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Immune Response of Bacterial Proteins of *Staphylococcus intermedius* from Canine Atopic Dermatitis

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Abstract : Bacterial infection of canine atopic dermatitis is largely caused by *Staphylococcus intermedius* and may be a superficial or deep pyoderma. The purpose of this study was to identify the major proteins of *S. intermedius* cell surface components in humoral immune response of atopic dermatitis dog. Sera samples were obtained from dogs with atopic dermatitis and superficial pyoderma referred to the Veterinary Medical Teaching Hospital, Konkuk University. An isolate of *S. intermedius* from a clinical case of canine atopic dermatitis was cultured in brain heart infusion broth overnight at 37°C in aerobic conditions on an orbital shaker. Following culture, Staphylococci were harvested by centrifugation, washed in PBS, and resuspended in PBS containing lysostaphin. The soluble components were separated by centrifugation and were collected. The soluble extract of *S. intermedius* was separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were electrophoretically transferred onto nitrocellulose membrane. Western blotting for the specificity of serum IgG antistaphylococcal antibody was performed with anti-dog-IgG and sera obtained from an atopic dermatitis case and a normal dog. The molecular masses of four major proteins of *S. intermedius* recognized by serum obtained from an atopic dermatitis case were 18, 31, 75, and 110 kDa as determined by Western blot analysis. The present study indicates that most dogs of *S. intermedius* infection with atopic dermatitis could have a significant humoral immune response to bacterial proteins of the causative organism.

Key words : *Staphylococcus intermedius*, atopy, dog

Introduction

Canine atopic dermatitis is a common pruritic skin disorder characterized by type I hypersensitivity to environmental allergens such as house dust mites, storage mites, animal epithelia, plant pollens and moulds[6]. Although the pathogenesis of atopic dermatitis is not fully understood, clinical and immunological studies have shown similarities between the human and canine condition[8-10]. In atopic dermatitis, bacterial infection is a very common and important complication. A major microbial pathogen involved is the pathogenic staphylococci, *S. intermedius*, in the dog. And, it is evident that *S. intermedius* adhered to keratinocytes and this is one possible explanation for the high incidence of staphylococcal pyoderma in atopic dermatitis [1].

The present study was conducted to prove the hypothesis that *S. intermedius* infection in skin can induce humoral immune response and its response may be mediated through the major bacterial proteins.

Materials and Methods

Case presentation

Sera samples were obtained from a dog with atopic dermatitis and superficial pyoderma referred to the Veterinary Teaching Hospital, Konkuk University. This dog with atopic dermatitis had clinical signs consistent with the criteria of Willemse (1986) and was positive in intradermal skin test. Control serum was obtained from clinically normal, 4-month-old, female Maltese dog. Sera were stored at 20°C until used.

Preparation of S. intermedius cell protein

An isolate of *S. intermedius* from the patient of atopic dermatitis and surperficial pyoderma was cultured in brain heart infusion broth (Difco, Detroit, MI, USA) overnight at 37°C in aerobic conditions on an orbital shaker. Following culture, staphylococci were harvested by centrifugation at 450 g for 10 minutes. The organisms were washed five times in PBS (pH 7.2) and resuspended in PBS containing lysostaphin (Sigma Chemical CO., St Louis, MO, USA) at 25 ug/ml. Following incubation for 15 min at 37°C with gentle agitation, formaldehyde at a final concentration of 0.1%, was added to stop further enzymatic reaction. The soluble components were separated by centrifugation at 10,000× g for 30 minutes. The supernatant containing soluble components was collected. The protein concentration of the staphylococcal extract was measured using a microbbicinchoninic acid assay (BCA) (Pierce, Rockford, IL, USA). The protein concentration was adjusted to 1 mg/ml and lysate aliquots were frozen at 20°C until used.

Western blotting

The soluble extract of *S. intermedius* was separated by sodium dodecylsulphate polyacrylamide gel electrophoresis

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(SDS-PAGE) using a protean II system (Bio-Rad, Hercules, CA, USA) under discontinuous, denaturing conditions as described by Johnstone and Thorpe (1987). The gel comprised a 3% stacking gel over a 10% resolving gel. The resolving gel contained 10% SDS in Tris-HCl buffer (pH 8.8), and the stacking gel contained 10% SDS in Tris-HCl buffer (pH 6.8).

Twenty micrograms of the bacterial extract was loaded on each well and one well was loaded with molecular markers (Bio-Rad) which included rabbit myocyte myosin. Electrophoresis was performed using 30 voltage through the stacking gel and 120 voltage through the resolving gel in buffer comprising glycine, Tris and SDS. Coomassie blue stain procedure was performed with Coomassie Blue R-250, Methol and glacial acetic acid.

The proteins were electrophoretically transferred onto nitrocellulose membrane (Trans-Blot Cell; Bio-Rad) at a constant voltage of 15 for 20 minutes. The transfer buffer contained glycine and Tris (pH 8.3) mixed with methanol at a ratio of 4 : 1. The nitrocellulose membrane (Bio-Rad) was removed and washed in Tris-buffered saline pH 7.5 (TBS) for 30 minutes with gentle rocking. In addition, the prestained molecular weight markers (Bio-Rad) were included.

The residual binding sites on the nitrocellulose membrane were blocked by incubation in TBS containing 1% w/v bovine serum albumin (BSA) for 60 minutes at room temperature with gentle rocking. The membrane was then cut into vertical strips (10 mm wide) and each strip placed in a small petridish. Each strip was incubated with a test serum at 1/25 in 0.5% w/v BSA in TBS for 2 hrs at room temperature. The membranes were washed with TBST, and then incubated for 2 hrs at 37°C with hydrogen peroxide (HRP)-conjugated rabbit anti-dog IgG (Sigma) at 1/1000 in the same diluent as above.

