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Mast Cell Distribution at Predilection Sites of Atopic Dermatitis in Normal Canine Skin

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Abstract: Mast cell distribution was quantified in acidified toluidine blue sections of normal skin from 8 different sites in 10 dogs and compared to the predilection sites of canine atopic dermatitis. Mast cell counts varied significantly from site to site ($p<0.0001$) and counts in the superficial dermis were significantly higher than the deeper dermis ($p<0.05$). The highest mast cells distribution sites were the concave surface of the ear (mean 74.88±17.93 per mm^2) and the interdigital skin of the forefeet (mean 28.326±6.24 per mm^2). Counts in these sites were 280% higher than all the other sites. Our results may provide some evidence that cutaneous mast cell distribution may be a factor in the frequent occurrence of ear and foot pruritus in atopic dermatitis. However, the low mast cell count in the predilection sites of atopic dermatitis did not explain the common occurrence of atopic lesions. Therefore, other factors or more complicated pathogenesis may be correlated with these predilection sites.

Key words : mast cell, atopy, dermatitis, dog.

Introduction

Mast cells play a major role in the pathogenesis of canine atopic dermatitis. Following Type I hypersensitivity reactions results in the release of a variety of inflammatory mediators and cytokines by cross-linking of receptor-bound IgE by multivalent allergens[4,11,12,16,19]. Therefore, mast cells are crucial initiation and maintenance effects on dog with atopic dermatitis[4,12,19].

Atopic skin disease is characterized by pruritus and predilection sites include the concave surface of the ear, upper eyelids, extensor surface of carpal joint, thorax, axillae, interdigital area, flexural surface of tarsal joint and lips[5,20]. Incidence of interdigital area and ears occur in approximately 70% and 55% of cases, respectively[19]. The reasons for these predilection sites have not been established but suggested hypotheses include variations in epidermal barrier function allowing transepidermal antigen penetration.

Although the obvious involvement of cutaneous mast cells in atopic dermatitis, the role of mast cell density in the development of these predilection sites has not been determined. Therefore, the purpose of this study was to quantify the number of mast cells in histological sections of normal canine skin from various body regions and to compare them with the predilection sites of canine atopic dermatitis.

Materials and Methods

Dogs

Ten dogs with no clinical signs of skin disease were included in the study. They were three-female and seven-male dog. Anesthesia was induced with xylazine (1.1 mg/kg) and ketamine (10 mg/kg) intravenously initially and maintained with a half dose IV as needed.

Skin biopsy

Biopsy specimens with 8-mm punch biopsies or no. 15 surgical blade were obtained from 8 different sites. Such sites were the concave surface of the ear, upper eyelids, extensor surface of carpal joint, thorax, axillae, interdigital space, flexural surface of tarsal joint and lips. Biopsies were excised and fixed in Bouin's solution. After fixation and routine processing, 3-4 μm sections were cut and stained with acidified toluidine blue.

Enumeration of mast cell

Each section was firstly assessed in order to confirm that there was no histological evidence of skin disease. In each section, mast cells were counted in the superficial dermis and deep dermis (objective ×40, eyepiece ×10) using an objective micrometer. The mast cells contained within ×400 high power fields were counted and then the graticule was moved to an adjacent field. This process was repeated 10 consecutive times in the superficial dermis and deep dermis, which positioned at least one high power field depth below the dermal-epidermal junction. Mast cell counts were performed by one investigator in a blind manner so as to preventing subjective prejudice.

Statistical analysis

The data were analyzed using SAS (GLM Procedure, Cary. NC. USA). Parametric tests were used to compare different sites. Differences between all 8 sites were assessed by nested design test. The data were reported as mean±SEM as indicated. A significance level of $p<0.0001$ was used except Table 1 of which level was $p<0.05$.

