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Increased Allergen-specific IgE Values and Lymphocyte Proliferation Response to House Dust Mites in Dogs with Atopic Dermatitis

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Abstract : We examined the responses of PBMCs to house dust mite (HDM) allergen in atopic and healthy, non-atopic dogs to identify differences in lymphocyte reactivity that might reflect the immunologic status of atopic dermatitis. Thirteen of 20 (65%) atopic dogs showed a positive lymphocyte proliferative response to HDM allergen. The rate of response was significantly higher in the atopic dogs than that in healthy, non-atopic dogs insensitive to the allergen ($P = 0.007$). The proliferative responses were positively correlated with the level of HDM-specific IgE in serum ($P = 0.035$), and were thereby confirmed to reflect the activity of lymphocytes competent to promote IgE production. These results suggest that HDM-specific lymphocytes were present in peripheral blood and played a role in the pathogenesis of canine atopic dermatitis.

Key words **:** atopic dermatitis, dog, house dust mites, specific IgE, lymphocyte stimulation test

Introduction

Canine atopic dermatitis (AD) is one of the most common clinical manifestations of IgE-mediated hypersensitivity in dogs. The generally accepted criteria for diagnosis include a compatible history and clinical signs, together with a positive reaction to an intradermal skin test (IDST)[14]. The most clinically important allergen for atopic dogs is house dust mite (HDM), and high positive IDST responses to mite extracts have been reported in atopic dog populations[5,6,7].

The detection of tissue-bound IgE using IDST and the quantification of circulating allergen-specific IgE have added to our understanding of the pathogenic role of allergen-specific IgE. The inflammatory skin lesions manifested in canine AD could be generated by the classical immediate hypersensitivity reactions, i.e., the cross-linking of allergen-specific IgE on mast cells and basophils, resulting in the release of many pharmacologically active compounds.

Recent human studies have demonstrated that allergen-specific lymphocytes are playing an important role in the regulation of the IgE synthesis[2,4,11]. IgE synthesis to aeroallergens is under T-lymphocyte control and partially regulated via some T cell lymphokines, such as IL-4 or IL-13[9,10]. Therefore, we assess the involvement of HDM allergy in atopic dogs, not only by IDST and allergen-specific IgE, but also by the HDM-allergen specific lymphocyte stimulation test.

In this study, we measured the responsiveness of canine peripheral blood mononuclear cells (PBMC) to HDM allergen. By comparing the responses of lymphocytes from atopic and normal non-atopic dogs, we tried to identify differences in lymphocyte reactivity that might reflect the immunologic status of AD.

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Materials and Methods

Dogs

Twenty dogs with atopic dermatitis (12 males, 8 females; mean 4.8 years old) were selected for this study. They were diagnosed as AD after fulfillment of Willemse's criteria[14]. IDST was performed with 12 common allergens. Seven dogs tested positive only to the HDM allergen, and 13 dogs reacted to 2 allergens including HDM.

Seven clinically healthy dogs (3 males, 4 females; mean 3.9 years old) were used as control. None of the control dogs were positive on IDST or had any history of skin disease.

Evaluation of skin response to IDST

Mixed HDM allergen (containing equal concentrations of *Dermatophagoides farinae* and *D. pteronyssinus*, Greer laboratory, Lenoir, NC) was diluted to a concentration of $1:10^4$ w/v in saline, and 0.05 ml of the diluted solution was injected intradermally.

The wheal diameters (wd) in animals injected with a negative control solution (0.9% sodium chloride), a positive control solution ($1:10^5$ w/v histamine acid phosphate), and HDM allergen were measured in horizontal planes. HDM IDST score was calculated by the following formula[12]:

$$\text{IDST score} = \frac{\text{HDM allergen wd}}{(\text{positive control wd} + \text{negative control wd})/2}$$

Measurement of allergen-specific IgE

The concentration of allergen-specific IgE in serum was measured using Immunodot test kits (CMG Laboratories, Fribourg, Switzerland) according to manufacturer's instructions. We tested the same series of allergens tested by IDST.

