Cutaneous Reactivity to *Malassezia Pachydermatis* in Dogs with Seborrheic Dermatitis

Masahiko Nagata

Animal Dermatology Center, ASC

Abstract

Intradermal testing using extracts from *Malassezia pachydermatis* was conducted in 50 dogs with seborrheic dermatitis. Immediate reactions to *M. pachydermatis* antigen were observed in 17 (34.0%) of 50 dogs, and delayed reactions to it in 3 (9.4%) of 32 dogs. None of 10 control dogs showed any positive reaction to the antigen. Shampoo, either selenium disulfide or chlorhexidine, was effective in 19 (90.5%) of the 21 cases with negative reactions. In contrast, the same treatment was effective for only 3 (33.3%) of the 9 dogs with positive reactions. The remaining cases showed improvement with a systemic anti-yeast agent, ketoconazole. This study indicates that *M. pachydermatis* may play a role as an allergen in some dogs with seborrheic dermatitis.

Key words: Dog, Intradermal testing, *Malassezia dermatitis*

INTRODUCTION

Seborrheic dermatitis is a common form of dermatitis in humans. This disorder has a distinctive morphology characterized by sharply demarcated erythematous lesions covered with oily-looking scales, and distinctive distribution on well-supplied areas of the sebaceous glands\(^1\). In dogs, seborrheic dermatitis is characterized by scaling and greasiness, with gross evidence of local or diffuse inflammation\(^2\). The skin lesions in dogs are similar to those in humans, though their distribution is more generalized since the entire body is completely covered with pilosebaceous units in dogs. *Malassezia* organisms are believed to play an important role in the development of human seborrheic dermatitis\(^3\), even though its pathogenesis is still unknown. *Malassezia*, a lipophilic yeast, is a member of the normal cutaneous flora in warm-blooded animals\(^4, 5\). *Malassezia pachydermatis* is found on normal skin and within the ear canal, anal sacs, vagina, and rectum of dogs\(^4, 5\). This organism is commonly isolated from seborrheic dermatitis, and its lipolytic nature could worsen a greasy skin condition\(^2\). Interestingly, some dogs have large number of *Malassezia* yeasts, whereas they may be difficult to isolate in others. It is suspected that inflammatory changes based on hypersensitivity reactions may be involved in its pathogenesis. The purpose of this study was to investigate cutaneous reactivity to extracts from *M. pachydermatis* in dogs with seborrheic dermatitis.

Correspondence to:
M. Nagata (Animal Dermatology Center, ASC)
1-3-2 Jindaijihigashi Chofu, Tokyo 182-0012, Japan
E-mail: nagata@so-net.ne.jp
MATERIALS AND METHODS

Cases

A total of 50 dogs with seborrheic dermatitis were studied. There were 24 males and 26 females (including 2 castrated males and 7 spayed females) with a mean age of 5.4 years (ranging from 9 months to 14 years old).

A diagnosis of seborrheic dermatitis was made based on both classical clinical features and successful management with either shampoo, which are anti-yeast or anti-seborrheic products, or systemic anti-yeast agents (Fig. 1). Although the clinical findings were fairly distinctive, similar dermatoses especially pyoderma, demodicosis, and dermatophytosis, were carefully ruled out based on clinical findings, standard diagnostic procedures, and antibiotics therapy. Ten clinically healthy dogs (mean age of 2.8 years, 9 female and 1 male) served as normal controls. Five were pet dogs of crossbreeds and the rest were research beagles.

Cytological samples for detection of the organism were taken from abnormal skin in the ear, the ventral cervical area, the axillary region, the ventral abdomen, and/or dorsal aspect of the web between the digits of the foot in all cases. Preparations were made by firmly pressing a 1 cm clean glass slide against 2 areas of the skin. These specimens were stained with new methylene blue (Brecher’s new methylene blue Sol; Muto Pharmaceuticals Ltd., Tokyo, Japan), and they were then carefully evaluated microscopically. Malassezia organisms were detected in all cases, although in some dogs the organism was not readily detectable in large numbers.