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Results

The statistical significances of mast cell counts in the superficial dermis were higher than the deeper dermis ($p<0.0001$, Fig 1). Mast cell counts from the 8 different body sites significantly varied when assessed by Nested design test ($p<0.0001$, Fig 2). Representative sections in Fig 3 revealed variation of mast cell density from different body regions. Mast cell counts in the deep dermis were consistently lower and showed much less variation than the superficial dermis (Fig 1). The regions of the highest mast cells distribution were the concave surface of the ear (74.88± 17.93 per mm^2)

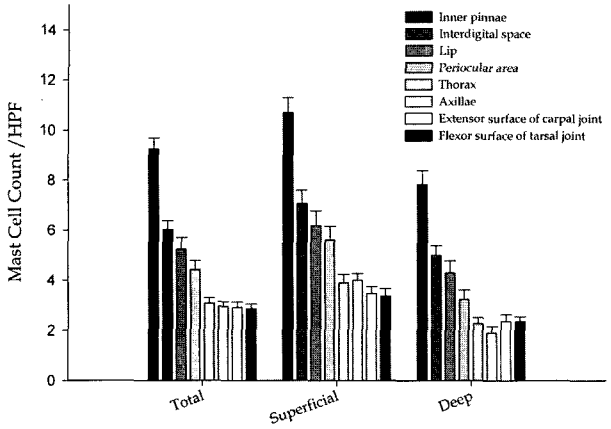

Fig 1. Mast cell count per high power field obtained from the superficial and deep dermis in normal canine skin. Wach data from superficial and total dermis showed a significant diffrence among all 8 sites ($p<0.0001$). All bars represents the mean± SEM of mast cells counts.

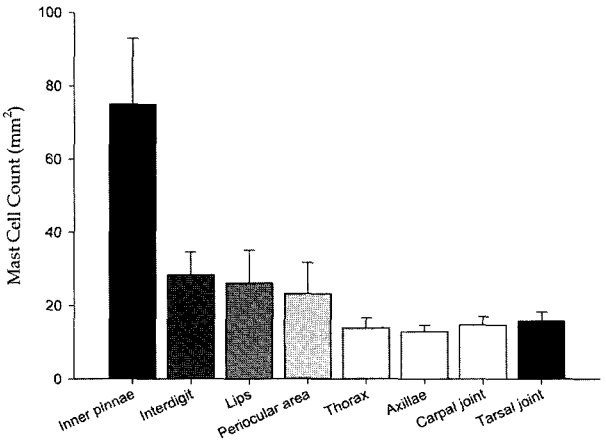

Fig 2. The mast cell count per mm^2 obtained from 8 different body sites. All data showed significant diffrences from 8 ($p<0.0001$). Each bars represents the mean±SEM of mast cells counts.

