Development of Enzyme-linked Immunosorbent Assay (ELISA) for allergen-specific IgG subclass Detection in Atopic Dogs

N. Khantavee1, C. Chanthick2, N. Saelim1, A. Tungtrongchitr3, N. Sookrung4, N. Prapasarakul*

1Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
2Dermatology Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, 50 Phaholyothin Road, Ladyyao, Chatuchak, Bangkok 10900, Thailand
3Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
4Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
*Corresponding author: Nuvee.P@chula.ac.th

Keywords: atopic dogs, ELISA, IgG1, IgG2

Introduction

Atopic dermatitis (AD) is one of allergic skin diseases in dogs. The disease involves complex factors including defect of inherited genes, health status, frequency of external allergen exposure and certain underlining diseases (6). The diagnosis is needed to rule out from other skin diseases such as ecto-parasitic infestation, microbial infection, food allergy, flea bite allergy that result the similar symptom (7). In practise, intradermal skin testing (IDST) and Allergen-specific IgE serology testing (ASIS) are utilized for allergen type detection in dogs and human. IDST is an in-vivo testing to detect local allergen-specific IgE on the effected skin. Unfortunately, many restrict conditions i.e. drug withdrawal periods, risk conditions of anaesthesia or systemic illness are the great obstacles for IDST (9). Whereas, ASIS is more convenient for practitioners due to lesser limitations. ASIS can quantitatively detect the level of circulating allergen-specific IgE in serum (4). However, ASIS is not available in Thailand and a lot of foreign allergens are included in the testing panel with still problematic interpretation. Serum IgG is generally circulated in blood circulation in larger amount compared to IgE. The level of IgG subclass has been proved to relate with stage of infection and allergy (5). A level of specific IgG and IgG subclasses at 1:540 to above 1:14,580 dilution were successful to differentiate specific IgE and IgG subclasses at 1:540-1:1,620 dilution, total IgG and allergens associated house dust mite by IDST and pool sera samples of five atopic dogs with positive to house dust mite by IDST and pool sera samples of five healthy dogs without skin problems were used. Three-fold serial dilution of sera samples were prepared, started at 1:20 dilution. Then, sera samples were added to the plated and incubated at 37°C 1 hr. After washing, the plate was reacted to anti-dog IgG1-HRP, anti-dog IgG2-HRP or anti-dog IgG-HRP. Lastly, ABTS® peroxidase substrate was added and measured OD intensity at 405nm.

Results and Discussion

We attempted to develop an ELISA assay for IgG and IgG subclass level detection in dog sera to four common allergens associated atopic dogs. Firstly, the proper allergen concentration and serum titer, were serially optimized until three-fold titer difference was found between healthy and atopic dog sera. For house dust mites, the sera at 1:180-1:1,620 dilution, total IgG and IgG subclass levels could detect the atopic dog associated house dust mite as the specific allergen. For allergen preparation, crude proteins of two house dust mites (Dermatophagoides farinae and Dermatophagoides pteronyssinus) were extracted by sonication and filtration. For microbial proteins, Malassezia pachydermatis strain 4108 and Staphylococcus pseudintermedius strain U4 were separately prepared by a mechanical disruption using glass bead and freeze-thaw assay at least 10 cycles in PBS buffer. The protein concentration was measured by Bradford assay (2). Next, a 96 well-plates was coated with 2 µg/well of each protein extraction. The micro-well plate was blocked with buffer contained 1%BSA and 0.05% Tween 20. For optimization of sera dilutions, pool sera samples of five atopic dogs with positive to house dust mite by IDST and pool sera samples of five healthy dogs without skin problems were used. Three-fold serial dilution of sera samples were prepared, started at 1:20 dilution. Then, sera samples were added to the plated and incubated at 37°C 1 hr. After washing, the plate was reacted to anti-dog IgG1-HRP, anti-dog IgG2-HRP or anti-dog IgG-HRP. Lastly, ABTS® peroxidase substrate was added and measured OD intensity at 405nm.
Optimization of in-house ELISA technique by serial serum dilution and optical density measurement of IgG subclass level against *D. farinae* (Df) using the sera from healthy and atopic dogs.

**Figure 1.**

Optimization of in-house ELISA technique by serial serum dilution and optical density measurement of IgG subclass level against *D. pteronyssinus* (Dp) using the sera from healthy and atopic dogs.

**Figure 2.**

Optimization of in-house ELISA technique by serial serum dilution and optical density measurement of IgG subclass level against *M. pachydermatis* (Mp) using the sera from healthy and atopic dogs.

**Figure 3.**

Optimization of in-house ELISA technique by serial serum dilution and optical density measurement of IgG subclass level against *S. pseudintermedius* (Sp) using the sera from healthy and atopic dogs.

House dust mites are the common external allergens in canine atopic dermatitis in Thailand (3). Secondary skin infections by *M. pachydermatis* and *S. pseudintermedius* often occur and become the target of treatment (7). They can also induce allergen-specific IgE (1,8) and acts as the common internal allergen. Consequently, but they are not included in commercial ASIS. Increasing of allergen-specific IgG level, especially allergen specific IgG1 was apparently related to allergic dermatitis in atopic dogs (5). Success of ELISA detection for allergen-specific immunological makers depended on optimized points among sera dilutions, antigen concentration and type of antigens. The results showed that IgG1 subclass concentration was a surrogate of allergic response in blood stream. This hypothesis was confirmed by consistency of IgG1 level to 3 tested allergens; *D. farinae, D. pteronyssinus, M. pachydermatis* but not *S. pseudintermedius*. In contrast, IgG2 level to allergens of house dust mites and the bacteria, was not different between healthy and atopy but only *M. pachydermatis* was distinguishable at high serum dilution. In conclusion, we proposed the in-house ELISA for specific allergen detection by measurement IgG subclass level in dog serum. To confirmation of its availability, this preliminary tool is still needed to detect in higher number of individual healthy and diseased dogs in further study.

**Acknowledgements**

We thank the Dermatology Clinic, Small Animal Hospital, Kasetsart University, Thonglor Pet Hospital providing serum samples. Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University for house dust mite antigen and their scientific facility. Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program for student scholarship fund, Thailand Research Fund (RSA5980056) and STAR; Detection and Monitoring Animal Pathogen, Chulalongkorn University

**References**

7. Hensel et al., 2015. BMC Veterinary Research. 11:196