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

CD3陽性細胞およびPax5陽性細胞は正常な犬胸背部皮膚の真皮からは検出されなかった

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Abstract: A small population of resident T-lymphocytes are present in the healthy epidermis of skin from humans, mice, cattle, sheep, and alpacas. In addition, small numbers of resident T-lymphocytes are present in the dermis of normal skin of humans, cattle, and sheep. The objective of this study was to determine the prevalence of lymphocytes, CD3+ cells (T-lymphocytes) and Pax5+ (B-lymphocytes) cells in the superficial and deep dermis of normal dog skin. Skin-biopsy specimens from the normal skin of the dorsolateral thorax from 26 dogs were examined histologically and immunohistochemically for the presence of CD3+ and Pax5+ cells in the superficial and deep dermis. All examinations were negative. It appears that lymphocytes rarely occur, or occur in very small numbers, in the superficial and deep dermis of normal dog skin.

Key words: dermal lymphocytes, dog, skin

要約: ヒト、マウス、ウシ、ヒツジおよびアルパカでは、少数の常在T細胞が常在な表皮に存在する。加えてヒト、ウシおよびヒツジでは、少数の常在T細胞が皮膚にも存在する。本研究の目的はリンバ球、CD3陽性細胞（Tリンパ球）ならびにPax5陽性細胞（Bリンパ球）が、常在犬の皮膚の真皮浅層および深層に存在するかを解析することであった。26頭の犬から採取された正常な皮膚の生検組織を対象として、真皮浅層および深層にCD3陽性細胞ならびにPax5陽性細胞が存在するかを組織学的および免疫組織化学的手法を用いて解析した。その結果、全ての検体において前述の細胞は認められなかった。この結果から、正常犬皮膚の真皮浅層および深層ではリンバ球がほとんど存在しないか、存在してもごく少数であることが示唆された。

キーワード: 真皮リンパ球、犬、皮膚

Introduction

Several studies have characterized the resident lymphocyte population of the epidermis of normal skin from a variety of species2,3,5-10,13,14,16-20. However, only a few studies have been performed to characterize the resident lymphocyte population in the dermis. CD3+ T-lymphocytes are found in very small numbers (not quantitated) – and predominately in a perivascular location – in normal human skin2,3,7,8,13. Resident dermal T-lymphocytes have also been described in cattle10) and sheep9), predominately in a perivascular location, and
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in larger numbers around superficial than deep dermal blood vessels. In dogs, resident dermal T-lymphocytes were found to make up only 3% of 200 dermal cells counted in a 0.155 m² sample[12]. In this very small study, normal skin was obtained from the dorsal paw, lateral thorax, axillary, and inguinal regions from three dogs (4 skin sections per site, 16 sections total). In a second study involving 10 healthy dogs, CD3+, CD4+, and CD8+ T-lymphocytes were described in the “subepidermal” dermis[15].

Although few studies regarding the resident dermal T-lymphocyte population have been performed, even fewer studies have investigated the presence of resident dermal B-lymphocytes. Resident dermal B-lymphocytes were not found in human or murine skin[3,18]. To the authors’ knowledge, no other studies have investigated the prevalence of B-lymphocytes in the dermis of normal skin. Paired box (Pax) protein 5 has been recently reported to be a B-lymphocyte (and not plasma cell) marker in humans and dogs[1,21].

The purpose of this study was to determine the prevalence of CD3+ and Pax5+ lymphocytes in the dermis of normal skin from the dorsolateral thorax of 26 dogs.

Materials and Methods

Sample Collection

Archival samples of normal skin from 26 dogs submitted to the Section of Anatomic Pathology for necropsy in 2010 were used in this study. The 26 dogs ranged in age from <1 month to 15 years old, included 14 males and 12 females, and represented several breeds and mongrels. All skin samples were taken from the dorsolateral thorax just after death 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 Examination

Sections stained with hematoxylin and eosin (1 section per dog) were examined by 2 of the authors (HE and JPK). Each microscopic field contained at least one vascular plexus and was separated into superficial and deep dermis. The superficial dermis was defined as the region superficial to the sebaceous glands. The deep dermis was defined as the region deep to the sebaceous glands. Three random 40X microscopic fields – one from each end of the slide and one in the center – were analyzed for the presence of CD3+ and Pax5+ superficial and deep dermal lymphocytes.