Antigen Preparations

M. pachydermatis harvested from canine ear was cultured at 30°C on Sabouraud’s agar medium. After harvesting, 1 vol. of the organisms was suspended in 50 vol. of phosphate-buffered saline (PBS; pH 7.4) and was sonicated for 15 minutes (VP-5, TAIKEK, Saitama, Japan). The suspension containing disrupted organisms was then centrifuged, and the supernatant was used as an antigen for intradermal testing after adjusting the protein concentration to 20 µg/ml in PBS. The protein concentration of the extract was determined using a protein assay kit (MPR A4, Toso Ltd., Tokyo, Japan).

M. pachydermatis extract, containing 380 µg/ml protein, was placed on SDS-polyacrylamide gels. Electrophoresis was carried out according to Laemmli with the following modifications. The separating gels were made of 12% acrylamide containing 0.1% sodium dodecyl sulfate. The gel was stained with Coomassie blue and photographed. Molecular weights were assumed to be those of the reference standards. The gel pattern of the M. pachydermatis extract is shown in Fig. 2. The most prominent band had a molecular weight of approximately 10 kDa.

Intradermal Testing (IDT) Procedure

IDT was carried out at the initial examinations. If the dogs were previously receiving doses of corticosteroids and/or antihistamines from referring veterinarians, those drugs were discontinued for a minimum of 21 days and 7 days, respectively. The lateral thorax was shaved, and the test
pattern was outlined with a felt-tipped marker. 0.04 ml of the extract as well as a positive control (1:100,000 W/V histamine phosphate) and a negative control (saline) were injected intradermally at individual sites. Injection sites were observed for immediate reactions 15 minutes after the last injection in all dogs, and were again checked for delayed reactions 72 hr after injection in 32 dogs. Reactions were determined subjectively (visually and by digital palpation) by comparing the test sites with the reactions seen at the positive and negative control sites. In this study, an IDT reaction equal to or exceeding the positive control was considered as an immediate positive reaction, and a persistent dermal nodule was considered as a delayed positive reaction. These data were statistically analyzed using the Chi-square test.

Efficacy of shampoo therapy

Thirty dogs with seborrheic dermatitis were initially treated with shampoo alone, either selenium disulfide (Canimal-one, Fujita Pharmaceutical Co. Ltd., Tokyo, Japan) or chlorhexidine (Nolvasan, Fort Dodge Animal Health, Iowa, USA), two or three times a week. The efficacy of the shampoo therapy was compared between the dogs with positive and those with negative reactions to the antigen.

Immediate reactions to *M. pachydermatis* antigen were observed in 17 (34.0%) of all 50 dogs, and delayed reactions in 3 (9.4%) of 32 dogs (Fig. 3 and Table 1). None of the control dogs showed any immediate or delayed positive reactions to the antigens. A significant difference was seen when comparing the incidences of positive reactions between dogs with seborrheic dermatitis and the healthy control dogs ($p<0.05$).
Skin reactivity to *Malassezia* in canine SD

**Table 2.** Efficacy of shampoo therapy in dogs with seborrheic dermatitis

<table>
<thead>
<tr>
<th></th>
<th>Improved cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seborrheic dermatitis</td>
<td>22/30 (73.3%)</td>
</tr>
<tr>
<td>Positive reactions to <em>M. pachydermatis</em></td>
<td>3/9 (33.3%)</td>
</tr>
<tr>
<td>Negative reactions to <em>M. pachydermatis</em></td>
<td>19/21 (90.5%)*</td>
</tr>
</tbody>
</table>

*Statistically significant difference from the population of dogs with the positive reactions to *M. pachydermatis* (Chi-square test, *p*<0.01).

Table 2 shows the results of the efficacy of the topical treatments. Shampoo was effective in 19 (90.5%) of 21 cases with the negative reactions. In contrast, the same treatment was effective in only 3 (33.3%) of 9 cases with positive reactions, confirming a significant difference between dogs with positive and those with negative reactions (*p*<0.01).

The symptoms in the remaining cases were completely improved with the systemic anti-yeast agent.

