CD3+ and Pax5+ Lymphocytes were not Found in the Epidermis and Adnexal Epithelia of Normal Skin from the Dorsolateral Thorax of Dogs

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Abstract: A small population of resident T-lymphocytes is present in the healthy epidermis of skin from humans, mice, cattle, and sheep. Resident lymphocytes were not found in the epidermis or adnexal epithelia of healthy skin from cats and horses. Skin-biopsy specimens from the normal skin of the dorsolateral thorax from 29 dogs were examined histologically and immunohistochemically for the presence of lymphocytes, CD3+ (T-lymphocytes), and Pax5+ (B-lymphocytes) cells in the epidermis and adnexal epithelia. All examinations were negative. It appears that lymphocytes rarely occur, or occur in very small numbers, in the epidermis and adnexal epithelia of normal dog skin.

Key words: dog, skin, resident epithelial lymphocytes

Introduction

Resident lymphocytes in human epidermis were likely first described by Kondo in 1922. Studies have demonstrated that resident epidermal lymphocytes in humans are CD3+ (pan T-cell marker) T-lymphocytes and occur rarely in the epidermis and adnexal epithelia9, 10, 24). Resident epidermal CD3+ T-lymphocytes have also been described in the mouse26), sheep11), and cow12), and are found in very small numbers. Two recent studies failed to identify resident lymphocytes in the epidermis and adnexal epithelia of normal skin from cats27) and horses28).

Lymphocytes have been immunophenotyped in numerous inflammatory dermatoses of the dog, and have been shown to be T-cells1, 7, 13, 15, 16, 18). However,
epidermal lymphocytes generally have not been demonstrated in normal skin (face, groin, axilla, thorax) save for the lip, from very small numbers of dogs. Normal skin (dorsal paw, lateral thorax, axilla, groin; 4 skin sections per site, 16 sections total) was studied from only 3 dogs. CD3+ cells were “rarely” seen in the epidermis in 1 of 16 sections (anatomic site not specified). To the authors’ knowledge, the occurrence of resident lymphocytes in the adnexal epithelia of normal dog skin has not been investigated.

B-lymphocytes have not been found in normal human and murine skin. To the authors’ knowledge, B-lymphocytes have not been detected in normal canine skin. Paired box (Pax) protein 5 has been recently reported to be a B-lymphocyte (not plasma cell) marker in humans and dogs.

The purpose of this study was to determine the prevalence of CD3+ and Pax5+ cells in the epidermis and adnexal epithelia in skin-biopsy specimens from 29 dogs with normal skin.

Materials and Methods

Sample collection

Archival samples of normal skin from 29 dogs submitted to the Section of Anatomic Pathology for necropsy in 2010 were used in this study. The 29 dogs ranged in age from <1 month to 15 years old, included 15 males and 14 females, and represented several breeds and mongrels. All skin samples were taken from the dorsolateral thorax using a 6-mm biopsy punch. Samples were then formalin-fixed and paraffin-embedded. Serial sections (4 μm thick) from each block were stained with hematoxylin and eosin and with antibodies against CD3 and Pax5.

Histological evaluation

Sections stained with hematoxylin and eosin (1 section per dog) were examined independently by 2 of the authors (MDC and DWS). Epidermis and epithelia of all hair follicles, sebaceous glands, and epiderchial sweat glands were examined for the presence of lymphocytes. The number of pilosebaceous units per section was recorded.

Immunohistochemical investigation

Immunohistochemistry for CD3 and Pax5 was performed as previously described. Briefly, sections were mounted (ProbeOn Microscope Slides, Fisher Scientific Co., Bridgewater, New Jersey, USA) and deparaffinized. Antigen retrieval was tested using a variety of methods. The best antigen retrieval method was to steam tissue sections in citrate buffer (0.01 mol/L, pH 9.0) for 5 minutes at higher power (800 W) and for 15 minutes at medium power (300 W). The sections were incubated with rabbit antihuman polyclonal CD3 antibody (Dako North America, Carpenteria, California, USA) at a 1:100 dilution of 500 μg/ml stock solution, and rabbit antihuman monoclonal Pax5 antibody (Thermo Fisher Scientific, Waltham, Massachusetts, USA) which was prediluted and in a ready-to-use form, and stained using a standardized streptavidin-biotin immunoperoxidase technique. The chromagen was Nova Red.

