

Case report

Seasonal atopic dermatitis in dogs sensitive to a major allergen of Japanese cedar (*Cryptomeria japonica*) pollen

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Abstract Three dogs were examined because of episodes of recurrent pruritic dermatitis in the spring, the season of Japanese cedar (*Cryptomeria japonica*, CJ) pollination in Japan. The dogs were shown to be sensitive to CJ pollen allergen using intradermal testing and antigen-specific IgE measurement. Fluorometric enzyme-linked immunosorbant assay (ELISA) showed increased concentrations of IgE specific to Cry j 1 and a negative result for Cry j 2 in the three dogs. The concentrations of IgE specific to Cry j 1 during the season of CJ pollination were higher than the concentrations found during the off-season in all the dogs, and the variation in the concentrations correlated with the variation in clinical signs. Peripheral blood mononuclear cells showed apparent proliferative responses to crude CJ pollen antigen and Cry j 1 during CJ pollination season. These findings indicated that Cry j 1 was the major allergen recognized by IgE and lymphocytes and resulted in the development of type I hypersensitivity to CJ pollen allergen in these atopic dogs.

Keywords: atopic dermatitis, Cry j 1, dog, IgE, Japanese cedar pollen, lymphocytes

INTRODUCTION

Japanese cedar (*Cryptomeria japonica*, CJ) pollinosis in humans is a major seasonal allergy in Japan, with rhinitis and conjunctivitis as clinical symptoms during the season of CJ pollination.^{1–3} Allergic responses to CJ pollen allergen in human CJ pollinosis have been investigated by means of various allergy tests such as the intradermal test, serum IgE measurement,^{1,3} histamine release test and lymphocyte proliferation test.⁴ The results from these tests have shown an involvement of type I hypersensitivity to CJ pollen and its two major allergens, Cry j 1⁵ and Cry j 2⁶ in the development of CJ pollinosis.

In addition to human CJ pollinosis, sensitivity to CJ pollen has also been found in monkeys⁷ and dogs.^{8,9}

The antigen-specific responses in monkeys were shown to be similar to those in human patients: positive responses to a crude extract of CJ pollen and the two major CJ allergens were documented using *in vivo* and *in vitro* methods such as intradermal testing, IgE measurement, histamine release test and lymphocyte proliferation test.⁷ In dogs, sensitivity to CJ pollen allergen was first demonstrated by *in vivo* tests including Prausnitz-Küstner testing, intradermal testing and provocation testing.⁸ We investigated *in vitro* reactivity to CJ pollen allergen in atopic dogs and found that histamine release and lymphocyte blastogenesis occurred against CJ pollen allergen in a manner similar to that seen in humans.⁹ However, responses to Cry j 1 and Cry j 2 have not been fully examined in dogs. We previously reported detection of dog IgE specific to CJ major allergens, Cry j 1 and Cry j 2 by enzyme-linked immunosorbant assay (ELISA) in atopic dogs sensitive to CJ pollen.¹⁰ The question is whether the sensitivity to these major allergens is associated with the clinical manifestation of atopic dermatitis in dogs. In this study,

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we report on three dogs sensitive to CJ pollen allergen and Cry j 1 that had seasonal atopic dermatitis.

CASE REPORTS

Case 1

A 7-year-old male Shiba Inu-mix dog was referred to the Veterinary Medical Center of the University of Tokyo because of seasonal dermatitis occurring from March to May, the season of CJ pollination in Japan. Pruritus and skin lesions including erythema, alopecia, hyperpigmentation and lichenification of the axillal, medial and lateral elbows, cranial thorax and inguinal area became apparent in spring (Fig. 1a). These clinical signs improved significantly with regard to pruritus and hair regrowth in the off-season of CJ pollination (Fig. 1b).

Based on Willemse's criteria for clinical diagnosis of canine atopic dermatitis,¹¹ the patient was diagnosed as having atopic dermatitis. Other skin diseases causing pruritus such as parasitic infestations (i.e. demodicosis, fleas or scabies) and infectious skin diseases (i.e. dermatophytosis and bacterial pyoderma) were ruled out by routine dermatologic examinations. Food allergy was also excluded because of the apparent complete seasonality of the dermatitis in the absence of dietary change.

