti-dog IgA, IgG and IgM did not interfere with anti-pollen allergen IgE activity in the ELISA. From these results, this assay is considered to have a high specificity for dog IgE.

Key words: allergen, dog, IgE, pollen.

Introduction

IgE-mediated allergies are common in dogs, which show pruritus, atopic dermatitis and other diseases, including rhinitis, asthma and conjunctivitis.[1,2] Several groups have reported the production of polyclonal antisera against dog IgE.[3–5] However, their use has been limited. Recently, production of mouse monoclonal antibodies to dog IgE was established.[6] The monoclonal antibodies were shown to induce a reverse cutaneous anaphylaxis reaction in dog skin and neutralized the Prausnitz-Küstner (PK) reactivity of atopic dog serum. Some anti-human IgE monoclonal antibodies reacted with similar epitopes on dog IgE.[7]

Japanese cedar (*Cryptomeria japonica*; CJ) pollinosis is one of the most important allergic diseases in Japan.[8] The number of patients with the pollinosis has increased.[9] Our seroepidemiological study demonstrated that approximately 30% of the general population aged 20–39 years and residing in the Tokyo area were IgE antibody positive to CJ allergens.[10] In another study, a periodical health examination of 892 university students, the percentage of subjects with CJ-specific IgE antibody was found to be 27% and the frequency of CJ pollinosis sufferers was 12%.[11]

Recently, Japanese cedar pollinosis has been reported to occur naturally in dogs in Japan.[12] Clinical signs in dogs with CJ pollinosis appear to differ from those of humans. These dogs showed allergic dermatitis and had a positive reaction to CJ allergen in the PK test. However, *in vitro* measurement of dog IgE to CJ allergens has not been reported.

In the present study we developed a fluorometric enzyme-linked immunosorbent assay (ELISA) for CJ-specific IgE with monoclonal anti-dog IgE, which has a sensitivity as high as radioimmunoassay.[13,14]

Methods

Antigen

Crude pollen extract was prepared as described previously.[15,16]

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Serum

Serum samples collected from two dogs with CJ pollinosis were used for the measurement of specific IgE antibodies to CJ allergens. Two dogs had allergic dermatitis during the CJ pollination season and had a positive reaction to CJ allergens in intradermal skin and PK tests. The sera used as a negative control in the allergen-specific IgE antibody assay were obtained from 10 dogs in the USA where there is no Japanese cedar growing.

Fluorometric indirect ELISA for specific IgE

Specific IgE antibodies to CJ allergens were assayed by fluorometric ELISA.[17,18] A white microplate (Microfluor W plate; Dynatech, Chantily, VA, USA) was coated with 10 μg/mL CJ crude antigen overnight at 4°C. After washing, several concentrations of serum were added to the wells and the plate was incubated for 3 h at room temperature. The plate was washed and then a mouse monoclonal anti-dog IgE antibody (0.5 μg/mL),[6] which was produced by one of us (DJDB), was added to the plate. The plate was incubated overnight at 4°C. After washing, biotinylated rat monoclonal anti-mouse IgG_1 (Zymed Laboratories, San Francisco, CA, USA) was added. The plate was incubated for 1 h at room temperature. After washing, β-D-galactosidase conjugated streptavidin (Zymed) was added. The plate was incubated for 1 h at room temperature. After final washing, 0.1 mmol/L 4-methylumbelliferyl-β-D-galactoside (Sigma Chemical Co., St Louis, MO, USA) was added to each well. The plate was incubated for 2 h at 37°C. The enzyme reaction was stopped with 0.1 mol/L glycine–NaOH (pH 10.2) and the fluorescence intensity was read as fluorescence units (FU) on a microplate fluorescence reader (Fluoroskan; Flow Laboratories, McLeane, VA, USA).

Sephacryl S-300 column chromatography

Sephacryl S-300 (Pharmacia, Uppsala, Sweden) column chromatography was performed as previously described.[19] Briefly, the pooled serum of dogs with pollinosis was applied to a Sephacryl S-300 column. The respective peak fractions of total IgA, IgG and IgM were determined by sandwich ELISA. Microplate (Immulon II; Dynatech) wells were coated with 4 μg/mL anti-dog IgA (Bethyl Laboratories, Montgomery, TX, USA), IgG or IgM (Nordic, Tilburg, The Netherlands). After washing, wells were incubated at room temperature with each fraction. Next, wells were incubated first with biotinylated anti-dog IgA (Bethyl Laboratories), IgG or IgM (Nordic) and then with peroxidase-conjugated streptavidin (Sigma). After the final washing, O-phenylenediamine dihydrochloride plus hydrogen peroxide were added. After the enzyme reaction was stopped, absorbance was measured on a colorimetric microplate reader.