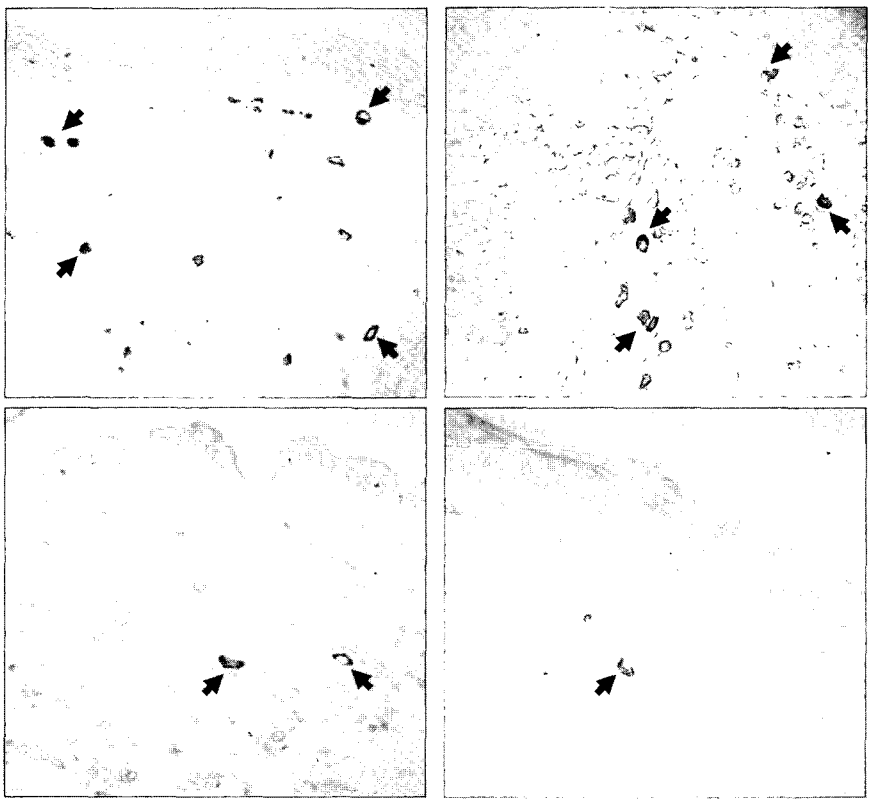

Fig 3. Photomicrographs of representative histological sections from various body sites: inner pinna (a), interdigital space (b), extensor surface of carpal joint (c), and flexor surface of tarsal joint (d). Mast cells are recognized by their metachromatic granules. Arrows show individual mast cells (Acidified toluidine blue stain, ×400 original magnification). bar=70 μm

Table 1. Mean mast cells counts in the superficial dermis from 8 different body sites. Counts are given per high power field (mean±SEM, magnification ×400) and per mm^2 (mean) in order to allow comparison with other studies ($p<0.05$).

Site	Mast cell count	
	per high power field	per mm^2
Inner surface of pinna	9.26±0.42*	74.88±17.93
Interdigital surface	6.03±0.33†	28.33±6.24
Lips	5.25±0.38	26.18±8.95
Periocular area	4.45±0.33	23.30±8.39
Thorax	3.09±0.21	13.94±2.68
Axillae	2.96±0.19	12.92±1.74
Carpal joint	2.92±0.19	14.73±2.28
Tarsal joint	2.87±0.18	15.78±2.55

*Significantly highly than counts from the interdigital surface below.
†Significantly highly than counts from the axillae below.

and the interdigital area of the forefeet (28.33±6.24 per mm^2). These body sites had mean mast cell counts in the superficial dermis that were at least 280% higher than all the other sites. The counts and significant differences observed between body sites are shown in Table 1 along with data converted from graticule area to high power microscopic fields and mm^2 ($p<0.05$).

Discussion

Atopic dermatitis is usually defined as an inherited predisposition to develop IgE antibodies to environmental allergens[10]. Changes in essential fatty acid metabolism in epidermis and decreased cutaneous cell-mediated immunity may also be involved leading to increased susceptibility to skin infections[4]. However, these mechanisms do not fully explain the typical clinical appearance of atopic dermatitis which shows marked predilection sites such as face, pinna, feet, distal extremities, thorax and axillae. These areas are common predilection sites for canine atopic dermatitis and clinical involvement of these regions is thought to occur in approximately 70% and 55% of cases, respectively[8,18,24]. In this study, the observed regions for mast cells density in normal skin were selected at the atopic dermatitis predilection sites based on Willemse's and Prélaud criteria[8,18,24]. Significantly high number of mast cells was found in the concave surface of pinna and interdigital surface. These results may therefore provide some evidence that cutaneous mast cell distribution may be a factor in the frequent occurrence of ear and foot pruritus in this disease.

Assessment of mast cell distributions in dogs has not involved predilection sites of canine atopic dermatitis in previous studies. It was reported the highest density of cutaneous mast cells in the ear followed by the vulva, prepuce, and scrotum. However, these data were not included interdigit and the skin around the feet the predilection sites of atopic dermatitis[7]. Since atopic criteria and predilection sites of atopic lesions had been established, it was investigated that only three sites such as lateral neck, dorsal rump, and craniolateral abdomen were not associated with the most common predilection sites[23]. In later study, it was revealed that pinna and interdigital skin had the highest density of mast cells in the 20 sites of atopic predilection and nonpredilection lesions[1].

