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Original article

# The prevalence of dogs with lymphocyte proliferative responses to food allergens in canine allergic dermatitis

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## Abstract

The aim of the present study was to examine the correlation between the results of lymphocyte proliferative test (LPT) specific to food allergens and allergic skin diseases in dogs. Investigations were performed in 138 dogs with allergic skin diseases diagnosed in a private animal hospital. Of the 138 animals, 97 cases had positive reactions in LPT specific to food allergens. Of these 97 dogs, 67 animals were diagnosed with canine atopic dermatitis (CAD), but 30 dogs did not have IgE antibodies to environmental allergens. As 14 dogs out of 30 animals showed a positive result, 12 dogs underwent elimination diet trial based on the test results and all of them showed improvement in the pruritus score. Therefore, we conclude that LPT is an effective diagnostic test for allergic skin disease. Results of the lymphocyte test are useful in the identification of food allergens for the elimination diet trial.

**Key words:** dog, allergy, dermatitis, IgE, lymphocytes

## Introduction

Allergic skin diseases in dogs can be broadly classified into the following two groups: atopic dermatitis and food allergy. Canine atopic dermatitis (CAD) can be diagnosed either by intradermal skin test (IDST) or the presence of allergen-specific immunoglobulin E (IgE) to environmental allergens in the serum in combination with clinical symptoms described in Fa-

vrot's diagnostic criteria (Favrot et al. 2010). In cases where allergen-specific IgE is not detected in the serum, the disease is reclassified as atopic-like dermatitis (Halliwell 2006). Over the years, the diagnosis of a food allergy is often challenging. Clinical symptoms of food allergy overlap with those of CAD (Chesney 2002, Loeffler et al. 2006). IDST or allergen-specific IgE test is neither sensitive nor specific enough to diagnose food allergy (Paterson 1995, Jeffers et al.

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1996, Ishida et al. 2004, Olivry et al. 2007). One of the reasons for this is that food allergy occurs through the combined mechanisms of IgE-mediated (type I) and non-IgE-mediated allergic reactions.

The lymphocyte proliferation test (LPT) is useful in the detection of non-IgE-mediated allergic reaction to food allergens in dogs (Ishida et al. 2004). Lymphocytes of dogs that are sensitized to food allergens will proliferate in response to the specific allergens *in vitro* (Ishida et al. 2004). Results of the LPT were consistent in dogs diagnosed with food allergy by food elimination and provocation tests where elimination of the food allergens improved the clinical symptoms of patients with reduced lymphocyte proliferative responses to the causative allergens and vice versa (Fujimura et al. 2011). An allergen-specific LPT for food allergy (Ishida et al. 2004) has recently become commercially available in Japan (Fujimura et al. 2011). This study describes a retrospective analysis of the correlation between allergic skin diseases diagnosed in 138 dogs and the results of LPT specific to food allergen.

## Materials and Methods

We reviewed the medical records of dogs that visited the Primo Animal Hospital with the main complaint of pruritus between June 2008 and September 2010. Dogs diagnosed with ectoparasitic infestations, such as sarcoptic and demodectic mange, by general dermatological examinations were excluded from the study. In addition, dogs with pyoderma, fungal infections, Malassezia dermatitis, and flea allergy dermatitis were also excluded from the study as these skin conditions hinder the accurate assessment of pruritus. Cases with a differential diagnosis of atopic dermatitis that met the requirements of the Favrot diagnostic standards (Favrot et al. 2010) and have not had any prior treatment at the time of the examination were selected. Serum and whole blood samples were collected for the allergen-specific IgE test and LPT, respectively (Fig. 1).

Allergen-specific IgE was measured with a new commercially available quantitative fluorometric enzyme-linked immunosorbent assay (Animal Allergy Clinical Laboratories, Inc., Sagami-hara, Kanagawa, Japan) (Okayama et al. 2011). The 40 allergens measured in this assay included 22 environmental allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, flea, mosquito, cockroach, mugwort, ragweed, goldenrod, dandelion, daisy, orchardgrass, sweet vernal, timothy, rye, bermuda, Japanese cedar, birch, alder, *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, and *Penicillium notatum*).

