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Canine Cutaneous Histiocytoma Is an Epidermotropic Langerhans Cell Histiocytosis That Expresses CD1 and Specific β_2-Integrin Molecules

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Canine cutaneous histiocytoma (CCH) is a common, benign neoplasm of the dog. Histiocytomas most commonly occur as solitary lesions that undergo spontaneous regression. The age-specific incidence rate for histiocytomas drops precipitously after 3 years, although histiocytomas occur in dogs of all ages. Langerhans cells (LCs) in humans and dogs express abundant major histocompatibility complex class II molecules and a variety of leukocyte antigens characteristic of dendritic cell differentiation including CD1a, CD1b, CD1c, and CD11c. The immunophenotype of CCH resembled that of cutaneous LCs by virtue of the expression of CD1 molecules (CD1a, -b, and -c), CD11c, and major histocompatibility complex class II. Furthermore, histiocytoma cells had a tropism for epidermis, which was also consistent with an epidermal LC lineage. The expression of adhesion molecules such as CD11b (variable), CD44, CD54 (ICAM-1), and CD49d (VLA-4) in CCH indicated that the infiltrating cells had some of the characteristics of activated LCs, as these molecules are not expressed by normal, resting canine epidermal LCs. CCH did not express Thy-1 or CD4. Thy-1 expression is a characteristic of human and canine dermal dendrocytes, which are perivascular dendritic antigen-presenting cells closely related to epidermal LCs. CD4 expression is prevalent in human LC histiocytosis, and in this respect CCH differed from human LC histiocytosis. Here we demonstrate that CCH is a localized form of self-limiting LC histiocytosis, which predominantly expresses an epidermal LC phenotype. CCH occurs as solitary or, less commonly, as multiple cutaneous nodules or plaques, which rarely may extend beyond the skin to local lymph nodes. Regression of CCH occurs spontaneously in the vast majority of cases in primary and secondary sites, and is mediated by CD8$^+$ αβ T cells. The high frequency of CCH within the general canine population offers the potential that the dog may provide an interesting model system to further the understanding of LC proliferative disorders, particularly the self-limiting, cutaneous form of human LC histiocytosis. (Am J Pathol 1996, 148:1699–1708)

Canine cutaneous histiocytoma (CCH) is a common, benign, cutaneous neoplasm of the dog.[1–7] Histiocytomas usually occur as solitary lesions that undergo spontaneous regression. The age-specific incidence rate for histiocytomas drops precipitously after 3 years, although histiocytomas do occur in dogs of all ages.[2] Reports of recurrence of histiocytomas at the same or other sites are rare, and the occurrence of multiple tumors is considered unusual. Epidermal invasion by cells of histiocytoma frequently occurs, and intra-epidermal nests of histiocytes resemble Pautrier's aggregates, characteristically found in epidermotropic lymphoma (mycosis

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Table 1. *Clinical Summary of Cutaneous Histiocytoma Cases*

Presentation	Number of cases	Age range (years; mean ± SD)	Epitheliotropism (n cases)	Lesion recurrence (n cases)	Lymphadenopathy (n cases)
Solitary lesions	16	0.3–12.0 (4.3 ± 3.5)	10/13	1	1
Multiple lesions	7	0.2–9.0 (2.5 ± 3.0)	6/7	1	3

fungoides). Epidermal invasion in histiocytoma or the presence of simultaneous multiple histiocytomas especially in aged dogs can present a diagnostic dilemma, and distinction from mycosis fungoides and non-epitheliotropic cutaneous lymphoma is difficult on purely morphological grounds.

