

Lymphocyte Blastogenic Responses to Inciting Food Allergens in Dogs with Food Hypersensitivity

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Lymphocyte blastogenic responses against food allergens in dogs with food hypersensitivity were evaluated in this study. Eleven dogs with food hypersensitivity, based on food elimination and oral food provocation tests and allergic responses to food allergens, were examined by various tests such as intradermal testing, antigen-specific IgE testing, and lymphocyte blastogenic responses. The number and kinds of food allergens identified as positive by these tests were compared with the offending food allergens that were found in an oral food provocation test. In 9 (82%) of the 11 dogs with food hypersensitivity, there was close agreement for positive allergens between the results of lymphocyte blastogenic responses and oral food provocation test; however, there was little agreement for intradermal and IgE testing of the positive allergens with those of the oral food provocation test (11% and 31%, respectively). In the 9 dogs, the stimulation indices of lymphocyte blastogenic responses increased to 2.0–10.1 upon food provocation but decreased significantly to 0.7–1.4 upon feeding the elimination diet until clinical signs disappeared. These results indicate that lymphocyte blastogenic responses may fluctuate because of exposure to offending food allergens in dogs with food hypersensitivity. Lymphocytes reactive to food allergens may play an important role in the pathogenesis of food hypersensitivity in dogs.

Key words: Elimination diets; Food provocation; Immunoglobulin E; Intradermal testing.

Food hypersensitivity in dogs is a common disease caused by allergic reactions to various kinds of food antigens, which provoke clinical signs such as pruritic skin disease,¹ diarrhea, and vomiting. Manifestation of clinical signs occurs after the intake of offending food antigens.² The pathogenesis of food hypersensitivity in dogs may be mediated by type I, type III, and type IV hypersensitivity³; however, the mechanism is not fully understood. Although intradermal testing and antigen-specific serum IgE testing showed positive reactions to a limited number of food allergens in food hypersensitivity of dogs, the sensitivity of these two tests for the identification of offending food allergens is not as high as that of the oral food provocation test.⁴ Therefore, it has been suggested that non-IgE mediated hypersensitivity might be associated with the pathogenesis of food hypersensitivity in dogs.

Various kinds of food ingredients induce allergic responses in dogs.² Beef antigen is the most common food allergen in dogs; 60% of the dogs with food hypersensitivity develop clinical signs after oral provocation of beef.² Other food allergens such as chicken, egg, soybean, milk, corn, and wheat are also allergenic in dogs with food hypersensitivity.⁵ Because 64% of dogs with food hypersensitivity develop clinical signs in response to >2 kinds of food ingredients during food provocation tests,⁵ complex diagnostic procedures may be needed.

The food elimination test followed by the oral food provocation test is considered necessary for the diagnosis of food hypersensitivity in dogs⁴; however, an alternative di-

agnostic test to identify offending food allergens in hypersensitive dogs is desirable to lessen the dependence on owner compliance for test procedures and results. Serum antigen-specific IgE testing and intradermal testing are diagnostic tests to identify allergens that induce IgE-mediated reactions in dogs with atopic dermatitis.⁶ The sensitivity of serum antigen-specific IgE testing with an enzyme-linked immunosorbent assay is low (14%), but its specificity is high (87%).⁴ The sensitivity and specificity of intradermal testing for identification of allergens in food hypersensitivity are 10% and 96%, respectively.⁴ Therefore, neither test is useful for identifying food allergens in dogs with food hypersensitivity.⁴

The lymphocyte stimulation test is useful for the diagnosis of food hypersensitivity in humans.⁷ Lymphocytes collected from human patients with food hypersensitivity proliferate in vitro and produce interleukin 2 after stimulation with the offending food allergens.⁸ The proliferating cells are helper T cells, which induce IgE production from B cells and effector T cells and initiate delayed type hypersensitivity.⁹ Thus, the lymphocyte stimulation test was considered to be useful for identifying any reaction associated with T-cell responses against antigens. In this study, a lymphocyte stimulation test was applied to identify offending food allergens in dogs with food hypersensitivity. The association between clinical manifestations and the degree of lymphocyte responses against food allergens also was investigated during food elimination and provocation.