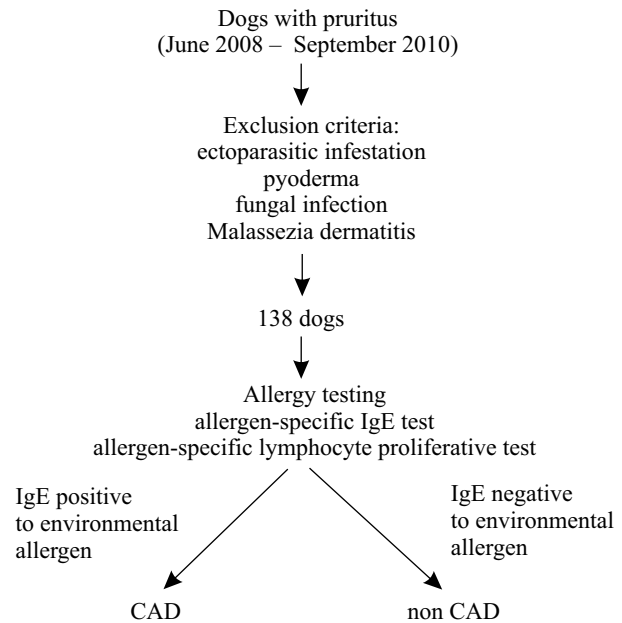


Fig. 1. Study design.

Each allergen was prepared as described in previous report (Okayama et al. 2011). The allergen solutions were diluted at concentrations of 10-20 µg/ml of total protein and immobilized in 96-well plates. A combination of rat anti-mouse IgE antibody (R35-72, BD, Franklin Lakes, NJ, U.S.A) immobilized in the well plate and purified mouse IgE (C38-2, BD) with pre-determined concentration was used for the standard curve. To determine the background reaction, pooled serum of normal BALB/c mice and healthy beagles (Zenoaq Nihon Zenyaku Kogyo Co., Ltd.) were used after subtracting the background fluorometric titers. All the results were calculated according to the standard curve and expressed in a scale of ng/ml.

Using sera from 46 clinically healthy dogs, the cut-off value of serum IgE was determined to be 100 ng/ml as reported (Okayama et al. 2011) and results above this value were considered as positive reactions.

The allergen-specific T lymphocyte reaction was measured with a commercially available system (Animal Allergy Clinical Laboratories, Inc.) (Fujimura et al. 2011). Briefly, peripheral blood mononuclear cells (PBMCs) were purified and cultured in RPMI-1640 medium (Sigma, St Louis, MO, U.S.A.) containing 10% fetal bovine serum (Equitech-Bio Inc., Kerrville, TX, U.S.A.) and antibiotics (100 µg/ml of streptomycin and 100 U/ml of penicillin) (Sigma). Each of the 18 food antigens used in the allergen-specific IgE test were added to the PBMCs and incubated at 37°C with 5% CO<sub>2</sub> in air for 4 days. After that, the cultures were continued for 3 days with the addition of human recombinant Interleukin-2 (PeproTech Inc., Rocky Hill,

NJ, U.S.A.). Concanavalin A (Sigma) was used as positive controls to examine the capability of cell proliferation in the culture. As described in the previous reports (Masuda and Yasuda 2008, Fujimura et al. 2011), cultured cells were finally stained with Alexa 647-labeled anti-canine CD4 antibody (Serotec Ltd, Oxford, UK) and PE-labeled anti-human CD25 antibody, ACT-1 (DAKO A/S, Denmark) for the detection of CD4<sup>+</sup>/CD25<sup>low</sup> cells, which indicates the proliferative fractions of lymphocytes in response to food allergens. The values of the cells cultured without the protein extract were subtracted as a background from each value of those cultured with different food proteins. The cut-off value of the percentage of CD4<sup>+</sup>/CD25<sup>low</sup> cells in CD4<sup>+</sup> lymphocyte in the lymphocyte proliferation test was 1.2%, as determined by samples from clinical healthy dogs (Fujimura et al. 2011) and any value above this was considered as a positive result. All aspects of this study were approved by the Animal Care and Use Committee of Zenoaq Nippon Zenyaku Kogyo.

