American Journal of Pathology, Vol. 144, No. 2, February 1994

Animal Model

Canine Cutaneous Epitheliotropic Lymphoma (Mycosis Fungoides) Is a Proliferative Disorder of $CD8^+$ T Cells

Peter F. Moore,* Thierry Olivry,† and Diane Naydan†

From the Department of Pathology and Veterinary Medical Teaching Hospital,† School of Veterinary Medicine, University of California, Davis, California*

Canine epitheliotropic lymphoma (mycosis fungoides [MF]) is a spontaneous neoplasm of skin and mucous membranes that occurs in old dogs (mean age 11 years) and has no breed predilection. The lesions evolve from a patch-plaque stage with prominent epitheliotropism into a tumor stage in which distant metastasis is observed. Unlike human MF, epitheliotropism of the lymphoid infiltrate is still prominent in tumor stage lesions. Tropism of the lymphoid infiltrate for adnexal structures, especially hair follicles and apocrine sweat glands, was marked in all clinical stages of canine MF. Twenty-three cases of MF were subjected to extensive immunophenotypic analysis in which reagents specific for canine leukocyte antigens and fresh frozen tissue sections of the canine lesions were used. Canine MF proved to be a T cell lymphoma in which the epitheliotropic lymphocytes consistently expressed CD3 (22 cases) and CD8 (19 cases); $CD3^+CD4^-CD8^-$ lymphocytes predominated in the remaining 4 cases. In this regard, canine MF clearly differed from human MF in which a CD4 immunophenotype predominates in the T cell infiltrate. Lack of expression of CD45RA by epitheliotropic T cells and intense expression of a β1 integrin (VLA-4-like) suggested that T cells in canine MF belonged to the memory subpopulation, as has been suggested for T cells in human MF. Pan-T cell antigen loss or discordant expression also proved useful as phenotypic indicators of neoplasia in canine MF. Loss of CD5 was observed in epitheliotropic T cells in 63% of cases. Discordance of neoplastic T cell Thy-1 expression was frequently observed between epithelial and dermal or submucosal compartments. We conclude that canine MF still represents a useful spontaneous animal disease model of human cutaneous T cell lymphoma, despite the immunophenotypic differences, which may reflect operational differences between human and canine skin-associated lymphoid tissue. (Am J Pathol 1994, 144:421–429)

Cutaneous T cell lymphomas (CTCLs) are a heterogeneous group of disorders usually of peripheral T lymphocytes. Mycosis fungoides (MF) and the related Sézary syndrome account for most cases of CTCL and are distinguished from other peripheral T cell lymphomas that affect skin by virtue of the prominent epidermotropism of the T cell infiltrates in MF.[1–4] Other rare forms of epidermotropic T cell lymphoma also exist. Pagetoid reticulosis or Woringer-Kolopp disease is a form of cutaneous lymphoma in which the lymphoid infiltrate is almost entirely confined to the epidermis.[5,6] Recently, it was suggested that some cases of pagetoid reticulosis represent lymphomas of γδ T cells, and more than 50% of cases of pagetoid reticulosis have either a $CD4^-CD8^-$ or $CD8^+$ phenotype.[6,7] Classical MF, by contrast, represents a tumor of αβ T cells that express CD4 in approximately 90% of cases;

Supported in part by the Laboratory for Companion Animal Health, School of Veterinary Medicine.

Accepted for publication October 5, 1993.

Address reprint requests to Dr. Peter F. Moore, Department of Pathology, School of Veterinary Medicine, University of California, Davis, CA 95616.

malignant T cell expression of CD8 occurs only in occasional cases of MF.[8–10]

Epidermotropic cutaneous lymphoma has been recognized in dogs for at least two decades, and diseases similar to MF, Sézary syndrome, and pagetoid reticulosis have been described.[11–20] Lesions closely resemble the equivalent human diseases except for the marked tropism of the lymphoid infiltrate for the hair follicles and apocrine sweat glands in canine epidermotropic lymphoma. This tropism is maintained even in tumor stage lesions. It is generally accepted that the cytological features of canine MF are more variable than human MF, and many more cases of canine MF present with histiocytic cytomorphology than is typical of human MF.[13,17] The clinical disease course of canine MF is approximately 1 to 4 years from initial diagnosis. Progression from erythematous patches and plaques to nodules and tumors, which are initially localized to the skin and mucous membranes but eventually metastasize to lymph nodes and beyond, is observed in canine MF.

Classification of canine MF as a T cell disorder has been largely by inference. Despite recent reports that document aspects of the immunophenotypic characteristics of canine MF,[18,19] detailed immunophenotypic studies have not been published. This is due primarily to the lack of well characterized immunological markers of T cell differentiation, and also the need to have unfixed, snap-frozen tissue for meaningful evaluation. We have recently produced a number of monoclonal antibodies (MAbs) specific for antigens expressed by canine T cells and other leukocytes.[21–23] The development of these reagents was driven by the need to accurately phenotype lymphoid and histiocytic disorders of dogs, particularly those associated with skin and mucous membranes. In this report, we present a phenotypic analysis of 23 cases of canine MF from which fresh tissue was harvested by surgical biopsy. We show that canine MF is a T cell lymphoma that is similar in many respects to human MF; however, the T cells in most cases of canine MF express CD8 rather than CD4.

Materials and Methods

Canine Patients

Fresh surgical biopsy specimens were obtained from 23 canine patients. Eighteen patients were referred to the Veterinary Medical Teaching Hospital of the University of California at Davis for evaluation and treatment of their lesions. Fresh surgical specimens were also received from 5 additional canine patients of several California private veterinary hospitals and from the University of Tennessee, School of Veterinary Medicine, Knoxville, TN. All specimens were obtained with the informed consent of the pet owners. Dogs had not received chemotherapy for their lesions before initial surgical biopsy.