Materials and Methods

Dog Selection

Eleven dogs were prospectively suspected to be food hypersensitive based on clinical signs such as pruritus and gastrointestinal symptoms associated with the individual history of food intake. The study was conducted during 2 years (1999 and 2000) in the Veterinary Medical Center (VMC) at the University of Tokyo. The diagnosis of food hypersensitivity was made based on the results of food elimination and provocation tests. Clinical signs of all the dogs disappeared during administration of elimination diets and relapsed upon oral exposure to food ingredients. Dogs that did not show improvement of clinical signs after food elimination were excluded from this study, although food hypersensitivity was suspected.

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Among the 11 dogs, 4 (dogs 1–4) had gastrointestinal signs, including chronic vomiting and/or diarrhea, and the remaining 7 (dogs 5–11) manifested pruritic skin disorder. In the 4 dogs with gastrointestinal signs, the dog owners reported association between clinical manifestation and the intake of specific food ingredients. Other diseases causing vomiting and diarrhea (except for inflammatory bowel disease) were ruled out by tests such as fecal examination, CBC, serum chemistry profile, and abdominal radiographs. There were no abnormalities in the test results in the 4 dogs. Inflammatory bowel disease was excluded after examination of the responses to food elimination and provocation tests.

The remaining 7 dogs had various kinds of skin lesions such as erythema with hair loss around the eyes and mouth, otitis externa, seborrhea on the back, angioedema, urticaria, papular eruptions on the ventral abdomen, and systemic pyoderma with persistent pruritus. Parasitic infestations with *Demodex canis*, scabies, and fleas were excluded by skin scraping and careful observation for flea feces. Fungal infection was excluded by negative results of ultraviolet light examination, and for suspected cases, fungal culture was further performed. Bacterial infection was excluded when clinical signs were not improved by a 2-week administration of antibiotics. The differential diagnosis of food hypersensitivity from atopic dermatitis was made based on the results of the elimination and provocation tests.

As a healthy control group, 6 castrated male Labrador Retrievers were used. The dogs were kept at the VMC for experimental purposes to obtain control samples. The dogs were not related to each other. The mean age of the 6 dogs was 9 years (range 8–10 years). None of the dogs showed clinical signs of skin or gastrointestinal problems.

Food Elimination Test and Oral Food Provocation Test

Commercial^a or homemade foods (potato or rice based) were used for the food elimination portion of the study. The optimal food for the elimination trial was selected according to the individual diet history. Food elimination trials were conducted for up to 8 weeks. When the clinical signs had completely disappeared (hair regrowth without pruritus in the lesion), the elimination trials were discontinued and food provocation tests were conducted. The period of food elimination was >3 weeks in all the dogs, with a maximum of 8 weeks, until complete resolution of clinical signs was obtained.

When the complete resolution of clinical signs was recognized by the dog owners during the food elimination, the dogs were then challenged with various kinds of food ingredients, including beef, chicken, cod, corn, chicken egg, milk, rice, and wheat. The food ingredients used for the food provocation test were selected based on the dietary history in each case. Each ingredient was fed at approximately 5 g on day 1, and the dose was gradually increased up to day 7 (day 2, 10 g; day 3, 20 g; day 4, 40 g; day 5, 80 g; days 6 and 7, 100 g). The food provocation test was discontinued immediately when clinical signs relapsed. After the relapse, food elimination was instituted again until the clinical signs were completely resolved. The next food provocation tests was then started.

The control dogs were fed 9 kinds of food ingredients (beef, chicken, milk, egg, rice, wheat, corn, cod, and tuna) as oral food provocation. Each food ingredient was fed for 1 week.

Antigen-Specific IgE Testing

Serum concentrations of IgE specific for 25 antigens were measured in the 11 trial dogs and the control dogs using 2 commercially available test kits^b as previously described.¹⁰ The Topscreen test is a 5-spot screening test using mixtures of allergens such as outdoor, indoor, food, and mold allergen groups. The Immunodot test is used to detect individual antigen-specific IgEs. The antigens examined for the antigen-specific IgE testing in this study were *Bos taurus* (meat and milk), *Sus scrofa* (pork), *Gallus gallus/domesticus* (egg), *Triticum aestivum*