For elimination diet trials, based on the results of LPT, the optimal diet, either Hill's Prescription Diet z/d Canine ULTRA Allergen-Free (Hill's Colgate (Japan) Ltd., Tokyo, Japan), Iams Veterinary Formulas Skin and Coat Response FP dry or KO dry (P&G Japan, Tokyo, Japan), was selected and was fed by owners at home. During the elimination diet trials, no diet was fed other than water and the selected optimal diet. Changes in clinical condition were carefully observed and noted by the owners. Pruritus was assessed by the owners using a vertical visual analog scale with grade descriptors (Rybnicek et al. 2009) at the first visit and at least 2 weeks after elimination of the diet trial.

The chi-squared test was used to evaluate the difference in the number of positive cases of each allergen-specific serum in the LPT. P values of less than 0.05 were considered as significant. Statistical analyses were performed with Microsoft Excel.

## Results

A total of 138 dogs met the inclusion criteria. Of the 138 dogs, 83 (60.1%) were intact or neutered females, and 55 (39.9%) were intact or castrated males. In total, 36 breeds of dog were represented, and, of these, the most commonly represented breeds were Miniature Dachshund (n = 23), Toy Poodle (n = 16), French Bulldog (n = 11), Shih Tzu (n = 10), Shiba Inu (n = 8), Chihuahua (n = 7), Miniature Schnauzer (n = 7), Miniature Pinscher (n = 6), and crossbreed (n = 11). The age of the dogs at admission ranged from 6 months to 14 years.

Of the 138 cases of allergic skin diseases examined, 104 (75.4%) had positive reactions in the IgE test that were specific to environmental allergens and 34 (24.6%) did not (Fig. 2a). These positive cases were diagnosed with CAD, but more than half of these cases (67 cases; 64.4%) also had positive reactions to food allergens in the LPT. Moreover, 30 of the 34 cases that did not have positive reaction in the IgE test specific to environmental allergens had positive reactions to food allergens in the LPT. The chi-square test showed that soybean was the most significant cause of food allergy ( $p < 0.01$ , Fig. 2b) among the food allergens that induced an increase in LPT against the food allergen. This was followed by rice, potato, and wheat.

Among the 30 cases that had positive lymphocyte proliferative reaction to food allergens in non CAD group (Fig. 2a, bottom column), 16 dogs also had positive reaction to food allergens in the IgE test, but 14 dogs did not. Out of 14 dogs showing only positive reaction to food in LPT, complete medical information was available in 12 cases for following-up investigations of the clinical symptoms after elimination diet trials to be carried out. All 12 dogs showed decrease in pruritus score after elimination diet trial without any other medication (Fig. 2c).

## Discussion

Both IgE and non-IgE-mediated hypersensitivity reactions are involved in food allergy in dogs (Ishida et al. 2004). In order to diagnose food allergy and identify the causative food allergens, canine patients conventionally undergo elimination diet trials and provocation tests. However, these tests are not typically performed in veterinary clinics due to the cumbersome nature. Hence, it has been difficult to identify the causative food allergen in these cases. It is not until recently that a commercially available allergen-specific LPT in Japan has eased the process of diagnosis of allergic skin diseases (Fujimura et al. 2011). However, there are no reports on the prevalence of cases with LPT-positive reaction to food allergens in dogs. In this retrospective study, we showed that an unexpectedly large proportion of dogs with allergic skin diseases (97/138; 70.3%) showed positive reactions in the LPT to food allergens. This finding may indicate that a high number of dogs with allergic skin diseases have unexpected allergic reactions to food allergens and that the existence of food allergen-sensitized lymphocytes in the peripheral blood may be the cause in cases of food allergy that were not diagnosed by the IgE-specific diagnostic technique. Furthermore, most of cases which were categorized to