Immunophenotypic analysis of histiocytic populations in humans has enabled differentiation of a variety of macrophages and dendritic/antigen-presenting cells (APCs).[8] The dendritic cells of human skin include epidermal Langerhans cells (LCs) and dermal dendrocytes, which are distinguished by their respective locations and antigen expression profiles.[8–12] LCs in humans and dogs express abundant major histocompatibility complex (MHC) class II molecules and a variety of leukocyte antigens characteristic of dendritic cell differentiation including CD1a, CD1c, and CD11c.[11,13–18] Dermal dendrocytes in humans and canines are perivascular dendritic cells located most prominently in the superficial dermis. They are identified by their expression of MHC class II, Thy-1, and factor XIIIa, which is an enzyme of the coagulation cascade; initial reports suggest that they did not express CD1a.[9,10] Subsequently, a detailed analysis of MHC class II^+ dermal cell suspensions revealed heterogeneous populations of cells of both the monocyte/macrophage lineage and dendritic/APC lineage. The dendritic/APCs expressed the phenotype $CD1a^{lo}$,CD1b,CD1c, CD11b,$CD11c^{hi}$; these cells also expressed intracytoplasmic factor XIIIa and were potent in alloantigen presentation to T cells.[12] This has recently been confirmed independently.[19] Hence, dermal dendrocytes are more closely related phenotypically to LCs than was originally thought, and expression of CD1 molecules by skin histiocytes is a marker of dendritic/APC lineage and serves to differentiate these cells from macrophages. Within the epidermis, CD1a expression is the most reliable marker of LC differentiation. LCs can up-regulate the expression of additional molecules, which are not evident or minimally expressed in resident epidermal LCs in unstimulated skin; this is indicative of activation or further differentiation. These molecules include CD4, very late activation antigen-4 (VLA-4 or CD49d), CD44, and intercellular adhesion molecule-1 (ICAM-1).[20,21]

We hypothesized that CCH was a localized LC histiocytosis (LCH) based on the cutaneous localization of the tumors, the morphological characteristics of the cells, and the epidermotropic tendency observed in many histiocytomas. In this paper, we describe the immunophenotypic characteristics of solitary and multiple histiocytomas and the lymphoid infiltrate that coincides with spontaneous regression. Furthermore, we present evidence that histiocytomas represent a proliferative disorder of epidermal LCs, which are of the dendritic/APC lineage.

Materials and Methods

Clinical Summary

Excised tumor tissues from 23 cases of CCH were obtained from regional veterinary practices and the Veterinary Medical Teaching Hospital at University of California, Davis, for evaluation (Table 1). The diagnosis of histiocytoma was based on characteristic morphological and clinical criteria.[2,5] The affected dogs were from 2 months to 12 years of age (mean, 3.7 ± 3.4 years). Two dogs had an original diagnosis of mycosis fungoides based on epidermal invasion by the infiltrates and the advanced age of the dogs. Their lesions regressed spontaneously shortly after surgical biopsy. In the majority of dogs, surgical excision was curative, although in two dogs, the tumors recurred at the original site several months after surgical excision and were again excised without further complication. Seven dogs had tumors in multiple sites. In three of these dogs, lesions were excised or regressed spontaneously. In the other four dogs, new lesions appeared over a 4- to 16-month period. The lesions eventually regressed spontaneously in two dogs, the third dog died from bacterial septicemia secondary to colonization of the ulcerated skin lesions, and the fourth dog was euthanized because of lymphadenopathy of the prescapular lymph nodes. In a related case, a 3-month-old Boxer presented with obliteration of the cervical lymph node chain, which occurred 2 weeks after excision of a solitary histiocytoma on the cheek. Two of these lymph nodes were excised and submitted for immunophenotyping. Lymphadenopathy in the