**DISCUSSION**

All cases in this study improved to some degree with either shampoo or systemic anti-yeast agent. A number of treatment studies with anti-yeast agents have demonstrated a correlation between *Malassezia* organisms and dermatitis in humans. Seborrheic dermatitis in dogs was found to correspond with its human counterpart, and *M. pachydermatis* is a likely causative agent in seborrheic dermatitis in both dogs and humans. Shuster argued that seborrheic dermatitis in humans meets Koch’s criteria for *Malassezia*. A recent study described seborrheic dermatitis as common among those suffering from acquired immunodeficiency syndrome, and that such patients are likely to have an increased susceptibility to yeast infections due to defective cell-mediated responses. Other authors have reported that some histological features of seborrheic dermatitis can be reproduced by inoculating animal skin with the dead yeast. *M. furfur* has also been shown to be capable of activating an alternative complement pathway, liberating C5a anaphylatoxin. It is suspected that the infectious etiology and/or inflammatory response to *Malassezia* products may trigger dermatitis in dogs.

In humans, the term *Malassezia* (Pityrosporal) dermatitis has been used synonymously with seborrheic dermatitis. *Malassezia* dermatitis has been described in dogs, and is known as ketoconazole-responsive pruritic dermatitis. *Malassezia* overgrowth is a common finding in *Malassezia* dermatitis, and clearing of the lesions is often paralleled by a reduction in the amount of yeast. However, it is reported that among dogs with classic clinical findings wherein various surface sampling techniques demonstrated little or no yeast, the dogs nevertheless responded completely to specific treatment. These cases emphasize that hypersensitivity rather than an infectious etiology underlies its pathogenesis. This study demonstrated immediate and delayed hypersensitivity reactions to *M. pachydermatis* in dogs. In addition, most of the dogs with positive reactions to the yeast antigen required systemic anti-yeast agent rather than shampoo alone for successful management. Based on these findings, it is suggested that *M. pachydermatis* likely acts as the allergen on the skin of some dogs. *Malassezia* dermatitis is descriptive of the etiological aspects of the condition. On the other hand, seborrheic dermatitis describes the morphological aspect, without defining its pathogenesis. Thus, it is suggested that this dermatitis should be carefully described as seborrheic dermatitis until the pathogenesis is more fully understood.

In conclusion, the present study indicates this study suggests that *M. pachydermatis* may be a causative agent of seborrheic dermatitis in dogs. Although proof of an infectious or enzymatic etiology would be important, it is clear from this study that *M. pachydermatis* acts as the allergen in some dogs. These findings warrant further study of immunologic reactions to the yeast.
ACKNOWLEDGEMENTS

The author is grateful to Dr. T. Ishida, H. Takeda, and Dr. J. Fukuoka of Nippon Veterinary and Animal Science University for their assistance with the antigen preparations and molecular weight analyses of *M. pachydermatis*, and to Dr. S. Ohba of Nihon University for the statistical analyses.

REFERENCES


犬の脂漏性皮膚炎における Malassezia pachydermatis に対する皮膚応答

永田雅彦
どうぶつ皮膚病センター

要約：犬の脂漏性皮膚炎の頭に対し Malassezia pachydermatis 抽出物を用いた皮内反応を実施した。M. pachydermatis 抗原に対する即時型反応が頭部（100%）に、また遅延型反応が頭部（50%）に認められた。健常犬頭部にも同様の抗原を用いた皮内反応を施行したが、陽性反応はみられなかった。供試犬にシャンプー療法（二硫化セレン、クロルヘキシジン）を実施したところ、皮内反応陰性犬頭部（20%）が改善した。一方皮内反応陽性犬では頭部に（頭部（50%））しか改善しなかった。シャンプー療法で改善しなかった症例は、その後使用した抗真菌剤（ケトコナゾール）が奏功した。以上より、M. pachydermatis は犬の脂漏性皮膚炎においてアレルギーとして関与しうることが明らかにされた。

キーワード：犬、皮内反応、Malassezia pachydermatis

連絡先：永田雅彦（どうぶつ皮膚病センター）
〒162-0824 東京都渋谷区渋谷表町1-7-6
電話: 03-5444-8888
 Fax: 03-5444-8888