Tissue Handling and Immunohistochemistry

The tissues were collected over an 8-year period from 1985 to 1993, and snap-frozen tissue specimens were maintained in a frozen tumor bank at −80 C. Tissues were fixed in 10% neutral buffered formalin or snap-frozen in dichlorodifluoromethane (Freon 22) or isopentane, which were cooled to their freezing point in liquid nitrogen. Formalin-fixed tissue was embedded in paraffin and 6-μm sections were stained with hematoxylin and eosin. Frozen sections were stained by a streptavidin-horseradish peroxidase method according to manufacturer's instructions (Zymed, South San Francisco, CA) and previously described methods.[21–23] MAb specific for canine leukocyte antigens were applied to sections as diluted tissue culture supernatants based on previous titration on frozen sections of normal canine spleen, tonsil, skin, or tongue. Negative controls consisted of substitution of specific MAb with isotype-matched nonspecific MAb (MOPC-21 IgG1, Sigma Chemical Co., St. Louis, MO) or omission of primary antibody. MAbs were also applied to frozen sections of normal spleen to ensure that specific and characteristic staining of leukocyte populations was achieved in each staining run. It should be noted that the epitopes recognized by most of the MAbs were destroyed by formalin fixation and paraffin embedding. Hence, it was imperative to use only snap-frozen tissues to develop a detailed immunophenotype of the epitheliotropic lymphocytes in canine MF.

Antibodies

MAb specific for canine leukocyte antigens were developed in the author's laboratory (PFM). They included MAb specific for CD1a (CA9.AG5, IgG1), CD1c (CA13.9H11, IgG1), CD4 (CA13.1E4, IgG1), CD8 (CA9.JD3, IgG2a), CD11a (CA11.4D3, IgG1), CD11c (CA11.6A1, IgG1), CD18 (CA1.4E9, IgG1), CD21 (CA2.1D6, IgG1), CD45RA (CA4.1D3, IgG1),

CD45 (CA12.10C12, IgG1), very late activation antigen-4 (VLA-4) or CD49d (CA4.5B3, IgG1), Thy-1 (CA1.4G8), and major histocompatibility complex class II (CA2.1C12, IgG1). These antibodies have been extensively characterized by immunohistology, multiparameter flow cytometry, antigen immunoprecipitation, and in some instances by functional studies.[21–23] A rat anti-canine CD5 MAb (YKIX.322, IgG2a) was characterized and generously provided by Steve Cobbold (Cambridge University, Cambridge, UK). Canine CD3ε was detected by a polyclonal rabbit antiserum (Dako, Carpinteria, CA), which was specific for a 13-mer peptide sequence from the cytoplasmic domain of human Cd3ε.[24] This sequence is highly conserved in several mammalian species including canine.[25] The antipeptide antiserum is a reliable marker of T cell differentiation in both frozen and paraffin sections of canine tissues (PFM, unpublished observations) and in the tissues of various mammalian species.[26]

Results

Clinical Summary

MF occurred in old dogs whose mean age was 11 years (range 6 to 14 years). Thirteen cases occurred in females and 10 cases occurred in males. A breed predisposition was not apparent, because 19 breeds were represented in the 23 cases. The lesions were present on average for 7 months before diagnosis, and the total course of the disease varied from 3 months to more than 48 months. The lesions were characterized by one or more of the following: erythema, scaling, pruritus, depigmentation, alopecia, plaque formation, ulceration and crusting, and nodule or mass formation. Lesions occurred throughout the skin and also had a marked tendency to involve either mucocutaneous junctions (lips, eyelids, anorectal junction, or nasal planum) or oral cavity (gingiva, palate, or tongue) in 17 cases (Table 1).

Table 1. *Selected Clinical and Pathological Features in Canine MF*

Case No.	Breed	Age (year)	Sex	Lesion Topography	Stage	Cytology	Tropism
1	Lhaso apso	12	M	Skin, tongue, gingiva, nasal philtrum, blood (Sézary syndrome)	PP	hyperchr	epi/ad
2	Chesapeake bay retriever	10	M	Skin (pagetoid features)	PP	hyperchr	EPI/AD
3	Shetland sheep dog	14	F	Lips	PP/TS	hyperchr/ histiocytic	EPI/AD
4	Springer spaniel	12	F	Skin	PP/TS	hyperchr/ histiocytic	epi
5	Beagle	12	F	Skin, lips, gingiva, hard palate	PP/TS	hyperchr/ histiocytic	EPI/AD
6	Poodle mix	10	F	Skin, lips	TS	histiocytic	EPI/AD
7	Golden retriever	10	M	Skin, diaphragm (metastasis)	PP/TS	hyperchr	EPI/AD
8	Labrador retriever	6	F	Skin, lips, nasal philtrum (pagetoid features)	PP	hyperchr	EPI/AD
9	Dalmation mix	12	F	Skin, buccal mucosa, submandibular LN	PP/TS	hyperchr/ histiocytic	EPI
10	Labrador retriever	6	F	Skin, tongue, gingiva, lips (pagetoid features)	PP	hyperchr	EPI/AD
11	Cockapoo	14	M	Buccal mucosa, lips	TS	histiocytic	epi
12	Briard	10	M	Lip, submandibular LN	TS	histiocytic	epi/ad
13	German shepherd mix	9	F	Skin	TS	histiocytic	AD
14	Spaniel	13	M	Skin, lips, buccal mucosa, submandibular LN, lung, kidney	TS	histiocytic	EPI/AD
15	Australian shepherd	12	F	Nasal skin, eyelids (pagetoid features)	PP	hyperchr	EPI/AD
16	Cocker spaniel	10	F	Lips (pagetoid features)	PP	hyperchr	EPI/AD
17	Shih tzu	12	F	Skin, lips, submandibular LN	TS	histiocytic	epi/AD
18	Boxer	13	M	Buccal mucosa, lips, eyelids, submandibular LN	TS	histiocytic	epi
19	Labrador retriever	11	F	Skin	PP	hyerchr	EPI/AD
20	West highland white terrier	11	M	Nasal philtrum	TS	histiocytic	epi/ad
21	German shepherd	11	F	Lower lip margins	PP	hyperchr	EPI/AD
22	Golden retriever	12	M	Skin	TS	histiocytic	epi/AD
23	Retriever cross	13	M	Anorectal junction, skin	TS	histiocytic	EPI/AD