(wheat), *Zea mays* (corn), *Glycine max* (soy bean), *Gadus morhua* (cod) and *Paralichthys olivaceus* (sole) mix, *Arachis hypogaea* (peanut), 6-grass/rye mix (*Dactylis glomerata*, cocksfoot; *Festuca elatior*, meadow fescue; *Lolium perenne*, perennial rye; *Phleum pratense*, timothy; *Poa pratensis*, Kentucky blue grass; *Holcus lanatus*, yorkshire), *Secale cereale* (rye), *Artemisia vulgaris* (mugwort), *Plantago lanceolata* (ribwort), *Cryptomeria japonica* (Japanese cedar), *Olea europea* (olive), *Parietaria judaica* (wall pellitory), a 3-tree mix of *Betula* spp.(birch), *Quercus* spp. (oak), and *Corylus avellana* (hazel), house dust mite mix (*Dermatophagoides farinae* and *D pteronyssinus*), *Acarus* spp. and *Tyroglyphus* spp. (storage mites), *Ctenocephalides felis* (cat flea), human dander, *Felis domesticus* (cat epithelia), *Ovis aries* (sheep wool), *Candida albicans*, *Penicillium notatum*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Alternaria alternaria*. Measurement of IgE against antigens of rice and chicken is not available in the test kits. Nitrocellulose strips each containing an allergen test mixture for the Topscreen or an individual allergen for Immunodot were prepared by the manufacturer.^b Serum samples were collected from all dogs with clinical signs at the first admission to the VMC. After serum samples were spotted on individual nitrocellulose strips and incubated for 2 hours at room temperature, peroxidase-conjugated monoclonal anti-canine IgE antibody and substrate solution were applied to the strips for colorimetric detection. An optical density (OD) value of >1.0 in the Topscreen and >2.0 in the Immunodot were considered positive for the allergen group and specific allergen, respectively.

Intradermal Testing

Intradermal testing for all treatment and control dogs was performed using the method previously established.¹¹ Crude extracts of 29 allergens were purchased from a commercial laboratory^c except for the extract of Japanese cedar pollen, which was prepared as reported previously.¹² The allergen extracts used in this study were classified into 4 groups: foods, outdoor and indoor allergens, and molds. The food allergens used in this study were *Bos taurus* (meat and milk), *Gallus gallus/domesticus* (meat and egg), *Oryza sativa* (rice), *Triticum aestivum*, *Zea mays*, *Gadus morhua*, and *Thunnus* sp. (tuna). The extracts of outdoor allergens consisted of sage mix (*Salvia azurea*, prairie sage; *Artemisia tridentata*, common sagebrush), *Rumex crispus* (yellow dock), national weed mix (*Xanthium strumarium* cocklebur; *Chenopodium album*, lambs quarter; *Amaranthus retroflexus*, rough pigweed; *Ambrosia trifida*, giant ragweed; *Ambrosia artemisiifolia*, short ragweed), a 7-grass/rye mix (*Poa pratensis*; *Dactylis glomerata*, orchard; *Agrostis alba*, redtop; *Phleum pratense*, timothy; *Anthoxanthum odoratum*, sweet vernal; *Festuca elatior*; *Lolium perenne*), eastern oak mix (*Quercus velutina*, *Q rubra*, *Q alba*), *Betula populifolia* (white birch), *Cryptomeria japonica*, pine mix (*Pinus taeda*, *P strobes*, *P echinata*), and elm mix (*Ulmus americana*, *U pumila*). The indoor allergens were *D pteronyssinus*, *D farinae*, *Ctenocephalides felis*, a 4-insect mix (*Periplaneta americana*, cockroach; *Musca domestica*, house fly; *Culicidae* spp., mosquito; *Heterocera* spp., moth), and *Felis catus/domesticus* (epithelia). The mold allergens included 6 extracts: *Alternaria tenuis*, *Curvularia spicifera*, *Rhodotorula rubra*, *Mucor* mix (*Mucor plumbeus*, *M racemosus*), *Aspergillus* mix (*A flavus*, *A fumigatus*, *A glaucus*, *A nidulans*, *A niger*), and a *Penicillium* mix (*P camembertii*, *P chrysogenum*, *P digitatum*, *P notatum*, *P roquefortii*). Each allergen extract was diluted with isotonic saline solution. The final concentrations of the 9 food allergens were 1:20,000 (w/v). The extracts of flea, *D pteronyssinus*, and *D farinae* were diluted to 1:5,000 (w/v). The extract of Japanese cedar pollen was used at a concentration of 200 ng/mL. The other 17 allergen extracts were used at a concentration of 1,000 PNU/mL. Histamine solution (0.0275 mg/mL) and 0.9% saline solution were used as the positive and negative controls, respectively. After the dogs were sedated by IM injection of medetomidine hydrochloride^d (0.02 mg/kg) and midazolam^e (0.3 mg/kg), 0.05 mL of each allergen solution was injected intradermally by a single clinician