Normal canine lymph node and canine brain from the same period served as positive and negative tissue controls, respectively. Additionally, diseased canine skin (Malassezia dermatitis with epidermal and dermal lymphocytes and dermal plasma cells present on hematoxylin and eosin-stained sections was processed in an identical fashion to the samples in question to serve as a positive tissue control. Diseased canine skin also served as a negative tissue control, when processed by substituting the primary antibody with nonimmune rabbit serum.

Results

The number of pilosebaceous units per hematoxylin and eosin-stained section ranged from 6–22 (total number examined = 313). No lymphocytes were seen in the hematoxylin and eosin-stained sections, and no CD3+ or Pax5+ cells were present in the epidermis and adnexal epithelia. The agreement between the 2 histological and immunohistochemical assessments was 100%. Positive and negative controls reacted appropriately (Figs. 1 and 2). The lymphocytes and plasma cells in the sections of Malassezia dermatitis were CD3+ (Fig. 3) and Pax5–, respectively.

Discussion

Resident epidermal T-lymphocytes are a component of the skin-associated lymphoid tissue and skin immune system in humans and mice. It has been suggested that, while present in very low numbers, resident
T-lymphocytes have varied important immunologic functions, to include disease mediation and protective roles (21).

In humans, most inflammatory dermatoses contain predominantly T-lymphocytes (9, 10, 23). In the dog, numerous reports have described T-lymphocytes in inflammatory dermatoses. CD3+ lymphocytes have been documented in canine epidermis and/or adnexal epithelium in graft-versus-host disease and erythema multiforme (11), exfoliative cutaneous lupus erythematosus (5), vesicular cutaneous lupus erythematosus (13), alopecia areata (17), Malassezia dermatitis (15), demodicosis (7), leishmaniosis (19), and atopic dermatitis (6).

Resident epidermal lymphocytes in humans and mice are not of the B-cell immunophenotype (4, 23, 26). Similarly, they are not a major component of most inflammatory dermatoses in humans or dogs. Pax5 has been evaluated as a pre-plasma cell, B-lymphocyte marker for canine lymphocytes (2, 29). We could find no reports on the occurrence of Pax5+ B-lymphocytes in normal skin or inflammatory dermatoses from dogs.

We elected to use Pax5 as a B-lymphocyte marker because it is not expressed by plasma cells (2, 29). Although CD79a and CD20 are also B-lymphocyte markers in dogs, they both can also be expressed by plasma cells (29).

To our knowledge, this is the first study to investigate normal canine skin for (1) the presence of lymphocytes in adnexal epithelia (hair follicle, sebaceous gland, epitrichial sweat gland), and (2) the presence of Pax5+ lymphocytes in epidermis and adnexal epithelia. In addition, our study concerns the largest cohort of dogs examined to date for the presence of resident epithelial lymphocytes. We found neither CD3+ nor Pax5+ cells in the epithelia of normal dog skin. In normal human epidermal sheet preparations and normal ovine epidermis, T-lymphocytes numbered <0.25/mm³ and approximately 10/cm², respectively (8, 11). It may be that resident epidermal lymphocytes occur in very small numbers in normal canine skin, but our sample size was not adequate to detect them.

In this study, only skin from the dorsolateral thorax was examined. It is possible that the presence and number of resident lymphocytes vary with body site. For instance, perhaps body regions with more environmental

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**Fig. 1.** Normal canine lymph node. CD3+ lymphocytes (arrow) are predominantly present outside germinal centers (Nova Red).

**Fig. 2.** Normal canine lymph node. Pax5+ lymphocytes (arrow) are predominantly present within germinal centers (Nova Red).

**Fig. 3.** Skin-biopsy specimen from a dog with Malassezia dermatitis. Note CD3+ lymphocytes (arrow) within hyperplastic epidermis (Nova Red).
exposure, such as glabrous regions, lips, or pawpads would yield different results. However, previous studies in humans generally detected no variation when multiple regions of skin were sampled, to include the breast, thigh, arm, chest, back, abdomen, eyelid, face, neck, scalp, and buttock. In addition, other investigators also failed to demonstrate epidermal lymphocytes in normal canine skin from various sites (e.g., face, groin, axilla, thorax, dorsal paw) in smaller numbers of dogs than investigated in the present study.

In conclusion, CD3+ and Pax5+ lymphocytes were not found in the epidermis and adnexal epithelia of normal canine skin from the dorsal lateral thorax. It appears that lymphocytes rarely occur, or occur in very small numbers, in the epidermis and adnexal epithelia of normal dog skin. The presence of lymphocytes in these structures should, therefore, be considered abnormal in canine skin-biopsy specimens.

References
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