In order to identify environmental allergens associated with the seasonal dermatitis observed in this case, allergy tests including intradermal testing and the antigen-specific IgE tests were performed according to procedures described previously.¹² Briefly, 19 allergen extracts for intradermal testing were purchased from a commercial company (Greer Laboratories, Lenoir, NC, USA) and an extract of CJ pollen was prepared as reported previously.⁵ There were seven groups of allergens used in this study: HDM (house dust mites;

Dermatophagoides pteronyssinus and *D. farinae*), arthropods (cockroach, housefly, mosquito, etc.), cat epithelia, moulds (*Curvularia spicifera*, *Penicillium camemberti*, etc.), grasses (Kentucky blue, orchard, redtop, etc.), weeds (cocklebur, lambs quarter, rough pigweed, etc.), and trees (Japanese cedar, white ash, red birch, etc.). The majority of the allergen extracts were used at a concentration of 1000 PNU after dilution with the diluent (0.9% sodium chloride with 0.4% phenol). The extracts of *D. pteronyssinus* and *D. farinae* were diluted to a concentration of 1:5000 w/v. The extract of Japanese cedar pollen was used at a concentration of 200 ng mL⁻¹. The diluent without allergen was used as a negative control. Histamine phosphate solution (0.0275 mg mL⁻¹) was used as a positive control. The hair coat on the lateral thorax of the dog was clipped and ≈ 0.05 mL of each allergen solution was injected intradermally using a skin test syringe with a 26-gauge needle without sedation. The diameters of wheals were measured 15–20 min post injection. The test sites were graded as follows: +3, equal to or greater than the diameter of the positive control; +2, equal to or greater than the mean diameter of the positive and negative controls; +1, larger than the diameter of the negative control, but smaller than the mean diameter of the positive and negative controls; 0, equal to or smaller than the diameter of the negative control. This dog showed a positive response only to CJ pollen extract, forming a reactive wheal estimated as +3, but was nonreactive to all other allergen extracts.

As the screening system for serum antigen-specific IgE, two commercially available test kits (Topscreen and Immunodot test kits, CMG Laboratories, Fribourg, Switzerland) were used as described previously.^{12,13} The Topscreen test is a five spot screening test using mixtures of allergens such as outdoor, indoor, food 1, food 2 and mould allergen groups. The Immunodot test is used to detect IgE specific to individual antigens. Briefly, for either test, patient serum samples were spotted on individual nitrocellulose strips each containing an allergen test mixture or individual allergen. After incubation, peroxidase-conjugated monoclonal anticanine IgE antibody and substrate solution were applied to the strips for colorimetric detection. The colour intensity was measured with a densitometer. An optical density (OD) value > 1.0 in the Topscreen and an OD > 2.0 in the Immunodot were considered positive. In this assay system, a total of 24 allergens belonging to nine allergen groups, house dust mite, fish, arthropods, epithelia, foods, moulds, grasses, weeds and trees including CJ pollen allergen were examined. The serum sample from this case was positive for IgE directed to CJ pollen and 6-grass mix, but negative for the other 22 allergens. Hence, on the basis of the positive reactions to CJ pollen in intradermal testing and the IgE tests, this dog was identified as being sensitive to CJ pollen.

For further investigation on the reactivity against major allergens of CJ pollen, we performed intradermal testing and IgE tests using purified major CJ pollen allergens, Cry j 1 and Cry j 2. In the intradermal testing,