Stage: PP, patch-plaque stage; TS, tumor stage; LN, lymph node. Cytology: hyperchr, small to medium sized lymphocytes with hyperchromatic, convoluted nuclei; histiocytic, large lymphocytes with vesicular nuclei and a histiocytic appearance. Tropism: EPI, marked tropism for epidermis; epi, some tropism; AD, marked tropism for adnexae (hair follicles, adnexal glands); ad, some tropism.

Morphological Features of Canine MF

The lymphoid infiltrate had tropism for the epidermis or oral mucosa in 22 of 23 cases (Table 1). Epitheliotropic lymphocytes were either diffusely distributed within the epithelium (Figures 1 and 2) or formed discrete focal aggregates (Pautrier's microaggregates) in some instances (Figure 3). In 5 cases, the lymphoid infiltrate was almost entirely confined to the epidermis (Figure 2), save for a few superficial dermal reactive cells of varied lineage (lymphocytes, plasma cells, histiocytes, and occasional granulocytes). These lesions resembled those observed in disseminated pagetoid reticulosis in humans. The dermal lymphoid infiltrate in the remaining 18 cases occupied the papillary dermis and often obscured the dermal-epidermal junction (Figures 1 and 2). If the dermal infiltrate did not extend deeper, the lesions were classified as plaque stage.[2,4] Patch stage lesions had scant superficial dermal infiltrates and usually occurred simultaneously with plaque lesions in multiple biopsy specimens from the same patient. In 15 cases the lymphoid infiltrate extended to the reticular dermis and subcutis. These lesions were classified as tumor stage lesions,[2] and lymph node metastases were observed in 3 of these cases at the time of presentation. Lymph node metastases developed with disease progression in 2 additional cases. Tumor stage lesions co-existed with plaque lesions in some patients (Table 1). Lymphoid infiltration of adnexal structures that included hair follicles, apocrine sweat glands, and sebaceous glands was observed in virtually all cutaneous lesions. Adnexal infiltration was usually of considerable extent even if epidermotropism was relatively inconspicuous.

The lymphoid infiltrates in canine MF were cytologically more diverse than those described for human MF. However, if the cytological features of the

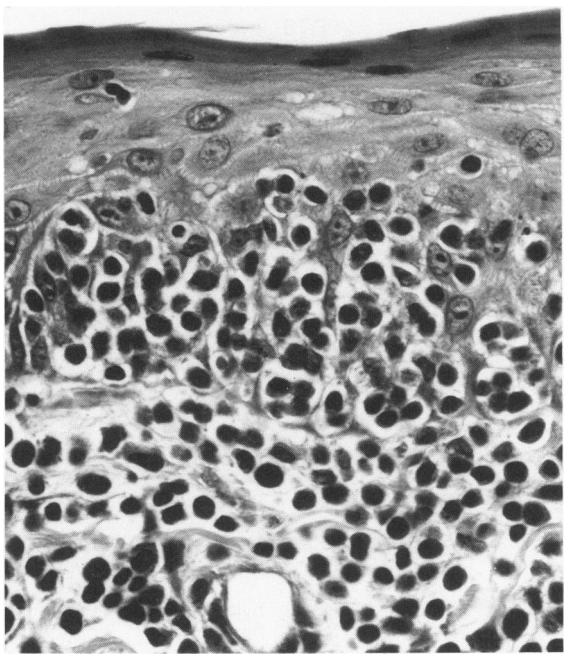

Figure 1. *Case 7: canine MF plaque stage lesion. Hyperchromatic pleomorphic small to medium-sized lymphocytes have infiltrated the lower epidermis and partially effaced the dermoepidermal junction (H&E, ×490).*

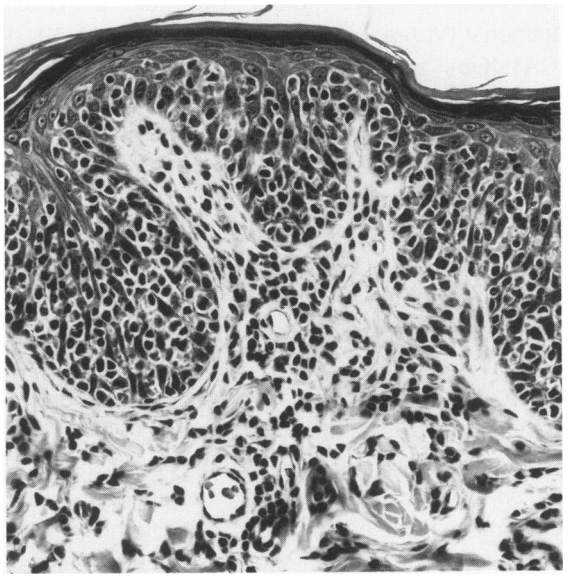

Figure 2. *Case 8: canine MF with pagetoid features. Hyperchromatic lymphoid infiltrate is almost entirely confined to the epidermis (H&E, ×240).*