Immunohistochemical Investigation

Immunohistochemistry for CD3 and Pax5 was performed as previously described[1,5,21]. 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 medium power (300W). The sections were incubated with rabbit anti-human polyclonal CD3 antibody (Dako North America, Carpenteria, California, USA) at a 1:100 dilution of 500 μg/ml stock solution, and rabbit anti-human monoclonal Pax5 antibody (Thermo Fischer Scientific, Waltham, Massachusetts, USA) which was prediluted and in a ready-to-use form. This was 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 dermal lymphocytes and plasma cells present on hematoxylin and eosin-stained sections[11]) 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

No lymphocytes were seen in the hematoxylin and eosin-stained sections, and no CD3+ or Pax5+ cells were present in either the superficial or deep dermis. The agreement between the 2 histological and immunohistochemical assessments was 100%. Positive and negative controls reacted appropriately (Fig. 1). The lymphocytes and plasma cells in the section of Malassezia dermatitis were CD3+ (Fig. 2) and Pax5 – (Figs. 3 and 4), respectively.
Resident epidermal T-lymphocytes play an important role in the skin-associated lymphoid tissue (SALT) or skin immune system (SIS) in humans and mice\(^3,17,18\). Resident epidermal T-lymphocytes have also been demonstrated in the normal skin of cattle\(^10\), sheep\(^9\), and alpacas\(^6\). Resident dermal T-lymphocytes have been demonstrated in the normal skin of humans\(^3,8\), cattle\(^10\), sheep\(^9\), and in 13 dogs\(^12,15\). Resident epidermal B-lymphocytes have not been demonstrated in humans\(^3,16\), rodents\(^8\), dogs\(^5\), cats\(^9\), horses\(^20\), or alpacas\(^6\). Further, resident dermal B-lymphocytes have not been found in humans\(^3\) or rodents\(^8\). Pax5 has been evaluated as a pre-plasma cell, B-lymphocyte marker for canine lymphocytes\(^1,21\). We could find no reports on the occurrence of Pax5+ B-lymphocytes in the normal dermis or inflammatory dermatoses from dogs. We elected to use Pax5 as a B-lymphocyte marker because it is not expressed by plasma cells\(^1,21\). Although CD79a and CD20 are also B-lymphocyte markers in dogs, they both can also be expressed by plasma cells\(^9\).

To our knowledge, this is the first study to investigate normal canine skin for the presence of B-lymphocytes in the dermis, and the largest cohort of dogs examined to date for the presence of resident dermal CD3+ T-lymphocytes. Resident CD3+ and Pax5+ lymphocytes were not found in the dermis of any sections examined.
Our failure to detect CD3+ resident dermal lymphocytes is contrary to the findings of two previous small studies. Olivry et al.\textsuperscript{12} found CD3+ dermal lymphocytes in very small numbers in the normal skin (paw, lateral thorax, axilla, groin) from only 3 dogs. Sinke et al.\textsuperscript{15} found CD3+, CD4+, and CD8+ dermal lymphocytes in the normal skin (chin, digit, axilla, carpus, tarsus, and periocular region) from 10 dogs. In the study by Sinke et al.\textsuperscript{15}, 10 mm horizontal sections through the “subepidermal” dermis were evaluated. It is impossible to compare our results with those of Sinke et al.\textsuperscript{15} as our methodologies (sample size and site, fixation and preparation of specimens) were different.

In our study, only skin from the dorsolateral thorax was examined. Thoracic skin was not examined in the study by Sinke et al.\textsuperscript{15}. Although lateral thoracic skin (3 dogs) was examined in the study by Olivry et al.\textsuperscript{12}, data specific to this site were not reported separately. Perhaps the number of resident dermal T-lymphocytes varies with body site.

Our failure to identify Pax5+ dermal B-lymphocytes is consistent with previous studies in humans\textsuperscript{3} and rodents\textsuperscript{18}.

In conclusion, CD3+ and Pax5+ lymphocytes were not found in the superficial and deep dermis of normal skin from the dorsolateral thorax of dogs. It appears that lymphocytes rarely occur, or occur in very small numbers, in the dermis of normal canine skin. The presence of dermal lymphocytes should, therefore, be considered potentially abnormal in canine skin-biopsy specimens.

References