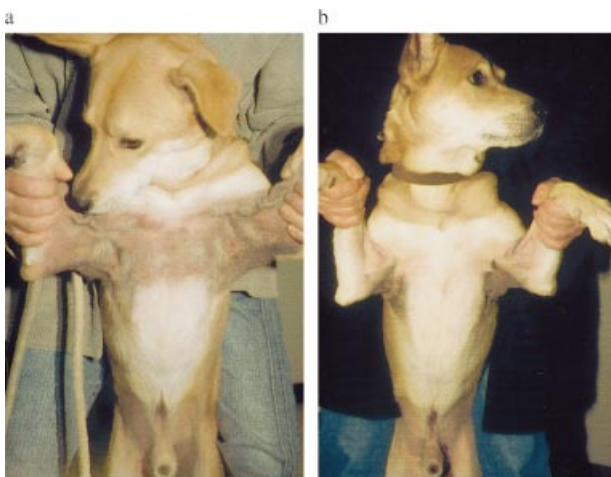


Figure 1. (a) Typical skin lesions in case 1. Marked erythema, alopecia, hyperpigmentation and lichenification were observed in the axillal, elbow, cranial thoracic and inguinal regions. These skin lesions were exacerbated each spring, the season of CJ pollination in Japan (this picture was taken in March). (b) Spontaneous improvement of skin lesions in case 1. The signs improved significantly in regards to pruritus and hair re-growth (this picture was taken in November).



Figure 2. Results of the intradermal skin test using CJ pollen extract in case 1. Reactive wheals were observed at the injection sites of crude CJ pollen extract (upper left) and Cry j 1 (upper centre): the sizes of these wheals were almost equal to that of histamine solution of the positive control (lower left). There was no wheal formation at the injection site of Cry j 2 (upper right), similar to that at the injection site of physiological saline as the negative control (lower right).

a positive response was observed after injection with Cry j 1 solution at a concentration of 200 ng mL^{-1} , showing a reactive wheal estimated as +3, but negative for Cry j 2 (also tested at 200 ng mL^{-1}) (Fig. 2).

The concentrations of IgE specific to Cry j 1 and Cry j 2 were assayed by a fluorometric ELISA using a method described previously.¹⁰ Several dilutions of serum from the dogs were added to the wells of a microplate (Immulon 2, Dynatech, Chantilly, VA, USA) coated with Cry j 1 and Cry j 2 ($10 \mu\text{g mL}^{-1}$), followed by incubation for 3 h at room temperature. After washing, the wells were incubated with mouse monoclonal antidog IgE antibody ($0.5 \mu\text{g mL}^{-1}$) at 4°C overnight.¹⁴ Using biotinylated rat monoclonal antimouse IgG₁ (Zymed Laboratories, San Francisco, CA, USA), β -D-galactosidase-conjugated streptavidin (Zymed Laboratories) and 0.1 mM 4-methylumbelliferyl- β -D-galactoside (Sigma Chemical Co., MO, USA), the enzyme reaction was carried out and stopped with 0.1 M glycine-NaOH (pH 10.2). The fluorescence intensity was read as fluorescence units (FU) on a microplate fluorescence reader (Fluoroskan, Flow Laboratories, McLeane, VA, USA). The concentration of specific IgE was expressed as an arbitrary unit (U mL^{-1}) calculated from the standard titration curves of pooled dog sera. In our dog, the concentration of serum IgE specific to Cry j 1 was 996 U mL^{-1} , whereas the IgE specific to Cry j 2 was below the detectable level ($< 5 \text{ U mL}^{-1}$) when marked skin lesions and severe pruritus were observed in March, coinciding with CJ pollination. The concentration of the IgE against Cry j 1 in November, the off-season of CJ pollination, decreased to 243 U mL^{-1} (Fig. 3). The seasonal change of the serum IgE concentration against Cry j 1 corresponded to the degree of skin lesions, suggesting an involvement of type I hypersensitivity to Cry j 1 in the development of atopic dermatitis in this case.

As a lymphocyte stimulation test, proliferative responses of peripheral blood mononuclear cells (PBMCs) to the crude antigen of CJ pollen and Cry j 1 were tested in March by a method described previously.⁹ PBMCs were collected by density gradient centrifuga-

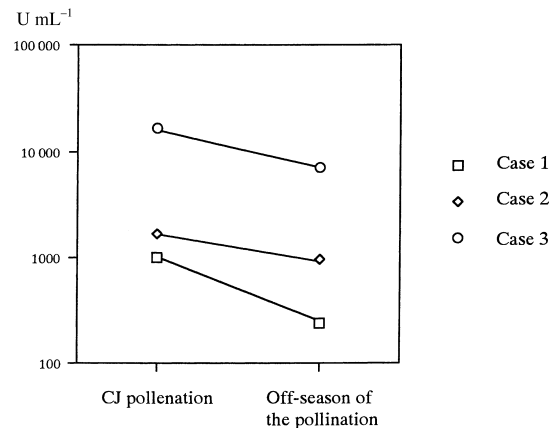


Figure 3. Seasonal change in the serum IgE specific to Cry j 1 in the three dogs sensitive to CJ pollen allergen. Serum samples of the dogs collected during the season of CJ pollination (March) and during the off-season of CJ pollination (September or November) were examined for the level of serum IgE specific to Cry j 1 with fluorometric ELISA.