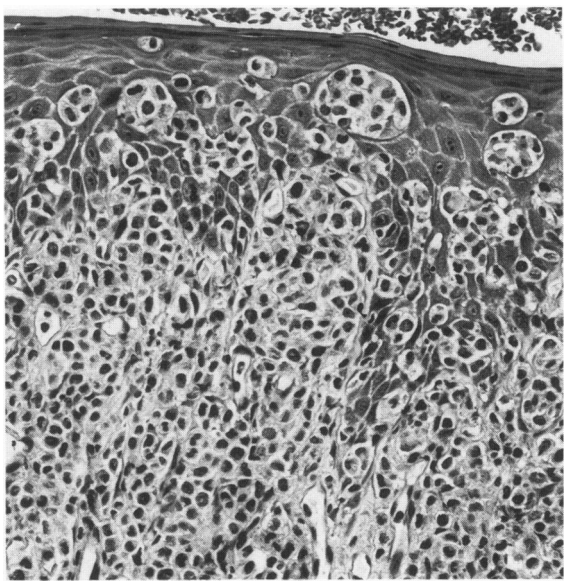

Figure 3. *Case 9: canine MF tumor stage lesion. Lymphoid infiltrate with histiocytic cytomorphology has partially effaced the dermoepidermal junction and has formed Pautrier's microaggregates within the epidermis (H&E, ×240).*

lymphoid infiltrates were correlated with the stage of the disease, then the lymphoid infiltrates of canine MF more closely matched those described for human MF, particularly in the patch-plaque stage. For instance, patch-plaque lesions in 13 cases were infiltrated by small to medium-sized hyperchromatic lymphocytes that had scant cytoplasm and marked nuclear contour irregularity (Figure 1). These lymphocytes closely resembled those described in human patch-plaque stage MF. However, tumor stage lesions in 14 of 15 cases were infiltrated by larger lymphoid cells with a histiocytic appearance. These cells possessed moderately abundant, eosinophilic to amphophilic cytoplasm and large oval to folded nuclei (Figure 3).

Immunophenotypic Features of Canine MF

The phenotypic characteristics of canine MF were strikingly different from those of human MF (Figure 4). In 19 of 23 cases, both the epitheliotropic lymphoid infiltrates and the dermal or submucosal lymphoid infiltrates in the lesions were predominantly $CD8^+$ (Figures 5 and 6). CD4 expression in the lesions was confined to dermal or submucosal reactive cells, which most often were macrophages and dermal dendritic cells. Epitheliotropic and dermal or submucosal $CD4^+$ lymphocytes were scarce in most lesions from these cases (Figure 7). In the remaining 4 cases, the epitheliotropic lymphocytes expressed neither CD4 nor CD8. Scattered lymphocytes that expressed either CD4 or CD8 occurred usually at low frequency among the dermal lymphoid infiltrates in these 4 cases, and probably represented reactive lymphocytes.

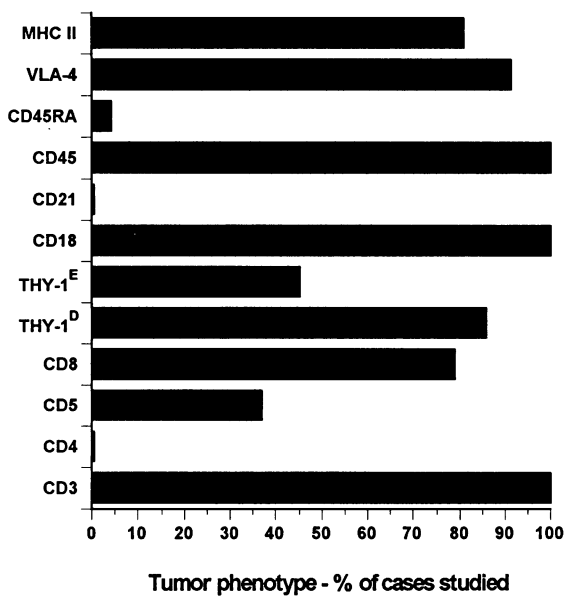

Figure 4. *Leukocyte antigen expression by the lymphoid infiltrate in canine MF. Thy-1^D and Thy-1^E refer to Thy-1 expression in the dermis and epidermis, respectively.*

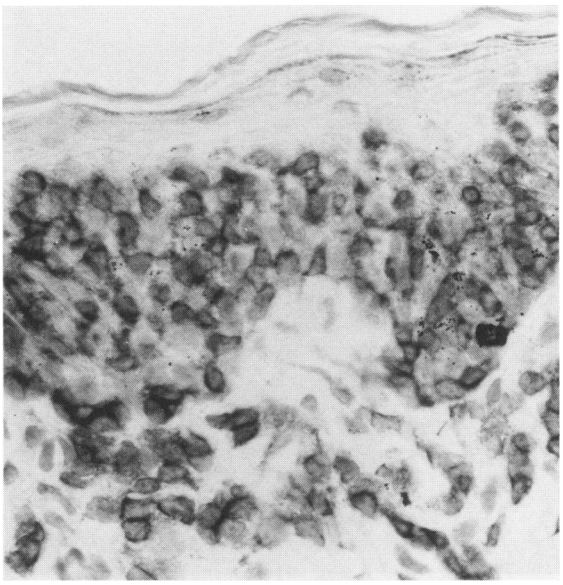

Figure 5. *Case 8: CD8 expression by epidermotropic T cells in canine MF with pagetoid features (immunoperoxidase, hematoxylin counterstain, ×490).*