into the clipped skin of the ventrolateral thorax using a skin test syringe with a 26-ga needle. The diameters of wheals were measured 15–20 minutes after the injection, and a reactive wheal was graded on the basis of size as follows: 3+, equal to or greater than the diameter of the positive control; 2+, equal to or greater than the mean diameters of the positive and negative controls; 1+, larger than the diameter of the negative control but smaller than the mean diameters of the positive and negative controls; –, equal to or smaller than the diameter of the negative control. A wheal size of 2+ or larger was considered indicative of a positive reaction.

Lymphocyte Stimulation Test

The lymphocyte stimulation test (LST) was performed at least 2 times (food provocation phase and food elimination phase) in each dog. Peripheral blood samples were collected from 1 day to 3 weeks after the relapse of clinical signs during the oral food provocation test (provocation phase). Clinical signs were still observable (of variable severity) in all the dogs when the samples were collected. After the 1st test, the 2nd test (elimination phase) was performed at a time when the clinical signs had disappeared after restarting the food elimination trial. In the elimination phase, there were no clinical signs in any of the dogs.

To confirm that lymphocyte blastogenic responses against food antigens were specific reactions, the blood samples of dogs 4, 6, 9, and 10 during provocation of nonoffending food ingredients were also collected within 3 weeks after the end of provocation with each food ingredient (dog 4, beef, milk, rice, and cod; dog 6, milk and egg; dog 9, chicken; dog 10, chicken, milk, and egg) because the owners of these dogs agreed to the blood collection for use as control samples. For the healthy controls, the LST was performed with 9 kinds of food ingredients (beef, chicken, milk, egg, rice, wheat, corn, cod, and tuna) on day 7 of the 1-week provocation, when no clinical signs had developed.

The procedure of the LST was described previously.¹³ Heparinized peripheral blood samples were diluted with an equal volume of phosphate-buffered saline and layered on Ficoll-Hypaque.[†] After centrifugation at $350 \times g$ for 40 minutes at room temperature, a layer containing the peripheral blood mononuclear cell fraction was obtained and suspended in Dulbecco's modified Eagle's medium (DMEM)[§] containing 5% heat-inactivated dog serum and antibiotics (penicillin,^h 100 U/mL; streptomycin,[†] 0.1 mg/mL) at a cell count of 1.25×10^6 cells/mL. Two hundred microliters of the cell suspension was allocated to each well of a 96-well plate and incubated at 37°C for 72 hours under stimulation with various crude food extracts^c at a concentration of 5 µg/mL. Blastogenic responses of the antigen-stimulated and unstimulated groups were measured by assaying the incorporation of ³H-thymidine (38 kBq/well) for an additional 18 hours. The incorporation was measured using a liquid scintillation counter.[†] The stimulation index was calculated with the following formula: counts per minute (cpm) of culture stimulated with food antigen ÷ cpm of unstimulated culture. A stimulation index of ≥ 2.0 was considered indicative of a positive reaction based on the criteria previously used.¹³

Data Analysis

The values for the stimulation indices were compared between dogs with food hypersensitivity and control dogs with the paired *t*-test at an α level of .05 (1-tailed). The difference in stimulation index value between the 1st and 2nd tests was also analyzed with the same method.

Results

Dogs

The breeds of dog included in this study were Beagle (2 dogs), Shih Tzu (2), Cavalier King Charles Spaniel (2), Bernese Mountain Dog (1), Labrador Retriever (1), French

Table 1. Comparison of results for various allergy tests in 11 dogs suspected of having food hypersensitivity and in 6 healthy control dogs.

Dog No.	Allergens ^a	IgE test	IDT	LST at provocation
1	Beef	+	+	3
2	Beef	+	+	2.9
	Cod	–	–	3.3
3	Beef	–	–	4.4
4	Chicken	NT	–	2.8
	Wheat	+	–	2.5
5	Rice	NT	–	10.1
6	Wheat	–	–	2
7	Beef	–	–	2.1
	Chicken	NT	–	2
8	Beef	+	–	1.2
	Milk	–	–	1.7
9	Beef	–	–	1.6
	Rice	NT	–	1.9
10	Beef	–	–	2.6
	Corn	+	–	2.8
11	Beef	–	–	2.1
	Milk	–	–	2.3
Control dogs (n = 6)	None ^b	–	Milk (1 dog)	0.4–1.5

IDT, intradermal test; LST, lymphocyte stimulation test; NT, not tested.

