

## 論文の内容の要旨

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### 論文題目

#### **Studies on the pathophysiology and control of allergic dermatitis in dogs**

(犬におけるアレルギー性皮膚炎の病態制御に関する研究)

Allergic dermatitis is a common pruritic skin disease in dogs. Dermatologic diseases mediated by allergic reactions in dogs include flea bite hypersensitivity, atopic dermatitis (AD), food hypersensitivity (FH), and contact hypersensitivity. Since inherited hypersensitive immune response is associated with the pathogenesis of AD and the number of predisposed pure breed dogs have been increasing as companion animals, AD is one of the most problematic diseases in small animal practice. Moreover, recent studies revealed that FH could be diagnosed by elimination dietary trial and provocative challenge in many dogs sometimes concurrent with AD. For these reasons, AD and FH are now accepted as clinically important diseases to be solved in veterinary medicine.

Diagnosis of AD in dogs is based on its characteristic clinical features including pruritus, typical appearance and distribution of the skin lesion, recurrence of skin symptoms with secondary bacterial infections, familial history or breed predisposition, and frequent elevation of serum IgE specific to environmental allergens. These clinical features are not specific to AD and can be also observed in dogs with FH. Diagnosis of FH in dogs can be made only after the positive results in elimination dietary trial and provocative challenge because allergen-specific IgE and intradermal skin test (IDST) do not have sufficient sensitivity and specificity to food allergens. For diagnosis of FH, highly efficient elimination diet is needed, however, there has been no elimination diet that can avoid both type 1 and type 4 hypersensitivity to food allergens. Recently, a novel hypoallergenic diet for dogs was developed as a collaborative project between the University of Tokyo and a pet food company.

The diet has been manufactured from a mixture of amino acids and potato. It is conceivable that amino acids do not induce either type 1 or type 4 hypersensitivity because of their small molecular weights unlike the proteins and peptides. Moreover, it has been shown that potato induces FH in few dogs. The study in Chapter 1 of the present thesis was carried out to examine the clinical efficacy of the newly developed hypoallergenic diet as an elimination diet to diagnose FH in dogs.

Although dogs with AD are frequently shown to be hypersensitive to environmental allergens, especially house dust mites (HDM), it is usually difficult to eliminate HDM from the environment. Therefore, treatments for AD in dogs have been carried out by administration of glucocorticoids and immunosuppressive drugs as well as allergen-specific immunotherapy (ASIT). But, many dogs are often subject to adverse effects or reactions during the treatments with these therapies. HDM avoidance measures in the environments have been carried out as a safe and effective treatment for controlling the symptoms in asthmatic human patients. But, there has been no report on the efficacy of the HDM avoidance measures on the control of AD. The study in Chapter 2 of this thesis was performed to know whether the HDM avoidance measures are effective to control the clinical symptoms and immune responses in dogs with AD that are sensitive to HDM.

In human patients with AD, complex immune responses are known to be associated with the pathogenesis to induce the skin lesion. It has been shown that, in addition to immediate hypersensitivity, late phase reaction is also involved in the formation of the characteristic lesion of AD. Moreover, predominance of Th2-skewed cytokines and chemokines has been demonstrated in the allergic skin lesions. Recently, many of these immunologic abnormalities were shown in the skin lesions of dogs with AD. Although a variety of immune cells are infiltrated conceivably to initiate and progress a series of immune reactions in the skin of humans and dogs with AD, many of the studies indicated significant roles of mast cells to develop the allergic reactions in the skin. Mast cells are considered to play not only as effector cells in type 1 hypersensitivity but also as immunoregulatory cells in acquired and innate immunity. Certain roles of mast cells in the late phase reaction have been also indicated in the experimental animal models. To establish a fundamental research system on the role of mast cells in AD, the study in Chapter 3 of this thesis was carried out to culture and characterize mast cells derived from the skin of dogs in comparison to those from the bone marrow.

## **Chapter 1. Usefulness of a new elimination diet composed of amino acids and potato for the diagnosis of canine food hypersensitivity**

Twenty dogs with suspected FH or FH concurrent with AD were enrolled in this study based on the history, onset of clinical signs at young age, non-seasonal pruritic chronic dermatitis, and rule-out of flea bite hypersensitivity, contact hypersensitivity and primary infectious skin diseases such as superficial pyoderma, mycotic skin disease and ectoparasitosis. After satisfactory explanation to the