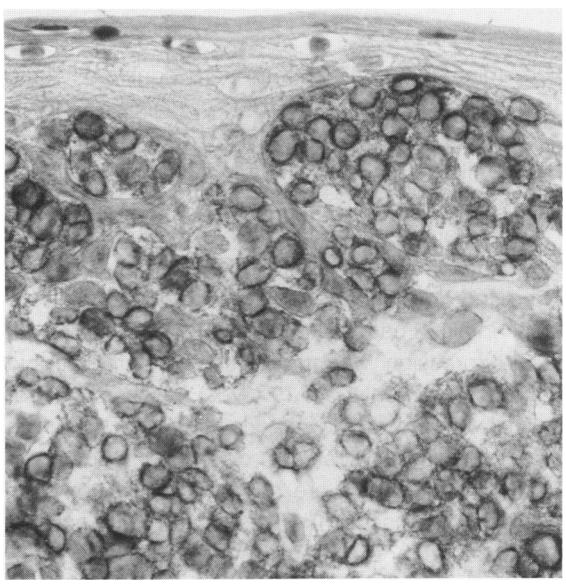

Figure 6. *Case 9: CD8 expression by epidermotropic and dermal T cells in tumor stage MF (immunoperoxidase, hematoxylin counterstain, ×490).*

The lymphoid infiltrates in virtually all cases intensely expressed CD45, β1 integrin (VLA-4-like), and β2 integrins (detected by anti-CD18) (Figure 4). Lymphocyte expression of CD45RA was observed in seven cases. In six cases $CD45RA^+$ lymphocytes

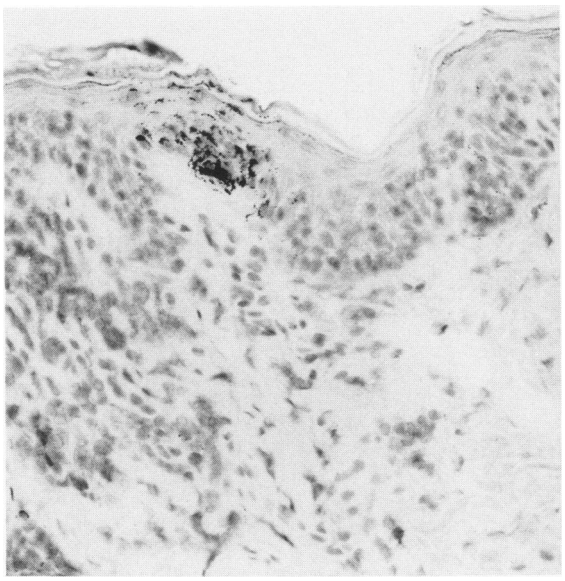

Figure 7. *Case 8: Paucity of CD4 expression by epidermotropic T cells in canine MF with pagetoid features. An aggregate of normal, pigmented melanocytes in the epidermis should not be confused with positive staining (immunoperoxidase, hematoxylin counterstain, ×240).*

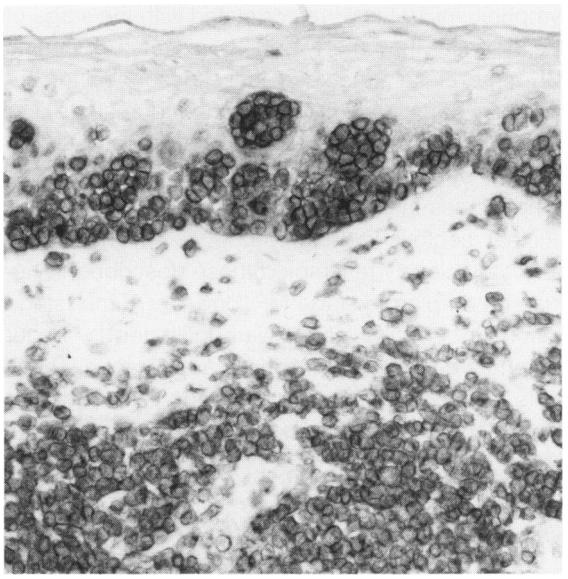

Figure 8. *Case 23: Tumor stage MF stained for CD3. Note the prominent epidermotropism and the Grenz zone between the dermal infiltrate and the epidermis (immunoperoxidase, hematoxylin counterstain, ×240).*

were minor populations that were confined to the dermis. Epitheliotropic, $CD45RA^+CD8^+$ lymphocytes were observed only in case 1; the dermal lymphoid infiltrate was also diffusely $CD45RA^+CD8^+$ in this instance.

Discordant pan-T cell antigen expression was observed in canine MF. Both epitheliotropic lymphocytes and dermal or submucosal lymphocytes were $CD3^+$ in all 22 cases examined (Figure 8). However, CD5 expression was observed in epitheliotropic lymphocytes in only 7 of 19 (37%) cases examined. Dermal lymphocytes were also diffusely $CD5^+$ in these cases. Only scattered dermal or submucosal $CD5^+$ lymphocytes were observed in lesions from the remaining cases. The absence of CD5 expression was seen at a similar frequency in patch-plaque and tumor stage lesions. The pattern of Thy-1 expression was even more curious. Thy-1 is expressed by all peripheral T cells in the dog.[21,23] Dermal or submucosal lymphocytes expressed Thy-1 in 19 of 22 cases examined. However, epitheliotropic lymphocytes expressed Thy-1 in only 10 of these cases.