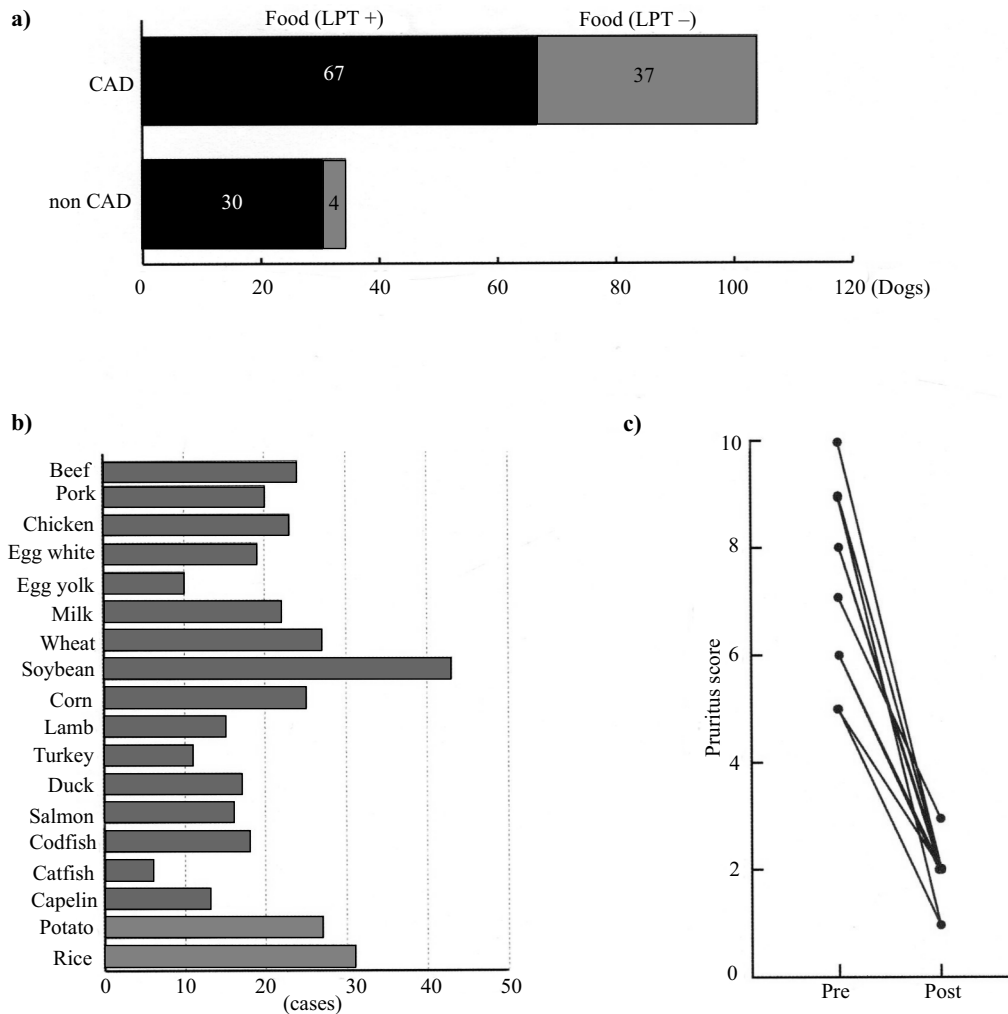


Fig. 2. The incidence of environmental allergen- and food allergen-positive cases: (a) The upper and lower bars indicate the numbers of cases with allergen-specific Immunoglobulin E (IgE)-positive canine atopic dermatitis (CAD) and those that were negative (non-CAD), respectively, to environmental allergens. The black bar indicates the number of cases that showed positive reaction to food allergens in the lymphocyte proliferation test (LPT), (b) Food allergens identified by the LPT, (c) Changes in pruritus score in 12 dogs between before and after food elimination test.

non CAD showed positivity to the food allergen in the LPT. Recently, dogs showing allergic skin diseases without elevated levels of IgE that are specific to environmental allergens have been reclassified as atopic-like dermatitis (Halliwell 2006). The cases (30 dogs) that were identified as LPT-positive without positive reactions to environmental allergens in the IgE tests in this study might be those cases classified as atopic-like dermatitis and this needs to be elucidated in another study.

Elimination diet trial and provocation tests based on the results of the LPT were only performed in some cases as shown in Fig. 2c. Even though these clinical tests are known as the gold standard in the diagnosis of food allergy in dogs, these tests, especially the provocation test, are often not performed due to owners' reluctance. Previous report (Ishida et al. 2004) indicated that LPT is a good test to identify the

causative food allergen. The high prevalence of LPT positive reactions in dogs with allergic skin diseases in this report prompted us to correlate the LPT results and the results of elimination diet trial. As expected, elimination diet trial performed based on the results of LPT alleviated the allergic skin disease.

We revealed that the high prevalence of LPT positive reactions in dogs with allergic skin diseases. This suggests that LPT can be used as an effective additional diagnostic tool for canine allergic skin disease.

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