Inhibition of ELISA for dog anti-CJ IgE by anti-dog IgA, IgG and IgM antibodies

A white microplate was coated with 10 μg/mL CJ antigen overnight at 4°C. After washing, the serum sample from a dog that had a high level of anti-CJ IgE was added to the wells and the plate was incubated for 3 h at room temperature. The plate was washed, then mouse monoclonal anti-dog IgE antibody (0.5 μg/mL) was mixed with 50 μg/mL anti-dog IgA, IgG and IgM antibodies as inhibitors and was added to the wells. The remaining procedures were the same as those for the indirect ELISA method described earlier.

Inhibition of ELISA for dog anti-CJ IgE activity by CJ antigen

A white microplate was coated with 4 μg/mL CJ antigen overnight at 4°C. After washing, the serum sample from a dog, diluted 1:50, was mixed with CJ antigen or crude mite (*Dermatophagoides farinae*) antigen (Torii Pharmaceutical Co., Tokyo, Japan; 40 μg/mL). The mixtures were added to microplate wells that had been coated with CJ antigen. After incubation for 2 h, the well was washed and anti-dog IgE was added to each well. The remaining procedures were the same as for the indirect ELISA method described earlier.

RESULTS

Anti-CJ IgE antibody in gel filtration

To determine the molecular size of the IgE antibody, a pooled serum sample from dogs with pollinosis was subjected to Sephacryl S-300 gel chromatography. The anti-CJ IgE antibody activity of each fraction was determined by the fluorometric ELISA and total IgA, IgG and IgM of each fraction was measured by the sandwich ELISA. As illustrated in Fig. 1, the peak of anti-CJ IgE

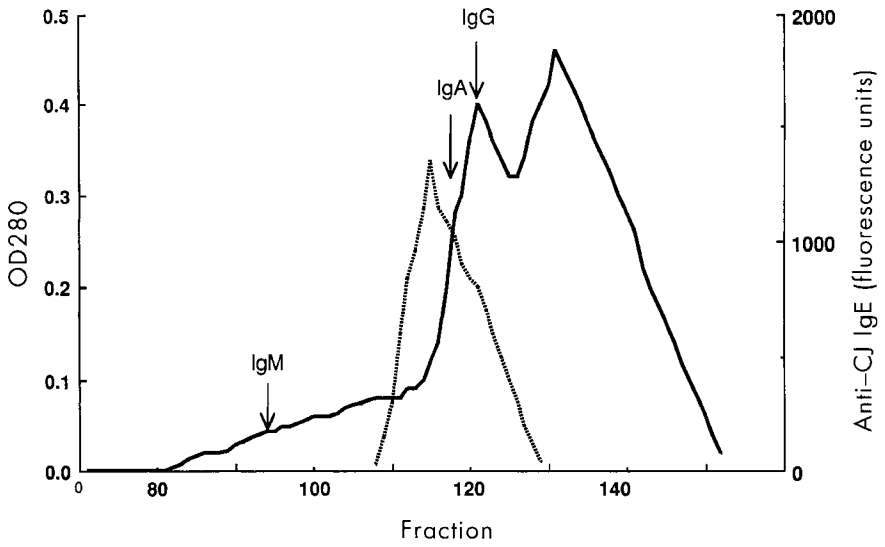

Fig. 1 Elution profile of anti-CJ IgE antibody in Sephacryl S-300. (—), OD280; (----), anti-CJ IgE.

antibody activity was eluted in fraction 115 and was different from the peaks of total IgA, IgG and IgM. Similarly, the serum from an American dog was subjected to Sephacryl S-300 gel chromatography. No fraction showed anti-CJ IgE reactivity (data not shown).

Effect of heating on the antibody activity detected by fluorometric ELISA

The serum sample from a dog with pollinosis had a high titer of anti-crude CJ IgE antibody (Fig. 2). Sera of 10 American dogs showed no IgE antibody reactivity (data not shown). The serum sample with anti-CJ IgE was heated at 56°C for 4 h and was then assayed for its specific IgE activity by fluorometric ELISA. The IgE antibody activity was markedly reduced.

Effect of anti-dog IgA, IgG and IgM on anti-dog IgE activity

The IgE-specificity of the fluorometric ELISA was assessed by inhibition experiments using polyclonal antibodies to dog IgA, IgG and IgM. As shown in Fig. 3, addition of 50 μg/mL of these polyclonal antibodies to monoclonal antibody to dog IgE did not inhibit anti-CJ IgE activity.