Following further washing, the bound antibody was visualized by incubation with substrate solution containing 4-chloro-1-naphthol (CN) (30mg/ml in methanol). A control strip was treated in the same way with normal dog serum in the first incubation. The molecular weight of the visualized bands was calculated by their relative mobility from the regression line of the $\log_{10}$ of the molecular weight of the standard proteins plotted against their relative mobility.

Results

After harvest of bacterial proteins, we conducted SDS-PAGE and observed the protein bands in gels after staining the gels with Coomassie brillient blue.

The immune reactivity of *S. intermedius* bacterial proteins identified by serum from a dog with atopic dermatitis was presented in Fig 1. Four major proteins of *S. intermedius* by serum obtained from an atopic dermatitis case were 18, 31, 75, and 110 kDa as determined by western blotting (Fig 1). However, when serum of a normal dog was used, additional protein bands were not identified in Western blot analysis.

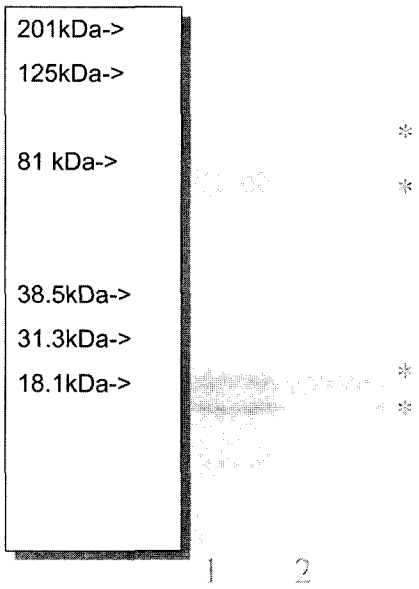

Fig 1. Western blot analysis after transferring bacterial surface proteins of Staphylococcus intermedius to nitrocellulose membrane (lane 1: prestained markers, lane 2 : 25 dilution of primary antibody, asterisk marker : major proteins).

Discussion

The present study has examined the humoral immune response to *S. intermedius* isolated from a dog with clinical signs of atopic dermatitis. According to the previous reports[2], serum IgG titers were elevated in atopic dermatitis without pyoderma, atopic dermatitis with pyoderma, deep pyoderma and pustular demodicosis. However, they suggested that the stage of disease may also influence the serum IgG concentrations.

In an earlier reports[11], the serum antistaphylococcal IgG concentrations in dogs with atopic dermatitis without pyoderma, atopic dermatitis with superficial pyoderma, idiopathic deep pyoderma and anal furunculosis were significantly higher than those of normal dogs. In addition, serum samples from dogs with recurrent idiopathic deep pyoderma, recurrent idiopathic superficial pyoderma and atopic dermatitis with recurrent pyoderma showed the elevated concentrations of antistaphylococcal IgG[3,7]. Therefore, the levels of antistaphylococcal IgG may reflect the extent and duration of exposure to staphylococci. The result of the present study support this hypothesis since antistaphylococcal IgG serum was present in serum of dogs with atopic dermatitis. For this reason, this result can also be induced from increased cutaneous permeability in dogs with atopic dermatitis[5], which may allow greater absorption of staphylococcal antigens, rather than being an increased number of staphylococci on the skin.

The western blotting analysis revealed that the presence of three major proteins involved in the IgG response to *S. intermedius* in dogs with pyoderma. This is in partial agreement

with study results of Halliwell and Campbell[3] who found that serum IgG was elevated in bacterial infection secondary to atopic dermatitis.

Conclusions

The present study indicates that most dogs infected with *S. intermedius* in atopic dermatitis could have a significant humoral immune response to a select number of proteins on the causative organism. This humoral response may be mediated through the major bacterial proteins. Future studies are going to address the role of such antibodies in phagocytosis by neutrophils and macrophages. In addition, it is necessary to identify the amino acid sequence of 4 major protein bands in *S. intermedius* infection secondary to atopic dermatitis.

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개의 아토피성 피부염에서 분리한 *Staphylococcus intermedius*균의 세균단백질의 면역반응

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요 약 : 개의 아토피성 피부염의 주된 세균성 감염은 *Staphylococcus intermedius*에 의해 발생한다. 본 연구의 목적은 아토피성 피부염을 가지고 있는 환견의 혈청과 정상견의 혈청을 이용하여 체액성 면역반응을 유발하는 주된 세균단백질을 규명하는데 있다. 건국대학교 수의과대학 부속동물병원에 내원한 아토피성 피부염 및 표재성 세균성 농피증을 앓고 있는 환견의 혈청 및 정상견의 혈청을 분리하여 본 실험에 사용하였으며 아토피성 피부염을 가지고 있는 환견에서 *S. intermedius*균을 분리하였다. Brain heart infusion 액체배지 조건 및 37°C 배양조건에서 호기상태로 증균시켰으며 증균후 포도상구균을 PBS완충액에 부유시킨후 원심분리하고 최종적으로 lysostaphin을 이용하여 세균단백질을 분리하였다. 수거한 세균단백질은 SDS-PAGE을 이용하여 단백질을 전기영동하였으며 영동후 nitrocellulose membrane을 이용하여 단백질을 이동시켰다. Western blot은 anti-dog-IgG, 아토피성 피부염의 혈청 및 정상혈청을 이용하였다. 결론적으로 아토피성 피부염의 혈청을 이용해 확인한 *S. intermedius*의 주요 세균단백질은 18, 31, 75, 및 110 kDa이었다. 현재의 연구결과로 볼 때 아토피성 피부염에 감염된 대부분의 환견은 *S. intermedius*의 세균단백질에 대한 체액성 면역반응이 유발된다고 생각된다.

주요어 : *Staphylococcus intermedius*, 위축, 개