Accessory cell populations (macrophages and dendritic cells) were abundant in 14 of 23 cases. These cells were recognized by their abundant cytoplasm or dendritic processes in sections stained with MAb specific for one or more of the following antigens: major histocompatibility complex class II, CD1a, CD1c, CD4 (weak), CD18, and Thy-1.

Discussion

In the past, there has been interest in documenting the comparative morphology of canine non-Hodgkin's lymphomas to explore the potential of utilization of spontaneous lymphomas in dogs as a preclinical model system for the evaluation of various therapeutic modalities in human lymphomas.[27–29] Also, numerous sporadic reports have documented the existence of canine epitheliotropic lymphomas that appeared to cover the spectrum presented by MF and pagetoid reticulosis in humans.[11–18] More detailed analysis of these canine lymphomas has previously been thwarted by the unavailability of MAbs specific for canine T cell antigens and the lack of molecular genetic probes specific for canine T cell receptor and immunoglobulin gene segments. In this study, we have unequivocally established that canine MF is a T cell lymphoma in which the epitheliotropic lymphocytes consistently express CD3 and CD8. In this regard, canine MF clearly differs from human MF in which a CD4 immunophenotype predominates in the T cell infiltrate.[2,8–10]

The clinical and pathological features of canine MF largely resemble the human disease.[2,4,5] The major differences are that epitheliotropism of the lymphoid infiltrate is prominent in canine tumor stage lesions, and tropism of the lymphoid infiltrate for adnexal structures, in particular, is marked in all clinical stages of canine MF. Also, there is marked variability in the cytomorphology of the lymphoid infiltrate in canine MF. Typical hyperchromatic, convo-

luted nuclei were only seen in patch-plaque lesions, whereas tumor stage lesions almost invariably exhibited a histiocytic appearance. The high frequency of cases (65%) that presented with tumor stage lesions probably reflects the tendency of canine MF to progress more rapidly from patch-plaque stage to tumor stage than is typical of human MF.

The immunophenotype of the epitheliotropic T cells in canine MF in our cases was strikingly different from that described for human MF. Of the 23 cases in our series, 19 cases were $CD8^+$ and 4 cases were $CD4^-CD8^-$. We have not encountered a case in which epitheliotropic lymphocytes expressed CD4. This situation contrasts markedly with human MF where less than 10% of cases express a $CD8^+$ immunophenotype and approximately 90% of cases express a $CD4^+CD45RO^+CD45RA^-$ phenotype.[9,10] Canine MF cases, in which the T cells expressed CD4, have been described previously.[19] However, in this study, much of the case material (10 cases) was formalin fixed and paraffin embedded. Fresh frozen material was available for only 5 cases; the T cell infiltrate was $CD4^+$ in 3 cases and $CD8^+$ in 2 cases. Antigen retrieval of surface antigens, such as leukocyte differentiation antigens, in formalin-fixed paraffin-embedded sections has proven to be difficult; even in successful instances it is often incomplete. For this reason we confined this study to sections of snap-frozen tissues from a large number of cases to totally remove the potentially confounding variables of type of fixative, time of fixation, and antigen retrieval conditions. Hence, we believe our canine MF phenotypic data is more likely to be truly representative.

Human MF is a disease of CD4 memory cells that express CD45RO and lack CD45RA.[10] CD45RA expression by epitheliotropic T cells was seen in only one case of canine MF, which implies that the remaining cases, which were $CD45RA^-$, expressed a memory cell phenotype. This impression was strengthened by the strong expression of a canine $\beta 1$ integrin (VLA-4-like) by T cells in most cases of canine MF. Memory T cells have been reported to express high levels of adhesion molecules, which include $\beta 1$ integrins.[30] Canine $CD8^+$ T cells are divisible into VLA-4^{low} and VLA-4^{high} populations by multiparameter flow cytometry,[23] and $CD8^+$VLA-4^{high} T cells predominate in peripheral blood of dogs in advanced age (PFM, unpublished observations). Expression of CD45RO could not be evaluated in canine MF due to the lack of reagents specific for canine CD45RO.

Even though $CD4^+$ CTCL predominate in humans, other phenotypic patterns have been observed in CTCL.[6,31–34] A review of these reports suggests that the lymphoid infiltrates in pagetoid reticulosis (both Woringer-Kolopp and Ketron-Goodman types), and in forms of MF in which marked epidermotropism was observed, more frequently expressed alternative phenotypes than would be expected in typical MF cases. In particular, an increased incidence of $CD8^+$ and $CD4^-CD8^-$ phenotypes was encountered. Our canine patient group, in which the lesions had pagetoid features, may be similar to these human patient groups. However, our remaining canine patients with more classical MF lesions and a $CD8^+$ T cell phenotype contrast markedly with the vast majority of human MF patients who have a $CD4^+$ T cell phenotype.

A hierarchy of pan-T cell antigen loss has been observed in epitheliotropic T cells in human MF and has been used as a phenotypic indicator of T cell neoplasia.[8] In our canine MF cases, T cells expressed CD3 in all cases tested and only expressed CD5 in 37% of the cases tested. Lack of CD5 expression was almost equally distributed between patch-plaque lesions and tumor stage lesions. Thy-1, which is expressed by all peripheral T cells in the dog,[21,23] was more often lost from epidermotropic T cells (55% of cases) than from dermal T cells (14% of cases). This phenomenon has been termed antigen discordance and has been previously observed in human MF with respect to expression of CD5, CD7, and T cell receptor-Cβ, although this occurred in only 9% of MF patients.[3,5] Expression of CD2, CD7, and T cell receptor-Cβ could not be assessed in canine lesions due to the lack of specific reagents. Our results suggest that pan-T cell antigen loss and discordant antigen expression is more frequently seen in canine MF than in human MF regardless of disease stage.