Table 2. *Monoclonal Antibodies Specific for Canine Leukocyte Antigens*

Antigen	MAb	Isotype	Reference	Source
CD1a	CA9.AG5	IgG1	Submitted	P. Moore
CD1b	CC20	IgG2a	22	C. Howard (Compton, UK)
CD1c	CA13.9H11	IgG1	Submitted	P. Moore
CD3ε	Polyclonal		24	Dako (Carpinteria, CA)
CD4	CA13.1E4	IgG1	48	P. Moore
CD5	YKIX.322	IgG2a	49	S. Cobbold (Oxford, UK)
CD8α	CA9.JD3	IgG2a	48	P. Moore
CD11a	CA11.4D3	IgG1	16	P. Moore
CD11b	CA16.3E10	IgG1	16	P. Moore
CD11c	CA11.6A1	IgG1	16	P. Moore
α_D	CA11.8H2	IgG1	29	P. Moore
	CA18.3C6	IgG1	29	P. Moore
CD18	CA1.4E9	IgG1	50	P. Moore
	CA16.3C10	IgG1	Unpublished	P. Moore
CD21-like	CA2.1D6	IgG1	48, 49	P. Moore
CD44	S5	IgG1	51	B. Sandmaier (Seattle, WA)
CD45	CA12.10C12	IgG1	48, 49	P. Moore
CD45RA	CA4.1D3	IgG1	48, 49	P. Moore
CD49d-like (VLA-4)	CA4.5B3	IgG1	48, 49	P. Moore
CD54 (ICAM-1)	CL18.1D8	IgG1	52	C. Smith (Houston, TX)
CD79a (MB-1)	HM57		26	M. Jones (Oxford, UK)
CD90 (Thy-1)	CA1.4G8	IgG1	49	P. Moore
MHC class II	CA2.1C12	IgG1	49	P. Moore
TCRαβ	CA15.8G7	IgG1	53	P. Moore
TCRγδ	CA20.8H1	IgG2a	54	P. Moore

remaining lymph nodes resolved over the next 4 weeks without treatment.

Antibodies

Monoclonal antibodies (MAbs) specific for canine leukocyte antigens were produced in the author's laboratory (CA prefix) or were obtained from listed sources (Table 2). CD1b was detected by a cross-reactive MAb (CC20) specific for bovine CD1b.[22] CC20 also reacts with COS cells transfected with human CD1B.[22] Specificity of CC20 for canine CD1b was confirmed by comparison of immunoprecipitates of biotin-labeled thymocytes with CA9.AG5 (CD1a, 49 and 12 kd), CA13.9H11 (CD1c, 43 and 12 kd), and CC20 (CD1b, 45 and 12 kd). The 12-kd subunit likely represented β_2-microglobulin, which associates with CD1 and MHC class I molecules.[23] Hence, canine CD1a, -b, and -c molecules were broadly similar to their human homologues with respect to antigen molecular weight and also with respect to cell and tissue distribution by flow cytometry and immunohistology[23] (manuscript submitted). Canine CD3ε was detected by a polyclonal rabbit antiserum (A452, Dako Corp., Carpinteria, CA) that is 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.[25,26] CD79a (MB-1) was detected by a MAb specific for a conserved intracytoplasmic peptide sequence derived from human cDNA, this sequence is also conserved in a variety of species.[26]

Tissue Handling and Immunohistochemistry

Tissues from histiocytomas or normal canine control tissues (skin, tongue, thymus, spleen, and tonsil) were fixed in 10% neutral buffered formalin; additional blocks were snap frozen in dichlorodifluoromethane (Freon 22) or isopentane cooled to their freezing points in liquid nitrogen. Formalin-fixed tissue was embedded in paraffin, and 4- to 6-μm sections were stained with hematoxylin and eosin (H&E). Frozen sections were stained by a streptavidin-horseradish-peroxidase method according to manufacturer's instructions (Zymed, South San Francisco, CA) and previously described methods.[16] MAbs specific for canine leukocyte antigens (Table 2) were applied to sections as diluted tissue culture supernatants based on previous titration on frozen sections of normal canine spleen and thymus. Negative controls consisted of substitution of specific MAb with isotype-matched nonspecific MAb 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 run. Normal epidermal LCs in skin adjacent to the histiocytoma served as a positive control for CD1a expression. This latter control