Briefly, patient serum samples were spotted on nitrocellulose strips containing the different allergens, the strips were incu-

bated, and peroxidase-conjugated monoclonal antibody against canine IgE and substrate solution were applied to the strips for colorimetric detection. The intensity of the colour was measured with a densitometer. An optical density (O.D.) of greater than 2.0 was considered as positive for the allergen.

Preparation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from heparinised venous blood samples of dogs by Ficoll density gradient centrifugation, as described previously[7]. The isolated cells were washed three times in phosphate-buffered saline, and resuspended at a concentration of 1Å~10^6cells/ml in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum(Gibco BRL, Grand Island, NY), penicillin (100 U/ml, Gibco BRL, Grand Island, NY), and streptomycin (100 μg/ml, Gibco BRL, Grand Island, NY).

Lymphocytes stimulation test (LST)

Cultures were performed in triplicate at 0.2 ml/well in round-bottomed 96-well microplates (Nunc, Roskilde, Denmark). PBMCs were stimulated with serially diluted HDM allergen or phytohemagglutinin (PHA) (10 mg/ml, Pharmacia Biotech, Uppsala, Sweden), and cultured at 37°C for 4 days in a humidified atmosphere containing 5% CO_2. Following culture, the cells were pulsed with 0.5 μCi [^{3}H]thymidine (Amersham, Buckinghamshire, UK) for 6 hours and harvested onto fibreglass filters with a multichannel-automated harvester. The filters were air-dried and transferred to plastic scintillation vials. Five ml of scintillation fluid (PICO-FLUOR 40, Packard, MA, USA) was added, and scintillation counts were measured with an automated liquid scintillation counter (Tri-Carb 2100TR, Packard). To compare the responses between individuals, we expressed the results as stimulation index (SI).

$$SI = \frac{\text{Mean cpm of samples stimulated with HDM allergens}}{\text{Mean cpm in unstimulated samples}}$$

Statistical analysis

Mann-Whitney's U test was used to compare the lymphocyte proliferative responses to HDM allergen (expressed as SI) and PHA between atopic dogs and control dogs. Spearman's correlation coefficient was used to evaluate the correlation between lymphocyte proliferation responses (expressed as SI) and serum HDM-specific IgE levels. A confidence level less than 0.05 was considered to be significant.

Results

Serum HDM-specific IgE levels

Serum IgE levels to HDM allergen were tested in 20 atopic dogs and 7 healthy control dogs. The IgE levels were positive in 14 atopic dogs and negative in 6 atopic dogs. The IgE levels in 7 all control dogs were negative.

Proliferative responses of canine PBMC to HDM allergen

The proliferative responses of PBMC for the whole series of atopic dogs and control dogs are shown in Figure 1. A proliferative response was detected in PBMC from 13 of 20 atopic dogs if the SI exceeded the SI+3 S.D. of controls. In most of atopic dogs, SI increased in a dose-dependent manner. In the control animals, the lymphocyte response was weak even after exposure to the highest concentration of HDM allergen.

The maximal proliferative responses of PBMC are presented in Table 1. When the responsiveness to HDM allergens was compared between the atopic dogs (n = 20) and control dogs (n = 7), the SI values of the atopic dogs were significantly higher (P = 0.007).

The SI values after PHA stimulation were 56.3± 78.6 in atopic dogs, and 74.3± 54.3 in control dogs respectively. However, there was no significant difference between them (P = 0.108).