The existence of a canine CTCL that is similar clinically and pathologically to human MF but differs inversely with respect to the T cell subset involved implies that the skin-associated lymphoid tissue (SALT) in dogs and humans are likely to have operational differences. Although the normal functions of canine SALT have not been extensively documented, cutaneous antigen-driven responses appear to differ between dogs and humans, particularly with respect to the ease of induction of contact hypersensitivity. Experimental induction of contact hypersensitivity in dogs with potent chemical inducers is difficult to achieve without resorting to extreme measures such as use of the maximization

technique.[36,37] Also, clinical recognition of contact hypersensitivity is uncommon in dogs.[37] The cellular basis of this response difference is largely unknown, although dogs possess appropriate accessory cell populations, such as epidermal Langerhans cells[38] and Thy-1$^+$ dermal dendrocytes,[19,39] which are thought to mediate antigen presentation to skin-seeking lymphocytes.

The balance between antigen presentation by Langerhans cells and dermal dendrocytes may favor preferential expansion of CD8 T cells over CD4 T cells in canine cutaneous immune responses; this would not promote development of contact hypersensitivity. A model based on this premise would predict that malignant transformation of T cells after chronic environmental antigen exposure in aged dogs would probably involve CD8$^+$ T cells in preference to CD4$^+$ T cells. Furthermore, the CD8$^+$ memory T cell subset should be recruited in canine CTCL, which appears to be the case. Studies that demonstrate a role for dermal dendrocytes in antigen presentation and delineate T cell subpopulation involvement have not been conducted in any species to our knowledge. These would be necessary to validate our proposed model.

Despite the immunophenotypic differences, canine MF in other respects may be a useful spontaneous disease model for preclinical testing of novel therapeutic strategies, and for studies concerning epidemiology of MF, because dogs, to an extent, share the environment of humans. Also, canine MF may be of value in the elucidation of the molecular mechanisms associated with T cell homing and tethering in epidermal and adnexal epithelial compartments and the abrogation of this behavior that accompanies tumor stage lesions. The rapid clinical progression of canine MF from patch-plaque to tumor stage would facilitate these investigations. We recently initiated these studies and have examined β_2-integrin and intercellular adhesion molecule-1 expression in our canine MF cases (TO et al, manuscript submitted). However, further realization of these goals awaits development of additional markers of specific canine lymphoid antigens such as T cell receptor-$\alpha\beta$, T cell receptor-$\gamma\delta$, Ki-1 (CD30), cutaneous lymphocyte antigen, and others.

Acknowledgments

We thank Barbara Atlee, Doug DeBoer, Janine Fidel, Pat Gilbert, Thelma Gross, Susan Kraegel, Bruce Madewell, Carlos Rodriguez, Ilse Silva-Krott, Alain Théon, and Melinda Upton for vigilance in identifying and providing case material for this study.

References

1. Kerl H, Cerroni L, Burg G: The morphologic spectrum of T-cell lymphomas of the skin: a proposal for a new classification. Semin Diagn Pathol 1991, 8:55–61
2. Hoppe RT, Wood GS, Abel EA: Mycosis fungoides and the Sézary syndrome: pathology, staging and treatment. Current Problems in Cancer. Edited by CM Haskell. St. Louis, Mosby Year Book, 1990, pp 297–361
3. Kuzel TM, Roenigk HH, Jr, Rosen ST: Mycosis fungoides and the Sézary syndrome: a review of pathogenesis, diagnosis, and therapy. J Clin Oncol 1991, 9:1298–1313
4. Nickoloff BJ: Light-microscopic assessment of 100 patients with patch/plaque-stage mycosis fungoides. Am J Dermatopathol 1988, 10:469–477
5. LeBoit PE: Variants of mycosis fungoides and related T-cell lymphomas. Semin Diagn Pathol 1991, 8:73–81
6. Mielke V, Wolff H, Winzer M, Sterry W: Localized and disseminated Pagetoid reticulosis. Arch Dermatol 1989, 125:402–406
7. Berti E, Cerri A, Cavichini S, Delia D, Soligo D, Alessi E, Caputo R: Primary cutaneous γ/δ T-cell lymphoma presenting as disseminated Pagetoid reticulosis. J Invest Dermatol 1991, 96:718–723
8. Picker LJ, Weiss LM, Meideros LJ, Wood GS, Warnke RA: Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. Am J Pathol 1987, 128: 181–201
9. Knowles DM: Immunophenotypic and antigen receptor gene rearrangement analysis in T-cell neoplasia. Am J Pathol 1989, 134:761–785
10. Ralfkiaer E: Immunohistological markers for the diagnosis of cutaneous lymphomas. Semin Diagn Pathol 1991, 8:62–72
11. Kelly DF, Halliwell REW, Schwartzman RM: Generalized cutaneous eruption in a dog, with histological similarity to human mycosis fungoides. Br J Dermatol 1972, 86:164–171
12. Shadduck JA, Reddy L, Lawton G, Freeman R: A canine cutaneous lymphoproliferative disease resembling mycosis fungoides in man. Vet Pathol 1978, 15: 716–724
13. McKeever PJ, Grindem CB, Stevens JB, Osborne CA: Canine cutaneous lymphoma. J Am Vet Med Assoc 1980, 180:531–536
14. Johnson JA, Patterson JM: Canine epidermotropic lymphoproliferative disease resembling Pagetoid reticulosis in man. Vet Pathol 1981, 18:487–493
15. Thrall MA, Macy DW, Snyder SP, Hall RL: Cutaneous lymphosarcoma and leukemia in a dog resembling Sézary syndrome in man. Vet Pathol 1984, 21:182–186
16. Magnol JP, Carlotti D, Cingia A, Olivry T: T-lymphome cutané chez un chien évoquant le mycosis fongoïde de l'homme. Pract Med Chir Animal Comp 1985, 20: 135–141