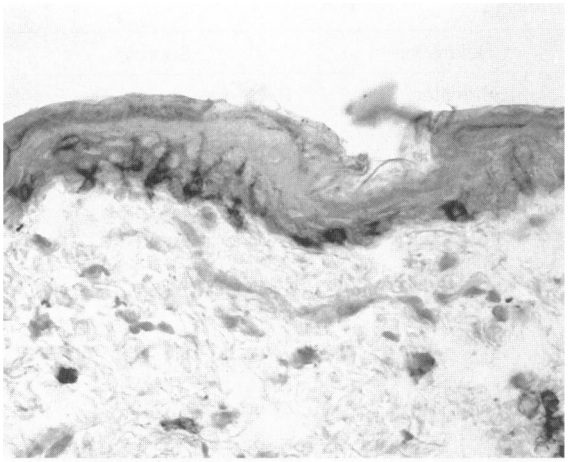

Figure 1. *Normal canine skin illustrating CD1a$^+$ dendritic cells within the epidermis. Immunoperoxidase; magnification, ×340.*

was particularly important, as MAb CA9.AG5 detects an allotypic variant of CD1a, which is expressed by approximately 80% of dogs (manuscript submitted); a negative staining result in the tumor had to be interpreted with caution. It should also be noted that the epitopes recognized by MAbs used in this study are destroyed by formalin fixation, with the exception of epitopes recognized by polyclonal anti-CD3ε, anti-MB-1, anti-α_D (CA18.3C6), and anti-CD18 (CA16.3C10). These latter reagents were useful in accurately differentiating cutaneous T cell lymphoma from histiocytoma in formalin-fixed, paraffin-embedded sections (data not shown).

Results

Immunophenotypic Characteristics of Canine Langerhans Cells

Canine LCs were identified in frozen sections of skin and mucosal sites, such as tongue and tonsillar epithelium, as MHC class II$^+$, CD1a$^+$, CD1b$^+$ (variable), CD1c$^+$ intra-epithelial dendritic cells, consistent with previous observations in humans (Figure 1).[12,17] Canine LCs had consistent, preferential expression of individual β_2-integrins (CD11/CD18) and isoforms of the leukocyte common antigen family (CD45) similar to that described for human epidermal LCs.[12,27,28] In particular, normal canine LCs expressed CD18 and the associated CD11 molecules CD11a and CD11c but did not express CD11b or α_D, the novel β_2-integrin expressed by splenic red pulp macrophages.[29] Canine LCs also expressed CD45 but did not express CD45RA. Thy-1 was not expressed by epidermal LCs, although Thy-1$^+$, MHC class II$^+$, CD1a, -b, and -c$^+$, and CD11c$^+$ dendritic

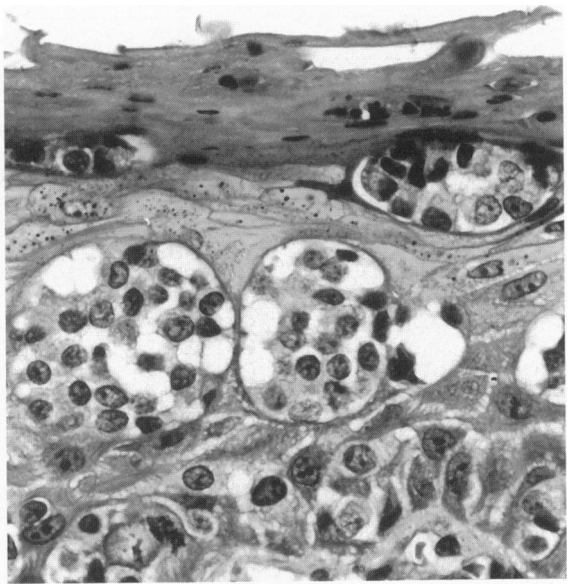

Figure 2. *Epidermal invasion by nests of tumor histiocytes in cutaneous histiocytoma. Tumor histiocytes possess abundant cytoplasm and irregular nuclear profiles, which are either oval, indented, or folded. H&E; magnification, ×485.*