^a Offending food allergens were identified by oral food provocation test.

^b Control dogs were challenged with beef, chicken, milk, egg, rice, wheat, corn, cod, and tuna.

Bulldog (1), Maltese (1), and mixed breed (1). The mean age of dogs was 4.7 years (range 1–13 years). The dogs included 6 females (4 spayed) and 5 males (4 castrated). Most of the dogs showed the clinical signs at an early age; mean age of 1st onset of clinical signs was 12.6 months. Five (46%) of the 11 dogs showed clinical signs at the age of <1 year, and 5 (46%) of the 11 dogs showed 1st onset of clinical signs at 1–3 years of age. The remained dog (dog 7) had shown clinical signs since 5 years of age.

Oral Food Provocation Test

Food allergens inducing the clinical signs in the dogs were identified by the oral food provocation test (Table 1). Beef was the most common food allergen to induce clinical signs; it was an allergen in 8 (73%) of the 11 dogs. Wheat and rice were offending food allergens in 2 (18%) of the 11 dogs. Regarding the number of offending food ingredients per dog, 4 dogs were reactive to 1 kind of ingredient, 6 dogs were reactive to 2 kinds of ingredients, and 1 dog was reactive to 4 kinds of ingredients. None of the control dogs showed any clinical signs during the provocation test, indicating that there was no food hypersensitivity in the control dogs.

Antigen-Specific IgE Testing

Antigen-specific IgE against various kinds of food antigens was detected for 5 (31%) of the 16 offending food allergens in the 11 dogs with food hypersensitivity (Table 1). Dogs 1 and 2 showed positive reaction to beef in the

IgE testing. In dog 4, 4 kinds of food allergens (cow milk, chicken egg, wheat, and corn) produced positive IgE results. In addition, allergens other than foods such as the mixture of 6 grasses and rye, mugwort and ribwort, olive and wall pellitory, Japanese cedar pollen, 3-tree mix, and house dust mite mix also produced positive reactions in dog 4. Dog 8 had IgE against antigens of house dust mite mix and storage mite mix. Dog 10 reacted to corn and to antigens of Japanese cedar pollen and the mixture of 6 grasses and rye. In the serum from the control dogs, the values of antigen-specific IgE against all of the tested food allergens were below the detectable level.

Intradermal Testing

The results of intradermal testing of food antigens are shown in Table 1. Strong reactions (estimated as 3+) against food antigens were found for only 2 (11%) of the 18 offending food allergens in the 11 dogs (dogs 1 and 2, beef). Moderate reactions (2+) against food antigens were observed in dog 4 for corn, dog 5 for rice, and dog 9 for egg. Dog 4 also showed positive reactions to 7 antigen extracts, including national weed mix, the 6-grass mix, the mixture of sage mix and yellow dock, pine mix, elm mix, eastern oak mix, white birch, and house dust mite mix. No positive reaction was observed against any kinds of food extracts in dog 11, but reactive wheals were found to antigens of *D pteronyssinus* and *D farinae*. In 1 of the 6 control dogs, a moderate reaction was observed for milk; however, this dog did not show any symptoms after a 1-week oral exposure to milk.

LST

The stimulation indices at the provocation phase ranged from 0.7 to 10.1 among the dogs with food hypersensitivity (mean, 2.7; SD, 2.0) (Fig 1). Nine (82%) of the 11 dogs showed lymphocyte blastogenic responses with a stimulation index of >2.0 (Table 1). The stimulation indices for nonoffending food allergens at the provocation phase ranged from 0.5 to 1.7, with a mean value of 1.1. Because the stimulation indices against the 9 food ingredients in the control dogs ranged from 0.4 to 1.5 (mean, 0.9), there was a significant increase of stimulation indices in the dogs with food hypersensitivity at the provocation phase ($P < .01$). Responses with a stimulation index of 2.0 were considered positive because there were no nonspecific responses greater than this value.

The 2nd LST was performed >3 weeks after the 1st examination in the 11 dogs when the clinical signs had completely resolved during the elimination diet phase (Fig 1). The mean stimulation index at the elimination phase was 1.0 (SD, 0.2), indicating a significant decrease of the indices compared with those at the provocation phase ($P < .01$).