The density of mast cells found in this study was 2.9-9.3 mast cells per HPF. One text said mast cells per 4-12 HPF and one reported 2.4-16.9 mast cells per HPF[7,19]. Some researchers found 8.3-10.1, and 2.9-11.3 mast cells per HPF, respectively[1,23]. The result of observation was limited only three body regions so their range does not take into consideration[23]. In our study, the lower density of mast cell may be due to the use of anesthesia[13,22], geographical difference, and subjective intervention of investigator in interpretation of cell counts. Therefore, more investigation of counting method of the mast cell density needs to be achieved.

In this study, the density of dermal mast cells was divided into superficial dermis just under the dermal-epidermal junction and deep dermis, at least one high power field depth below the dermal-epidermal junction. Mast cell counts in superficial dermis have the statistical significance, but not that in deep dermis. Meanwhile, one reported that mast cell counts in the superficial were significantly higher than the deep dermis, but the statistical significance both in superficial dermis and deep dermis was observed[1]. The reason for the differences in mast cell distribution in deep dermis is not known but it is likely to be related to the defense role of mast cells in the interfacing environment. It was demonstrated that mast cell play a critical role in neutrophilic response and bacterial clearance[6,15]. Therefore, higher density of mast cells could be expected in the superficial dermis and further studies would be required to investigate the disparity of mast cell in dermis depending on the amount of allergen and bacteria.

Our results may therefore provide some evidence that cutaneous mast cell distribution may be a factor in the frequent occurrence of ear and foot pruritus in this disease. These results confirmed previous research that shown a significant positive correlation between the magnitude of lesions of atopic model and the change in the number of mast cells[3]. However, clinical manifestations of canine atopy are varied among investigators. According to Scott, percentage of ventral and facial atopic lesions which have normally a low mast cell distribution is 89% and 79% of cases, resepectively[20].

In addition, the low mast cell counts in the predilection sites of atopic dermatitis does not explain the common occurrence of atopic lesions such as the lips, periocular area, thorax, axillae, extensor surface of carpal joint and flexor surface of tarsal joint. Therefore, other factors or more complicated pathogenesis may be correlated with these predilection sites. Such factors might include autonomic nervous system disturbance, partial defect in the skin immune system, and the den-

sity of IgE molecules on epidermal Langerhans cells, changes in fatty acid metabolism, disruption in the skin barrier function and phosphodiesterase activity.

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개의 아토피성 피부염의 피부증상 호발부위의 비만세포분포조사

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요 약: 개의 아토피성 피부염의 피부증상이 호발하는 8부위에서 비만세포의 분포를 조사 비교하였다. 피부증상을 보이지 않는 10두의 개의 8부위의 피부조직을 채취하여 비만세포에 특이적인 acidified toluidine blue 염색을 하여 현미경하에서 계수비교하였다. 비만세포수는 표재진피에서 각각의 부위간에 유의한 변화가 관찰되었으며 ($p<0.0001$) 표재진피가 심재진피에 비해 유의하게 높았다 ($p<0.05$). 호발 부위중 비만세포가 가장 높은 부위는 내측이개와 지간으로 각각 74.88 ± 17.93 per mm^2, 28.33 ± 6.24 per mm^2이었다. 이 부위는 다른 부위에 비해 비만세포가 280% 높게 분포하였다. 따라서 개의 아토피성 피부염의 호발증상인 귀와 발의 소양증의 발생은 비만세포의 수가 다른 부위에 비해 높은 것이 하나의 요인으로 사료된다. 그러나 비만세포의 수가 낮게 나타난 다른 호발부위에 대해서는 다른 요인이 관계하는 것으로 사료되며 이에 관한 연구가 필요하다.

주요어: mast cell, atopy, dog