owner, the elimination trial was carried out using the newly developed hypoallergenic diet (Aminoprotect care<sup>®</sup>, Sankyo Lifetech, Co., Ltd. Japan) for 2 months. During the period of elimination dietary trial, some dogs were treated using systemic antibiotics and anti-microbe shampoos for control of secondary skin infections, and received systemic glucocorticoid therapy for reducing their pruritus. Before and after the dietary trial, the dogs' clinical symptoms were evaluated using the pruritus score, medication score of systemic glucocorticoid, and canine atopic dermatitis extent and severity index (CADESI). Fifteen of the 20 dogs could complete the elimination dietary trial. The other 5 dogs were suspended because of diet unpalatability (3 dogs), progression of skin disease (1 dog) or diarrhea (1 dog). Based on the comprehensive evaluation of the clinical scores, improvement of the clinical scores was seen in 10 of the 15 dogs that completed the trial. Food provocation tests were then applied to 9 of the 10 improved dogs, and 6 dogs were confirmed to be hypersensitive to certain food allergens such as pork, beef and chicken from the recurrence of the clinical symptoms. The results of the food elimination tests using the newly developed hypoallergenic diet and succeeding provocative challenge revealed that the diet was useful for the diagnosis of FH in dogs.

## **Chapter 2. Effect of house dust mite avoidance measures on the clinical symptoms in dogs with atopic dermatitis**

For the study in this chapter, six dogs with AD sensitive to HDM were enrolled. Clinical diagnosis with AD was based on Willemse's criteria. All of the 6 dogs were shown to be sensitive to HDM by measurement of allergen-specific serum IgE and/or IDST. HDM avoidance in which the decrease of a representative HDM allergen, Der 1, was performed by withdrawing conventional bedding and encasing furniture and bedding in covers allowing no mite penetration (Danizerock<sup>®</sup>, Yamasei Corp., Tokyo, Japan). The clinical symptoms of the dogs were quantitatively evaluated by recording CADESI, pruritus score, and glucocorticoid medication score before and after the HDM avoidance measures for approximately 20 weeks. Der 1 concentration was measured by fluorescence sandwich ELISA using monoclonal antibodies directed to the allergen. Seven to 23 weeks after the intervention, Der 1 concentration was shown to be significantly decreased on floors (from 4.73 to 0.39  $\mu\text{g/g}$  dust,  $p=0.0306$ ) and furnitures (from 9.78 to 0.17  $\mu\text{g/g}$  dust,  $p=0.0082$ ), but not on dogs' mats (from 1.32 to 0.09  $\mu\text{g/g}$  dust,  $p=0.1425$ ). After the HDM avoidance measures, 4 of the 6 dogs showed overall improvement of their clinical symptoms. Moreover, decrease of the level of serum IgE specific to HDM was shown in 4 of the 6 dogs after the intervention. The results of this study indicate that decreasing the amount of HDM in the environment can have an efficacy on the control of clinical symptoms and hypersensitive immune reactions in dogs with AD.

## **Chapter 3. Cultivation and characterization of skin-derived mast cells in comparison to the**

### **bone marrow-derived mast cells in the dog**

In this chapter, I carried out a study on the cultivation and characterization of skin-derived mast cells in comparison to the bone marrow-derived mast cells in the dog. Skin biopsy samples from healthy dogs were enzymatically digested to obtain single cells. After cultivation for 1 to 2 months in serum-free AIM-V medium supplemented with recombinant canine stem cell factor, the proliferating mononuclear cells were purified using anti-c-Kit antibody with magnetic cell sorting system. Purity of the cells was assessed by co-expression of the surface c-Kit and Fc epsilon receptor 1 by using flow cytometry. The cell morphology was examined by toluidine blue staining and transmission electron microscopy. Immunocytochemistry for tryptase and enzyme cytochemistry for chymase were employed to examine the protease phenotype. A beta-hexosaminidase assay was performed to examine mast cell-specific degranulation induced by  $\text{Ca}^{2+}$  ionophore, A23187, and anti-canine IgE antibodies. These characteristics of skin-derived mast cells (skin MC) were compared with those of bone marrow-derived cultured mast cells (BMCMC). The skin-derived culture showed the purity of mast cells as high as  $91.7 \pm 6.6\%$ . These cells were 10 to 20 micrometers in diameter and contained a round or oval nucleus and metachromatic granules. Electromicroscopic examination revealed numerous electron-dense granules in the cytoplasm. The cells were positive for both tryptase and chymase. Release of beta-hexosaminidase induced by  $\text{Ca}^{2+}$  ionophore and anti-canine IgE antibodies was seen in a dose-dependent manner. In the morphological observation, the skin MC were larger than BMCMC and had longer cytoplasmic projections and larger numbers of cytoplasmic granules than BMCMC. The amount of degranulation induced by anti-canine IgE antibodies was smaller in skin MC than BMCMC. These results indicate the usefulness of the culture method employed in this study to obtain canine skin-derived mast cells with its typical characteristics that will function to develop allergic reactions in the skin.

A series of the studies in this thesis were carried out for understanding the mechanisms to initiate and progress allergic dermatitis in dogs and for development of novel therapeutic strategies to the disease. Nowadays, there is a rapid increase of the number of patients with allergic diseases in dogs as well as in humans. The present studies provide basic and applied knowledge on the future development of novel therapies that will efficiently control the problematic allergic diseases in dogs and humans.