Correlations of proliferative responses to HDM with serum IgE levels to HDM and immediate IDST sensitivity

The correlation between proliferative responses to HDM and HDM-specific IgE levels in 14 IgE-positive and 6 IgE-negative atopic dogs is presented in Figure 2. Maximal SI and serum IgE levels to HDM allergens were significantly correlated (r=0.652, P=0.035). These results indicate that SI reflects

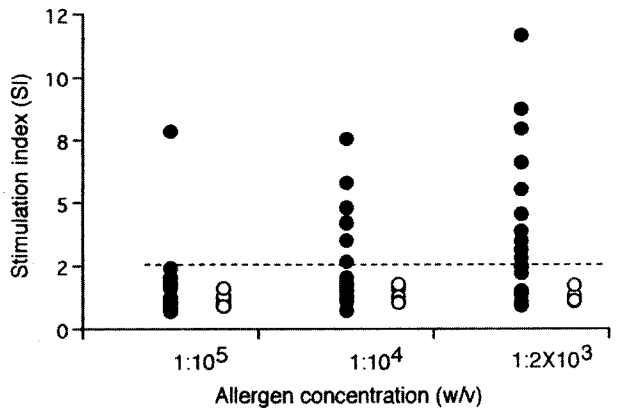

Fig. 1. Dose response for PBMC proliferative responses of atopic dogs (solid circles) and non-atopic control dogs (open circles) after stimulation with HDM allergen.

Table 1. Proliferative responses of PBMCs to HDM allergen and PHA in atopic and control dogs

		Maximal SI (mean±SD)	
	n	HDM	PHA
Atopic dogs	20	4.0±2.9	56.3±78.6
Control dogs	7	1.6±0.3	74.3±54.3
		P=0.007*	P=0.108*

*Maximal proliferation responses to HDM allergen of two groups were analyzed by the Mann-Whitney's U test.

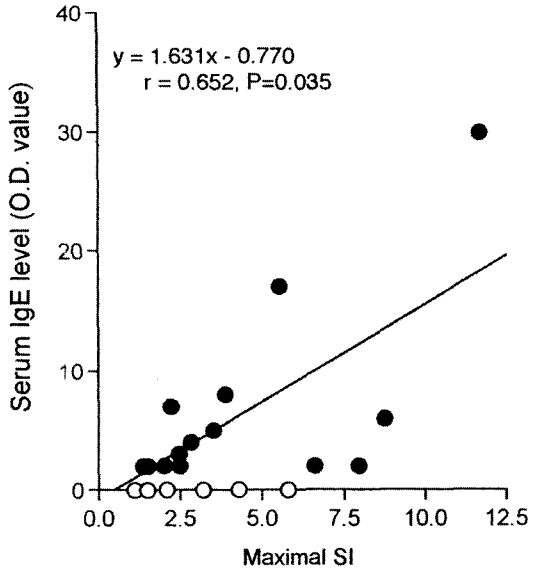

Fig. 2. Specific IgE levels (O.D. value) plotted against lymphocyte proliferative responses to HDM allergen (maximal SI) in 14 IgE-positive (solid circles) and 6 IgE-negative atopic dogs (open circles). Spearman rank correlation coefficient was significant (P=0.035).

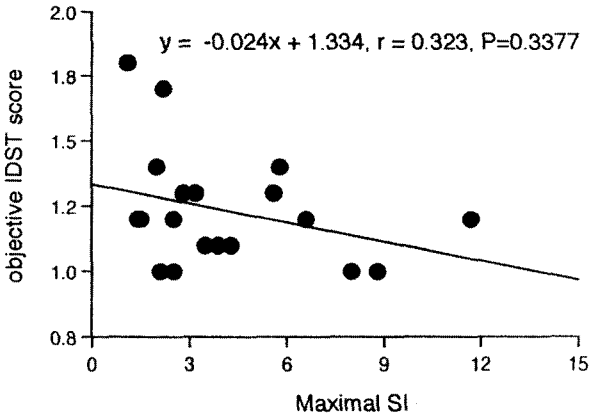

Fig. 3. Intradermal skin test score plotted against lymphocyte proliferative responses (maximal SI) to HDM allergen in 20 atopic dogs.

the activity of lymphocytes competent in IgE-positive atopic dogs promoting the production of IgE. Additionally, elevated SI in IgE-negative atopic dogs suggests that the sensitization of lymphocytes to HDM allergen begins prior to the development of HDM-specific IgE antibody.