tion using Ficoll-Hypaque (Lymphoprep, NYCOMED PHARMA AS, Oslo, Norway) at 350 g and suspended in phosphate-buffered saline at 4°C overnight. The cell suspension was overlaid on heat-inactivated fetal bovine serum and centrifuged at 400 g , and the cells were finally suspended in Dulbecco's modified Eagle's medium (DMEM) containing 5% pooled dog serum and antibiotics (penicillin 100 U mL^{-1} and streptomycin $100 \mu\text{g mL}^{-1}$) at a cell count of $1.25 \times 10^6 \text{ mL}^{-1}$. A $200\text{-}\mu\text{L}$ aliquot of cell suspension was dispensed into a well of a 96-well plate and incubated for 72 h at 37°C with either crude CJ pollen extract or Cry j 1 at a concentration of $3 \mu\text{g mL}^{-1}$. Cell proliferative responses were measured in triplicate cultures by incorporation of ^3H -thymidine (37 kBq mL^{-1}) for 18 h and stimulation index was calculated (cpm in antigen-stimulated PBMCs/cpm in nonstimulated PBMCs). A stimulation index of > 2.0 was considered to be positive in this study. In this patient, apparent proliferative responses of PBMCs collected in March were detected by stimulation with crude CJ pollen extract and Cry j 1; the stimulation indices for the crude CJ pollen extract and Cry j 1 were 3.1 and 3.2, respectively (Fig. 4). To examine mitogenic responses of healthy dog PBMCs against the crude antigen of CJ pollen and Cry j 1, blastogenic responses to the allergens were measured by the same method in three healthy Beagles negative for IgE against CJ pollen allergen by the Topscreen and Immunodot kits. Stimulation indices against either the crude antigen of CJ pollen and Cry j 1 in the three dogs were < 2.0 with the mean stimulation indices of 0.7 and 1.1 against the crude antigen of CJ pollen and Cry j 1, respectively. Stimulation indices in PBMCs simultaneously cultured with concanavalin A ($5 \mu\text{g mL}^{-1}$) were markedly high ($48.0\text{--}268.6$), indicating that the cell culture was accomplished appropriately in the three dogs. From these data, we concluded that there were no mitogenic effects on PBMCs from dogs in the crude antigen of CJ pollen and Cry j 1, that the proliferative response in the allergic patient was above normal.

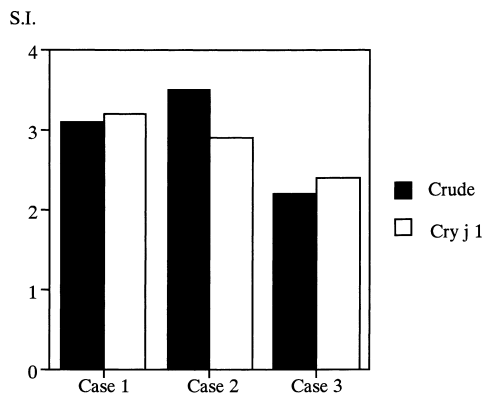


Figure 4. Lymphocyte proliferative responses to crude CJ pollen extract and Cry j 1. PBMCs from the three dogs sensitive to CJ pollen collected during the season of CJ pollination. The PBMCs were cultured in the presence of crude CJ pollen extract and Cry j 1 at a concentration of $3 \mu\text{g mL}^{-1}$. Stimulation indices were calculated from the cpm of incorporated ^3H -thymidine in the stimulated PBMCs and unstimulated PBMCs. As a negative control, PBMCs from three healthy Beagles negative for IgE against CJ pollen allergen did not proliferate against the stimulation of those antigens, showing all the stimulation indices were < 2.0 . No statistical analysis on stimulation indices between the cases and normal control was carried out because of the small sample size.