Inhibition of ELISA for dog anti-CJ IgE activity by CJ antigen

The antigen-specificity of the ELISA was evaluated by inhibition experiments using CJ antigen (Fig. 4). When pooled serum of dogs with pollinosis was incubated with

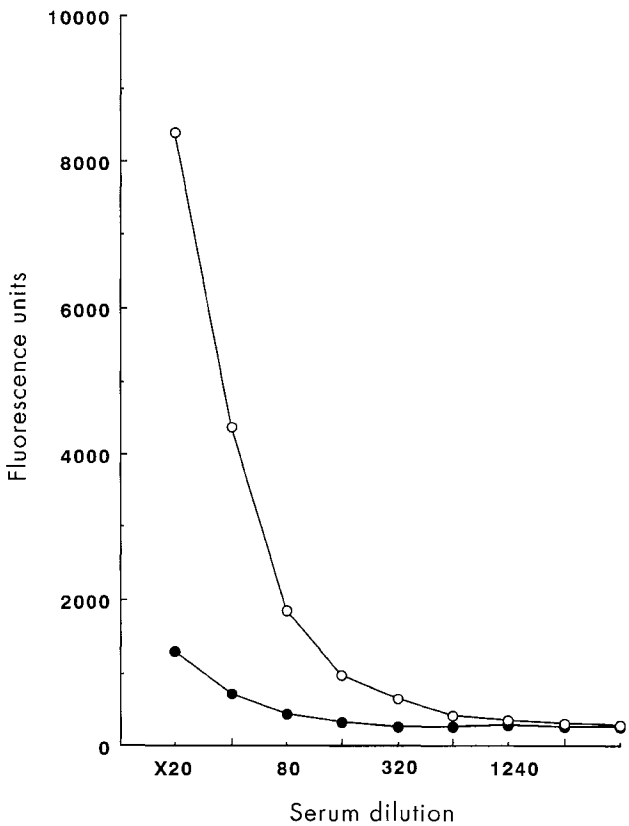

Fig. 2 Effect of heating (56°C, 4 h) on anti-CJ IgE activity. (O), without heating; (●), with heating.

CJ antigen, the anti-CJ IgE activity in the serum was markedly reduced. When the serum was incubated with mite antigen, the anti-CJ IgE activity was not changed.

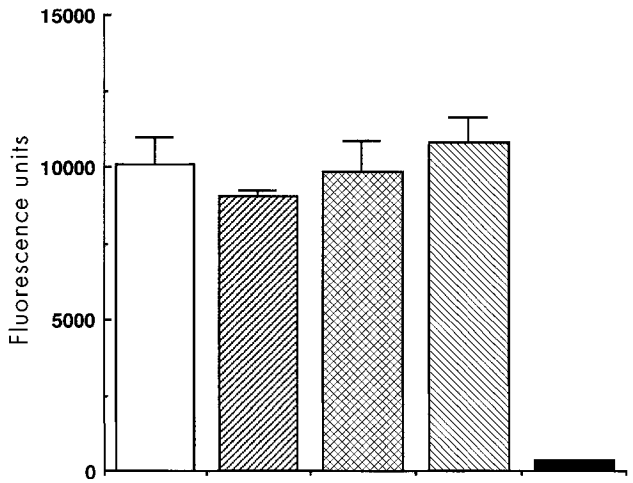

Fig. 3 Effect of anti-dog IgA (▨), IgG (▩), and IgM (▧), on anti-dog IgE activity. (□), no inhibitor; (■), blank.

Antibody titration curves from dogs with and without pollinosis

Figure 5 shows anti-CJ IgE titration curves in serum samples from a dog with a high titer of anti-crude CJ IgE antibody as a positive control and from an American dog as a negative control. The serum of the dog with pollinosis gave a high level of anti-CJ IgE. Sera from American dogs failed to react with CJ antigen. The mean (±SD) FU of the sera from the 10 American dogs was 294±65 FU. Thus, the cutoff value was determined as 489 FU (mean + 3SD).

Discussion

IgE-specificity of this ELISA was confirmed as follows. First, the peak of activity of IgE on Sephacryl S-300 gel chromatography was eluted between the peaks of IgA and IgM fractions. The molecular weight of IgE is between IgA and IgM.[20] Second, polyclonal antibodies to dog IgA, IgG and IgM did not inhibit anti-CJ IgE activity in this ELISA. Third, the IgE antibody activity determined by the fluorometric ELISA was markedly reduced after heat treatment of serum at 56°C for 4 h. However, this activity did not completely disappear, even after heat treatment. The epitopes of dog IgE recognized by this monoclonal anti-dog IgE antibody may not be as heat-labile as the mast cell-binding activity of IgE. We have also found that the mouse IgE epitope detected by anti-mouse IgE monoclonal antibody was not completely lost after the same treatment.[21] Furthermore, sera from 10