17. Doe R, Zackheim HS, Hill JR: Canine epidermotropic cutaneous lymphoma. Am J Dermatopathol 1988, 10: 80–86
18. DeBoer DJ, Turrel JW, Moore PF: Mycosis fungoides in a dog - demonstration of T cell specificity and response to radiotherapy. J Am Animal Hosp Assoc 1990, 26:566–572
19. Fivenson DP, Beck ER, Dunstan RW, Nickoloff BJ, Moore PF: Dermal dendrocytes and T cells in canine mycosis fungoides: support for an animal model of human cutaneous T cell lymphoma. Cancer 1992, 70: 2091–2098
20. Walder EJ, Gross TL: Malignant lymphoma. Veterinary dermatology: a macroscopic and microscopic evaluation of canine and feline skin diseases. Edited by TL Gross, PJ Ihrke, EJ Walder. St. Louis, Mosby Year Book, 1992, pp 476–482
21. Moore PF, Rossitto PV, Danilenko DM: Canine leukocyte integrins: characterization of a CD18 homologue. Tissue Antigens 1990, 36:211–220
22. Danilenko DM, Moore PF, Rossitto PV: Canine leukocyte cell adhesion molecules (LeuCAMS): characterization of the CD11/CD18 family. Tissue Antigens 1992, 40:13–21
23. Moore PF, Rossitto PV, Danilenko DM, Wielenga JJ, Raff RF, Severns E: Monoclonal antibodies specific for canine CD4 and CD8 define functional T lymphocyte subsets and high density expression of CD4 by canine neutrophils. Tissue Antigens 1992, 40:75–85
24. Mason DY, Cordell J, Brown M, Palleson G, Ralfkiaer E, Rothbard J, Crumpton M, Gatter KC: Detection of T cells in paraffin embedded tissue using antibodies against a peptide sequence from the CD3 antigen. J Clin Pathol 1989, 42:1194–1200
25. Nash RA, Scherf U, Storb R: Molecular cloning of the CD3 epsilon subunit of the T-cell receptor/CD3 complex in the dog. Immunogenetics 1991, 33:396–398
26. Jones M, Cordell JL, Beyers AD, Tse AGD, Mason DY: Detection of T and B cells in many animal species using cross-reactive anti-peptide antibodies. J Immunol 1993, 150:5429–5435
27. Applebaum FR, Sale GE, Storb R, Charrier K, Deeg HJ, Graham T, Wulff JC: Phenotyping of canine lymphoma with monoclonal antibodies directed at cell surface antigens: classification, morphology, clinical presentation, and response to chemotherapy. Hematol Oncol 1984, 2:151–168
28. Carter RF, Valli VEO, Lumsden JH: The cytology, histology and prevalence of cell types in canine lymphoma classified according to the National Cancer Institute working formulation. Can J Vet Res 1986, 50: 154–164
29. Greenlee PG, Filippa DA, Quimby FW, Patnaik AK, Calvano SE, Matus RE, Kimmel M, Hurvitz AI, Lieberman PH: Lymphomas in dogs: a morphologic, immunologic, and clinical study. Cancer 1990, 66:480–490
30. Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, Shaw S: Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN-gamma production. J Immunol 1988, 140:1401–1407
31. Agnarsson BA, Vonderheid EC, Kadin ME: Cutaneous T cell lymphoma with suppressor/cytotoxic (CD8) phenotype: identification of rapidly progressive and chronic subtypes. J Am Acad Dermatol 1990, 22:569–577
32. Smoller BR, Stewart M, Warnke RA: A case of Woringer-Kolopp disease with Ki-1 (CD30)+ cytotoxic suppressor cells. Arch Dermatol 1992, 128:526–529
33. Heald P, Buckley P, Gilliam A, Perez M, Knobler R, Kacinski B, Edelson R: Correlations of unique clinical, immunotypic and histologic findings in cutaneous gamma/delta T-cell lymphoma. J Am Acad Dermatol 1992, 26:865–870
34. Sperling M, Kaudewitz P, Braun-Falco O, Stein H: Reactivity of T cells in mycosis fungoides exhibiting marked epidermotropism with the monoclonal antibody HML-1 that defines a membrane molecule on human mucosal lymphocytes. Am J Pathol 1989, 134:955–960
35. Michie SA, Abel EA, Hoppe RT, Warnke RA, Wood GS: Discordant expression of antigens between intraepidermal and intradermal T cells in mycosis fungoides. Am J Pathol 1990, 137:1447–1451
36. Nobreus N, Magnusson B, Leandoer L, Attstrom R: Induction of dinitrochlorobenzene contact sensitivity in dogs: transfer of sensitivity by thoracic duct lymphocytes and suppression of sensitivity by anti-thymocyte serum. Monogr Allergy 1974, 8:100–109
37. Conroy JD: Immune mediated diseases of skin and mucous membranes. Textbook of veterinary internal medicine: diseases of the dog and cat. Edited by SJ Ettinger. Philadelphia, W.B. Saunders, 1983, pp 2146–2147
38. Moore PF, Mariassy AT: Dendritic (Langerhans) cells in canine epidermis: ultrastructure and distribution. Anat Histol Embryol 1986, 15:178–179
39. Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ, Headington JT: Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. Br J Dermatol 1989, 121:421–431