Based upon the positive test results above, the dog was diagnosed as sensitive to CJ pollen. The major allergen was Cry j 1 identified by both IgE and lymphocytes reactive to CJ pollen allergen. Seasonal variations in anti-CJ IgE levels were correlated with seasonal onset of atopic dermatitis in this dog.

Case 2

A 7-year-old spayed female West Highland White Terrier dog was presented because of skin lesions similar to those seen in case 1. These skin lesions became apparent during the spring. Clinical signs were considered suggestive of atopic dermatitis, based on Willemse's criteria.¹¹ Other pruritic skin diseases including parasite infestation and infectious skin diseases were excluded by the negative results of routine dermatologic examinations. Food allergy was not suspected from clinical history in this case because of the apparent seasonality of the symptoms not related to changing in diet.

The intradermal testing and IgE tests were performed as described in case 1. The intradermal testing in this dog showed a positive reaction to crude allergen of CJ pollen with the wheal size estimated as +3. The measurement of antigen-specific IgE by the Topscreen and Immunodot test kits showed that the dog was positive only for CJ pollen. Intradermal reactions to Cry j 1 and Cry j 2 were positive with the wheal sizes estimated as +3. The ELISA for IgE against Cry j 1 and Cry j 2 showed an increased concentration of IgE against Cry j 1 at 1688 U mL^{-1} in March (Fig. 3), but IgE against Cry j 2 was not detectable ($< 5 \text{ U mL}^{-1}$). The IgE concentration against Cry j 1 decreased to 975 U mL^{-1} when measured in September (Fig. 3).

Cell proliferative responses of PBMCs against crude antigen of CJ pollen and Cry j 1 were examined using

the same method described in case 1 in March. Stimulation indices were found to increase against both the crude antigen of CJ pollen and Cry j 1 at 3.5 and 2.9, respectively (Fig. 4). It was suggested that most of the lymphocytes reactive to crude antigen of CJ pollen would be reactive to Cry j 1.

Thus, the dog was diagnosed as being sensitive to CJ pollen, particularly Cry j 1, which may explain the exacerbation of the skin lesions during spring, the season of CJ pollination.

Case 3

A 2-year-old female Yorkshire Terrier dog showed seasonal pruritus with mild skin lesions such as papules and excoriation in the regions from medial thighs to the lower ventral abdomen during spring. Atopic dermatitis was diagnosed, based on the same criteria used in the two previous cases and other pruritic skin diseases were also excluded by routine skin examinations and clinical history, similar to the previous cases.

In this case, the intradermal testing and IgE tests were carried out using the same method as used for case 1. A positive reaction was found only against the crude antigen of CJ pollen, with wheal sizes estimated as +3. The Topscreen and Immunodot test kits showed IgE only against CJ pollen allergen. As for reactions to the major allergens of CJ pollen, a reactive wheal was formed only against Cry j 1 with a wheal size of +3. The ELISA for IgE against Cry j 1 and Cry j 2 showed an IgE concentration against Cry j 1 of $16\,660 \text{ U mL}^{-1}$ in March, while IgE for Cry j 2 was not detected. The concentration of IgE for Cry j 1 in September decreased to 7227 U mL^{-1} .

Proliferative responses of PBMCs against crude antigen of CJ pollen and Cry j 1 were measured using the method described in case 1. Positive cell proliferation was also seen in this case and stimulation indices were 2.2 and 2.4 against crude antigen of CJ pollen and Cry j 1, respectively.

Because of the apparent seasonality of clinical signs and the data of *in vivo* and *in vitro* allergy tests, the dog in this case was also considered to be sensitive to CJ pollen allergen, especially recognizing Cry j 1.

DISCUSSION

We report here that three atopic dogs with a history and clinical findings consistent with atopic dermatitis caused by CJ pollen were reactive against one of its major allergens, Cry j 1, in *in vivo* and *in vitro* allergy tests, indicating the occurrence of type I hypersensitivity to Cry j 1 in these canine patients, similar to what has been reported in humans and monkeys with CJ pollinosis.¹⁵ As additional evidence for sensitization to Cry j 1 in this study, the degree of proliferative response of PBMCs to crude CJ pollen extract was similar (Cry j 1 in all the cases) with the higher stimulation indices than those in healthy dogs (statistical comparison was not carried out because of the small sample size). This