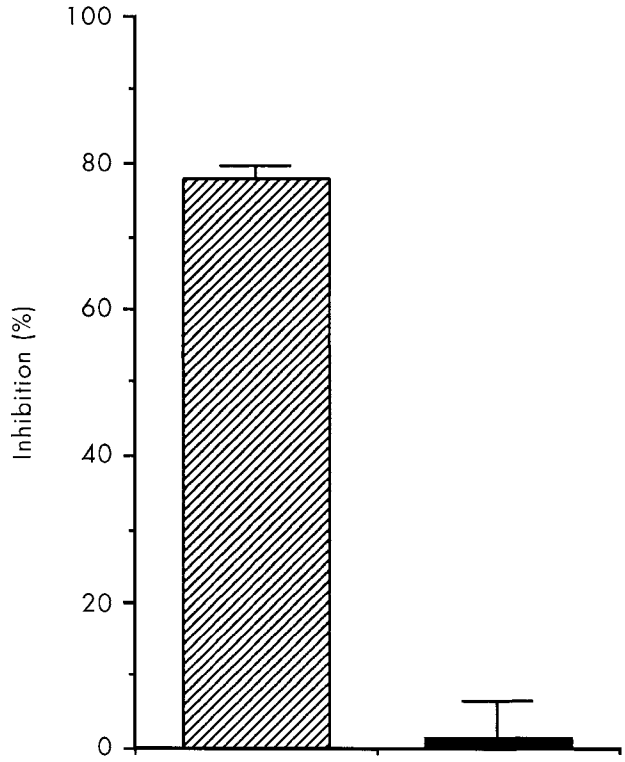

Fig. 4 Effect of *Cryptomeria japonica* (CJ) pollen allergen (▨) on anti-CJ IgE activity. (■), mite.

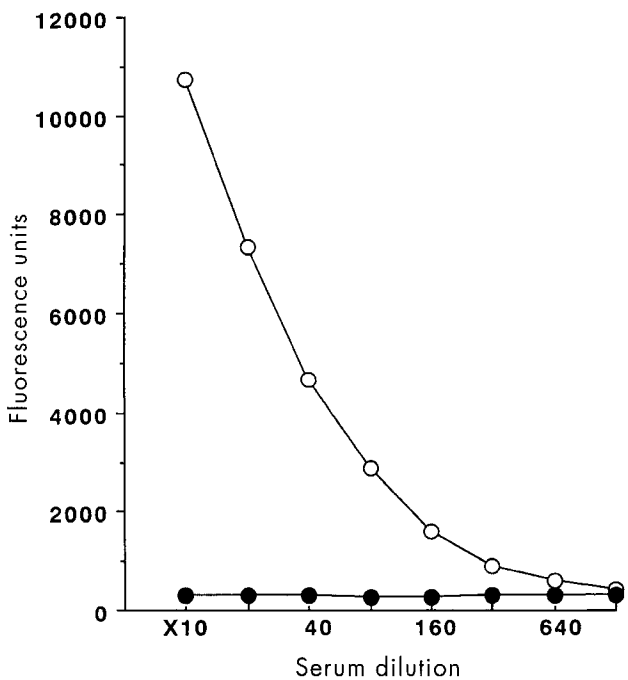

Fig. 5 Antibody titration curves of sera of a dog with pollinosis (○) and an American dog (●).

American dogs that were unexposed to CJ pollens did not react with CJ allergens. These findings confirmed the IgE-specificity of the ELISA.

The *in vivo* specificity of anti-dog IgE monoclonal antibodies has been reported.[6] This antibody induced wheal and flare reactions after injection into the skin of normal dogs. The minimum amount of anti-dog IgE monoclonal antibodies necessary to induce a wheal reaction was approximately 4 ng. Furthermore, in inhibition of PK tests using dog serum absorbed with anti-dog IgE, the PK titer was reduced 160-fold. These *in vivo* findings indicated that the monoclonal antibodies specifically react with dog IgE.

In humans, CJ pollinosis is one of the most common immediate-type allergic diseases in Japan.[8] The occurrence of CJ pollinosis has been reported in the Japanese monkey.[22] We measured anti-CJ IgE in Japanese monkeys and found that 45 of 276 (16.3%) Japanese monkeys in nine troops throughout Japan had CJ-specific IgE.[23–25] Recently, we found that dogs had allergic disease to CJ pollen in Japan.[12] However, there have been no *in vitro* reports of the measurement of dog IgE to CJ allergen.

The dog is one of the species that spontaneously develops atopy like diseases in a relatively large proportion of the population.[2] Allergic disease is one of the most important diseases in veterinary medicine for dogs. Furthermore, the use of dog models appears to benefit the study of human allergy.

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