However, comparing the proliferative response with IDST score, no correlation was observed between the proliferative response and IDST score (r=0.323, P=0.337) (Fig. 3).

Discussion

In this study, we investigated canine PBMC proliferative responses to HDM allergens in atopic dogs and clinically healthy dogs.

We found that the PBMCs in atopic dogs responded to HDM allergen. This result suggests that the peripheral blood of the atopic dogs contained allergen-specific lymphocytes that proliferated when exposed to allergens. However, it remains unclear whether this response to the allergen was induced in the atopic dogs by the increased frequency of HDM-specific lymphocytes in their peripheral blood, or suppressed in the control dogs by inhibitory cytokines such as transforming growth factor-$\beta 1$[13] and IL-10[3]. The establishment of a direct method to measure the frequency of allergen-specific lymphocyte, a method akin to HLA-tetramer staining in humans[1], is necessary for dogs in this present study.

In our study, the *in vitro* proliferative responses were positively correlated with the level of allergen-specific IgE, despite of unable to assess phenotype of the responsive lymphocyte subsets present in our cultures. Our result demonstrates that it is not the type of the Th response, but the frequency of the allergen-specific lymphocyte response that plays a crucial role in the pathogenesis of canine AD. Th2 type response may strengthen the IgE production from allergen-sensitised B cells.

In our final experiments to compare the relation between proliferative responses and skin test sensitivity, no correlation was demonstrated, though we found that the *in vivo* IDST yielded poorer quantitative results than HDM-specific IgE levels. We were also unable to find any correlation between the IgE level and skin sensitivity.

Skin reaction to allergen is a complicated process that can emerge when serum IgE is present in only low levels or even completely absent or can be mediated by other classes of antibodies. It would be best to employ a variety of diagnostic procedures for canine AD.

In conclusion, we showed a significant increase in the lymphocyte proliferative responses to HDM allergen in atopic dogs, suggesting a proliferation of allergen-specific lymphocytes in these animals. The proliferative responses were positively correlated with the level of HDM-specific IgE in serum, and thus reflected the production of circulating IgE by lymphocytes. These results suggest that HDM-specific lymphocytes are present in peripheral blood in atopic dogs and may play a role in stimulating the HDM-specific IgE synthesis in these animals.

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개의 아토피성 피부염에 대한 집먼지 진드기 항원 특이적 IgE와 림프구 증식 반응의 증가

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요 약 : 혈중 항원 특이적 IgE 검사와 피내시험으로 집먼지 진드기 (house dust mites, HDM)에 양성 반응을 보인 20두의 아토피성 피부염으로 진단된 개를 대상으로, 말초혈단핵구 (peripheral blood mononuclear cells, PBMC)를 채취하여 HDM항원에 대한 반응을 검토하였다. PBMC를 분리하여 HDM항원으로 자극한 결과 20두중 13두 (65%)에서 항원 특이적인 림프구의 증식반응을 확인할 수 있었다. HDM 항원에 증식반응은 아토피성 피부염군에서 대조군에 비해 유의적으로 높은 반응이 확인되었다 (P=0.007). 또한, HDM에 대한 반응은 혈중의 IgE 농도와 유의적으로 상관관계를 나타내었으며 (P=0.035), 이는 체내에서 항원 특이적인 IgE의 생산을 촉진하는 작용을 반영하는 지표가 될 수 있다고 생각되었다. 이러한 결과로, 말초혈액중에 존재하는 HDM 항원 특이적인 림프구는 개의 아토피성 피부염의 병태생리에 관여하고 있는 것으로 시사되었다.

주요어 : 아토피성 피부염, 집먼지 진드기, 항원 특이적 IgE, 림프구 증식 반응