suggests that most of the lymphocytes reactive to the crude antigen of CJ pollen were recognizing Cry j 1. Cry j 1 is the major allergen in human patients with CJ pollinosis, and T-cell epitopes were identified in Cry j 1.¹⁶ Our recent study measuring IgE against Cry j 1 and Cry j 2 indicated that atopic dogs sensitive to Cry j 1 were seen more frequently than those sensitive to Cry j 2,¹⁰ and it was therefore suspected that Cry j 1 was a major allergen in the dogs sensitive to CJ pollen with regard to antigen recognition. Thus, because of the apparent recognition of Cry j 1 by both IgE and lymphocytes, we demonstrated that Cry j 1 is the crucial component of CJ pollen allergen for the sensitization to CJ pollen in these dogs. Similar to human CJ pollinosis, T- and B-cell epitopes of CJ pollen allergen should be identified in Cry j 1 in atopic dogs.

The concentrations of IgE specific to Cry j 1 during the season of CJ pollination (spring) were higher than those measured in its off-season (autumn) in all the dogs in this study. This seasonal fluctuation of the antigen-specific IgE concentrations correlated well with the deterioration and amelioration of atopic dermatitis in these patients. Because similar seasonal fluctuations of IgE specific to CJ pollen allergen were reported in humans with CJ pollinosis and correlated with the onset of clinical signs,¹⁷ it is strongly suspected that the development of IgE against Cry j 1 is involved in the pathogenesis of atopic dermatitis in these dogs. The seasonal pattern of IgE concentration change, corresponding with clinical signs, supports a role for IgE-mediated hypersensitivity in CJ pollinosis. In our previous study carried out in Japan, 7 (16.7%) of 42 atopic dogs examined were found to be positive to CJ pollen allergen by serum testing for allergen-specific IgE, showing that a relatively high population of atopic dogs were sensitive to CJ pollen allergen.¹²

Atopic dermatitis was the predominant clinical manifestation of CJ pollinosis in these canine patients. In human CJ pollinosis, the major clinical signs are rhinitis and conjunctivitis associated with a seasonal increase in the levels of serum CJ-specific IgE and histamine release against CJ pollen allergen.⁴ There is conflicting evidence concerning the differences in clinical signs of CJ pollinosis between humans and dogs. Nasal discharge was reported previously in CJ pollen-sensitive dogs with atopic dermatitis when they were experimentally exposed to CJ pollen extract.⁸ This indicates that dogs sensitive to CJ pollen allergen experimentally may show both skin and nasal signs when challenged with the antigen, however, typically dermatitis may be the chief complaint. A recent study in our laboratory indicated that healthy dogs experimentally sensitized to CJ pollen allergen with alum adjuvant did not show clinical signs of atopic dermatitis in spite of increased level of IgE against CJ pollen allergen.¹⁸ This suggests that additional factor(s) other than increased IgE antibody concentrations against the allergen is required to cause clinical atopic dermatitis in dogs. In humans with atopic dermatitis associated with CJ pollinosis, a report indicated a correlation between *in vitro*

IgE testing and patch testing against CJ pollen allergen, suggesting that both type I hypersensitivity and type IV hypersensitivity could be involved in the development of atopic dermatitis.¹⁹ A similar pathogenesis may be concerned in the development of atopic dermatitis in dogs sensitive to CJ pollen. Because the significant proliferation of PBMCs against Cry j 1 in this study could also occur as a reaction of type IV hypersensitivity, it is possible that type IV hypersensitivity to the allergens, in part, plays a role in the onset and exacerbation of atopic dermatitis together with the reactions of type I hypersensitivity in the spontaneous CJ pollinosis in dogs.

Disagreement of results between intradermal testing and IgE testing was seen in case 2 in terms of reactivity to Cry j 2. This could be mainly due to different sensitivity and specificity between both tests. Intradermal testing has a high sensitivity, although IgE testing shows a high specificity with a cut-off value.²⁰ Intradermal reaction would occur against Cry j 2 if IgE against Cry j 2 existed at a low concentration undetectable by ELISA. Besides, one might consider contamination of Cry j 1 in Cry j 2 solution as another possibility for the intradermal reaction to Cry j 2. However, a concentration of Cry j 1 contaminated in Cry j 2 solution should be quite low and would not provoke the strong intradermal reaction graded as +3 only in case 2.

