CD3+ and Pax5+ Lymphocytes in the Dermis of Normal Skin from the Dorsolateral Thorax of Cats

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Abstract: Very small numbers of resident T-lymphocytes are present in the dermis of normal skin of humans, cattle, sheep, dogs, and alpacas. The objective of this study was to determine the prevalence of lymphocytes, CD3+ cells (T-lymphocyte), and Pax5+ cells (B-lymphocyte) in the dermis of normal cat skin. Skin-biopsy specimens from the normal skin of the dorsolateral thorax from 29 cats were examined immunohistochemically for the presence of CD3+ and Pax5+ cells in the superficial and deep dermis. Two CD3+ lymphocytes were found in the superficial dermis in 1 of 29 cats. B-lymphocytes were not found.

Key words: cat, dermal lymphocytes, skin

Introduction

Few studies have examined the resident lymphocyte population in the dermis of normal skin. CD3+ T-lymphocytes were found in very small numbers (not quantitated), and in a predominately perivascular location in normal human skin2–5,13). Resident dermal T-lymphocytes were also described in cattle9), sheep9), and alpacas7), predominately in a perivascular location, and in larger numbers around superficial than deep dermal blood vessels. CD3+ T-lymphocytes were found in very small numbers in the dermis of normal dog skin in two small studies11,15), but were not detected in a third larger study6). In two small studies, dermal lymphocytes were found in 0 of 1012), and 2 of 616) normal cats.

Previous publications have documented the reliability of immunophenotyping cat lymphocytes using CD3 (T-lymphocytes)17), and paired box protein 5 (Pax5) (B-lymphocytes, not plasma cells)1). 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 29 cats.

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Materials and Methods

Archival samples of normal skin from 29 cats submitted to the Section of Anatomic Pathology for necropsy in 2006 were used in this study. The cats had no gross skin lesions or history of skin disease. All 29 cats were domestic shorthairs, but specific age and gender were not recorded. Specimens were collected from the dorsolateral thorax just after euthanasia using a 6-mm biopsy punch and were then formalin-fixed and paraffin-embedded. The skin over the dorsolateral thorax was used because (1) it is commonly biopsied and (2) it was used in previous studies in dogs and alpacas. Serial sections (4 μm thick) from each block were stained with antibodies against CD3 and Pax5.

Two of the authors (DCF and JPK) independently examined the specimens (1 section per cat). Each section was examined at 40×. The superficial dermis was defined as the area superficial to the sebaceous glands. The deep dermis was defined as the region deep to the sebaceous glands. Within each section, the authors evaluated microscopic fields in the superficial and deep dermis at each end of the section and in the middle. Hence, 6 fields were examined in each section. The presence of CD3+ and Pax5+ lymphocytes was recorded.

Immunohistochemistry for CD3 and Pax5 was performed as previously described in similar studies. Briefly, sections were mounted (ProbeOn Microscope Slides, Fisher Scientific Company, Bridgewater, New Jersey, USA) and paraffin was removed. Tissue sections were steamed in a citrate buffer (0.01 mol/L pH 9.0) for 5 minutes at medium power (300 W). Rabbit antihuman polyclonal CD3 antibody (Dako North America, Carpenteria, California, USA) was used in incubation at 1:100 dilution of 500 μg/mL stock solution. A standardized streptavidin-biotin immunoperoxidase technique with Nova Red chromagen was used to stain sections incubated with a prediluted, ready-to-use form of Pax5 antibody (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Positive and negative tissue controls were feline lymph node and feline brain, respectively. In addition, tissue sections from a cat with plasma cell pododermatitis were stained in identical fashion.

Results

Two CD3+ lymphocytes were found in one field in the superficial dermis in 1 of 29 cats (Fig. 1). No Pax5+ lymphocytes were seen in either the superficial or deep dermis of any specimen evaluated by the authors. The agreement between the two microscopic evaluations was 100%. Positive and negative controls (Figs. 2 and 3) reacted appropriately. The plasma cells in plasma cell pododermatitis were Pax5−.

Discussion

The importance and presence of resident lymphocyte populations have been evaluated within the dermis and epidermis of humans, mice, cattle, sheep, alpacas, horses, and dogs. The epidermis and adnexal epithelia of normal cats has been shown to contain no lymphocytes positive for CD3 (expressed on T cells) or BLA36 (expressed on B cells). To the authors’ knowledge, this is the first study to exclusively evaluate normal feline dermis for the presence of CD3 and Pax5 positive lymphocytes. In our study, there was 1 of 29 cats in which two CD3+ lymphocytes were found in one 40× microscopic field in the superficial dermis. These findings are consistent with two small studies evaluating the skin of allergic cats. In these studies, the authors found that in the normal (control) cat groups there were 0 of 10 (CD4 and CD8 immunophenotyping) and 2 of 6 (CD3 immunophenotyping) individuals with T-lymphocytes. The CD3+ lymphocytes reported in the study by Taglinger et al. were not quantitated. Hence, we cannot numerically compare their results with ours. Immunohistochemical markers unique to B-lymphocytes were not used in previous studies of dermal lymphocytes in normal cat skin. In our study, B-lymphocytes (Pax5+) were not detected in any specimen, which is consistent with findings in humans and dogs. In conclusion, resident T-lymphocytes apparently occur in very small numbers in normal feline skin. In our study, the maximum number of CD3+ lymphocytes was 2 in a 6-mm skin-biopsy specimen. Hence, larger numbers should be considered abnormal. It appears that B-lymphocytes are not present in the dermis of normal cats and could be considered abnormal if seen.
Fig. 1. Normal feline skin. Two CD3+ lymphocytes (arrows) are present in the superficial dermis (diaminobenzidine; 60×).

Fig. 3. Normal feline lymph node. Pax5+ lymphocytes are predominantly present within germinal centers (red arrow) (diaminobenzidine; 20×).

Fig. 2. Normal feline lymph node. CD3+ lymphocytes (black arrow) are predominantly present outside germinal centers (red arrow) (diaminobenzidine; 20×).

References