Discussion

Diagnosis of food hypersensitivity in dogs has been made based on results of food elimination and provocation tests, which are considered to be the "gold standard."¹⁴ Antigen-specific IgE testing and intradermal testing have been reported as unreliable for detecting offending food allergens

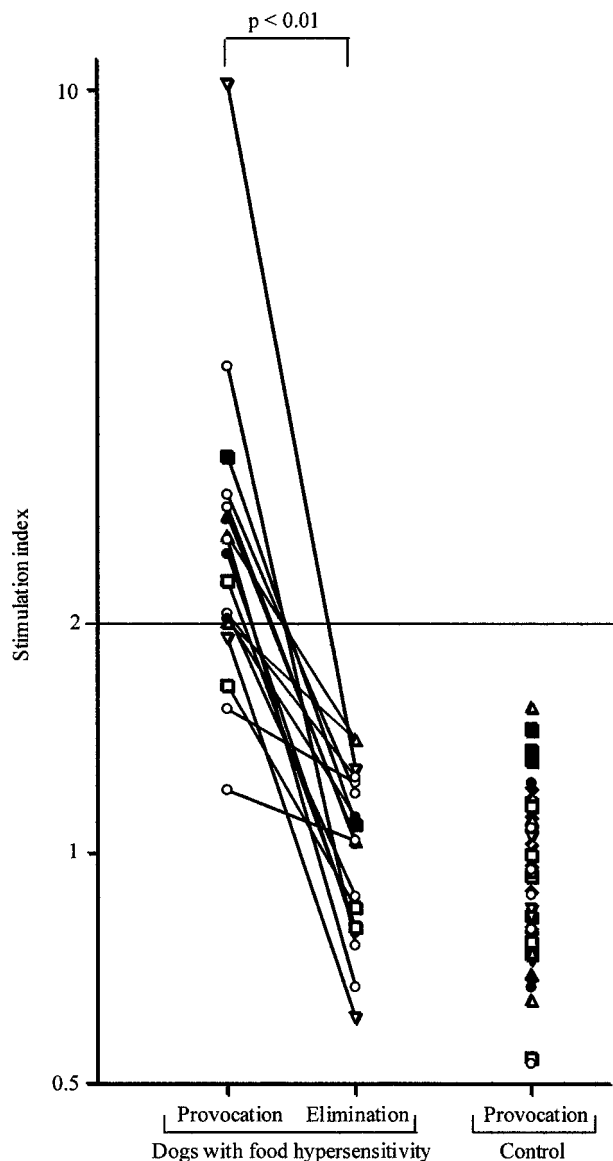


Fig 1. Stimulation indices for the lymphocyte stimulation test against various kinds of food antigens at 2 phases of the food provocation and elimination tests in 11 dogs with food hypersensitivity. Stimulation indices against the food antigens in 6 healthy dogs were used as controls for the food provocation phases. Food extracts used were beef (○), chicken meat (△), milk (□), chicken egg (◇), rice (▽), wheat (●), corn (▲), cod (■), and tuna (◆).

in humans¹⁵ and dogs⁴ with food hypersensitivity. In agreement with those previous studies, the results of intradermal testing and antigen-specific IgE testing in this study showed low correlation with results of oral food provocation tests. This might be due to the presence of non-IgE-mediated mechanisms such as type IV hypersensitivity in the pathogenesis of food hypersensitivity in dogs.⁴ Food intolerance in the dogs in this study is not likely because the results of the LST suggested that the dogs had allergic reactions to food antigens. An association between type IV hypersensitivity against food antigens and manifestation of clinical signs is possible based on the fact that anaphylaxis was not observed in these dogs. The sensitivity of antigen-specific

IgE testing was 40–72% in humans with food hypersensitivity¹⁶ and 14% in dogs with food hypersensitivity.⁴ Because antigen-specific IgE testing is thought to indicate a possible involvement of immediate reactions of type I hypersensitivity, type I hypersensitivity against food ingredients may be less frequently seen in dogs with food hypersensitivity than in humans with food hypersensitivity. Antigens used for the intradermal testing and IgE testing might be inappropriate for inducing the binding of IgE specific to food antigens in patients with food hypersensitivity. Because the structure of food antigens may be changed during digestion in the gastrointestinal tract, IgE that is produced against the altered food antigens in patients with food hypersensitivity might not recognize the crude antigens of these same foods.¹⁷ The effects of digestion on the structure of food antigens must be considered in the tests of offending food allergens.