In conclusion, this study described canine patients with atopic dermatitis showing type I hypersensitivity to Cry j 1, a major allergen of CJ pollen. Evidence of sensitivity was provided by intradermal testing, *in vitro* allergen-specific IgE testing and lymphocyte stimulation testing. Seasonal clinical signs appeared to correlate with allergen-specific IgE levels. A larger number of CJ-sensitive dogs should be studied over a long period to see if this seasonal fluctuation is characteristic of CJ pollinosis in dogs.

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Résumé Trois chiens ont été présentés pour des épisodes récidivants de dermatite prurigineuse pendant le printemps, période de pollinisation du cèdre du japon (*Cryptomeria japonica*, CJ) au Japon. La sensibilisation de ces animaux aux allergènes de pollen du CJ a été démontrée par test intradermique et mesure des IgE spécifiques. Un test ELISA a montré des concentrations augmentées d'IgE sépcifiques de Cry j 1 et un résultat négatif pour Cry j 2 chez ces trois chiens. Les concentrations en IgE spécifiques de Cry j 1 pendant la saison de pollinisation du CJ étaient plus élevées que les concentrations mesurées en dehors de cette période. La variation des concentrations était corrélée avec l'intensité des signes cliniques. Les cellules sanguines périphériques mononucléées ont montré une réponse proliférative apparente à la stimulation par un antigène brut de pollen de CJ et par le Cry j 1 pendant la saison de pollinisation. Ces observations indiquent que Cry j 1 est l'allergène majeur reconnue par les IgE et les lymphocytes de ces chiens atopiques, à l'origine du développement d'une hyeprsensibilité de type I.

Resumen Se examinaron tres perros debido a episodios de dermatitis prurítica recurrente en primavera, la estación de polinización en Japón del cedro japonés (*Cryptomeria japonica*, CJ). Los perros mostraron sensibilidad al antígeno de polen de CJ utilizando la prueba intradérmica y la medición de IgE antígeno-específico. La prueba ELISA mostró un incremento en la concentración de IgE específico a Cry j 1 y negativo a Cry j 2 en tres perros. Las concentraciones de IgE específico a Cry j 1 durante la estación de polinización de CJ fueron más altas que las concentraciones halladas durante las otras estaciones en todos los perros, y la variación en las concentraciones se correlacionaban con la variación de los síntomas clínicos. Las células sanguíneas mononucleares mostraron respuestas proliferativas aparentes a antígeno de polen de CJ puro y a Cry j 1 durante la estación de polinización de CJ. Estos hallazgos indicaban que Cry j 1 era el alérgeno más reconocido por IgE y por linfocitos, y resultaron en el desarrollo de una hipersensibilidad de tipo I a alérgeno de polen de CJ en estos perros atópicos.

Zusammenfassung Drei Hunde wurden auf Grund von Episoden rezidivierender, juckender Dermatitis im Frühling, der Blütezeit der Japanischen Zeder (*Cryptomeria japonica*, CJ) in Japan vorgestellt. Die Hunde waren gegen CJ Pollen sensibilisiert, wie mit Intrakutantest und Bestimmung von Antigen-spezifischem IgE gezeigt wurde. Fluorometrischer "Enzyme-linked Immunosorbent Assay" (ELISA) zeigte erhöhte Konzentrationen von IgE spezifisch für Cry j 1 und ein negatives Resultat für Cry j 2 bei drei Hunden. Die Konzentration von für Cry j 1 spezifisches IgE war bei allen Hunden in der Pollensaison von CJ höher als ausserhalb dieser Saison und die Änderung der Konzentrationen korrelierte mit der klinischen Symptomatik. Periphere mononukleäre Blutzellen zeigten eine proliferative Reaktion gegen CJ Pollen Antigen und Cry j 1 während der CJ Pollensaison. Diese Befunde deuten darauf hin, dass Cry j 1 das von IgE und Lymphozyten erkannte bedeutendste Allergen war und in der Entwicklung einer Typ 1 Überempfindlichkeitsreaktion gegen CJ Pollenallergen bei diesen atopischen Hunden resultierte.