The LST was considered a more reliable diagnostic tool than IgE testing and intradermal testing in dogs with food hypersensitivity in this study. Because the results of the LST are thought to reflect reactions of both type I and type IV hypersensitivity,¹⁸ the LST should be useful for detecting allergic responses associated with T cells reactive to allergens. Nine (82%) of the 11 dogs with food hypersensitivity in this study showed lymphocyte blastogenesis against offending food allergens, suggesting that the LST would be useful as an objective marker for food hypersensitivity in dogs. The stimulation indices of all the dogs in this study significantly decreased to levels similar to those in the control group when the elimination diet was administered. Similarly, in humans with food hypersensitivity the lymphocyte blastogenic responses were maximally increased 5–8 days after oral food provocation and decreased to a normal level with elimination diet therapy.¹⁹ Therefore, LST may be useful as an alternative to the oral food provocation test for detecting offending food allergens in dogs, although false-negative results may occur unless freshly isolated samples of lymphocytes are available. Because the specificity, sensitivity, and predictive values of LST are still unknown, further studies using diseased and breed-matched control dogs will be necessary to determine the efficacy of the LST for the diagnosis of food hypersensitivity in dogs.

Rice was found to be an offending food allergen in 2 (18%) of the 11 dogs with food hypersensitivity in this study. Although rice has been found to be a common food allergen in canine food hypersensitivity,^{5,20} it is often used as a component of commercial elimination diets²¹ and homemade elimination diets. The chance of exposure to rice is higher in Japan than in the United States or Europe because rice is a common food in Japan. In New Zealand where sheep meat is a common food, lamb is the 2nd most common food allergen in cats with food hypersensitivity,²² but this food is not a common allergen in other countries.²³ Therefore, allergy to rice may be a specific feature of food hypersensitivity in dogs in Japan, reflecting the common food ingredients used for dogs. A major allergen in rice that causes problems for humans in Japan is rice protein 16 KD (RP16KD).²⁴ Further studies on hypersensitivity of dogs to rice will provide insight into the similarities of the allergic responses of humans and dogs.

The exact mechanism of food hypersensitivity is not yet

fully understood. We previously reported food antigen-specific histamine release from peripheral basophils in a population of dogs with food hypersensitivity, indicating an involvement of type I hypersensitivity in these dogs.²⁵ However, in the present study, peripheral lymphocytes were associated with the development of clinical signs of food hypersensitivity in dogs, suggesting that type IV hypersensitivity is involved in this clinical manifestation. An involvement of type IV hypersensitivity is also implied by a previous study indicating that food exposure up to 14 days in duration is required to detect clinical signs.⁵ Therefore, the mechanism of food hypersensitivity appears to be complex and to involve both type I and type IV hypersensitivity. For more accurate diagnosis of food hypersensitivity in dogs, further studies should be conducted to dissect the differences in the induction of these 2 types of food hypersensitivity in dogs.

Lymphocyte blastogenic responses to food allergens were increased in the dogs with food hypersensitivity, indicating the presence of circulating lymphocytes reactive to food antigens in the peripheral blood. Most of the offending food allergens detected by the oral food provocation test produced positive reactions in the LST. Although the sensitivity, specificity, and predictive values for the LST must be examined in a larger study that includes disease controls, the present findings suggest that the LST might be useful for identification of offending food allergens in dogs with food hypersensitivity.

Footnotes

- ^a Prescription Diet Canine d/d Rice & Duck, Canine d/d Lamb & Rice or Canine z/d ULTRA Allergen Free, Hill's Pet Products, Topeka, KS
- ^b Topscreen and Immunodot tests, CMG Laboratories, Fribourg, Switzerland
- ^c Allergen extracts, Greer Laboratories, Lenoir, NC
- ^d Domitor, Orion Corporation, Espoo, Finland
- ^e Dormicum, Yamanouchi, Tokyo, Japan
- ^f Ficoll-Hypaque, Nycomed Pharma AS, Oslo, Norway
- ^g Dulbecco's modified Eagle's medium, Sigma Chemical Company, St Louis, MO
- ^h Penicillin, Sigma Chemical Company, St Louis, MO
- ⁱ Streptomycin, Sigma Chemical Company, St Louis, MO
- ^j LSC-5100, Aloka, Tokyo, Japan

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