cells were observed in the canine dermis. These were likely dermal dendritic/APCs, which also have been reported to express factor XIIIa.[9,10,12,30,31]. However, Thy-1 was broadly expressed; vascular endothelium and dermal fibroblasts also expressed Thy-1. Additionally, canine epidermal LCs did not express CD4, CD44, CD54 (ICAM-1), or CD49d. These molecules have been demonstrated, although sometimes inconsistently, on normal epidermal LCs or freshly isolated epidermal LCs from humans or mice.[14,15,20,21,28,32,33] Hence, expression of CD1 molecules, particularly CD1a and CD1c, were the best criteria of LC differentiation in canine epidermis. However, expression of CD1a by epidermal LCs was detectable in only approximately 80% of dogs with MAb CA9.AG5, as this MAb recognized an allotypic variant of CD1a. The presence of epidermal LCs in CD1a-allotype-negative dogs was confirmed by their expression of MHC class II and CD1c in serial sections of the respective tissues.

Morphological and Immunophenotypic Characteristics of CCH

Histiocytomas occurred as nodular lesions that infiltrated the superficial and deep dermis and subcutis. Epidermal invasion by histiocytes was observed in 16 of 20 cases, and in 11 cases this feature was prominent (Figure 2). Epidermal invasion was not assessable in 3 cases; in 2 of these, complete epi-

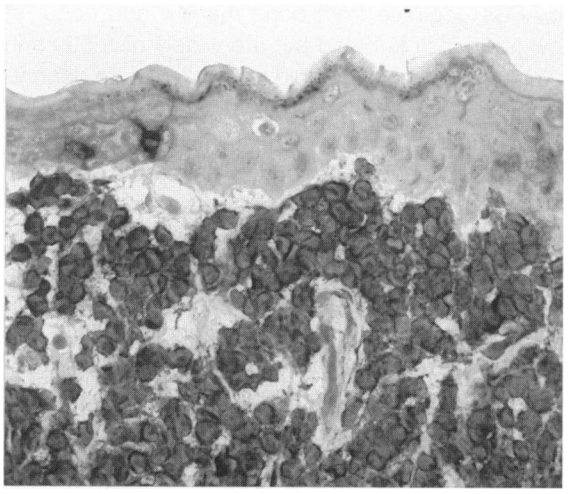

Figure 3. *CD1a expression by tumor histiocytes in the dermis in cutaneous histiocytoma. Normal CD1a$^+$ LCs are present within the epidermis. Immunoperoxidase; magnification, ×340.*

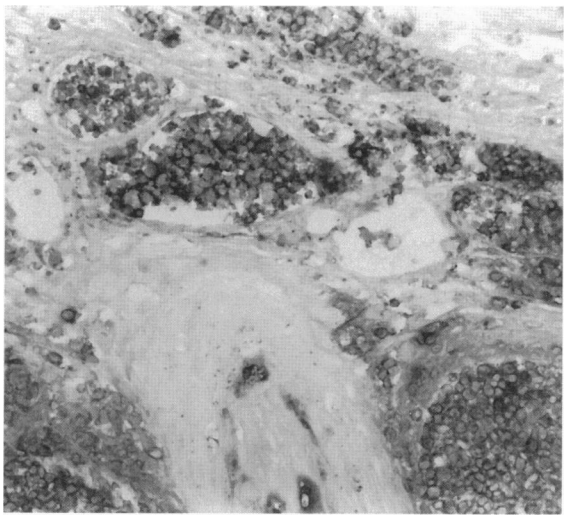

Figure 4. *Epidermal invasion by nests of CD1a$^+$ tumor histiocytes in cutaneous histiocytoma. Immunoperoxidase; magnification, ×250.*

dermal ulceration prevented evaluation. Histiocytes within tumors possessed abundant eosinophilic cytoplasm and either round, indented, or folded nuclei (Figure 2). In the 2 cases of lymph node involvement, tumor histiocytes obliterated the paracortex and follicular domains and the cortical and medullary sinuses.

The tumor cells within CCH consistently and diffusely expressed CD1b, CD1c, CD11a, CD11c, and MHC class II in all instances. Tumor cells expressed CD1a in 19 of 23 cases (Figure 3). Normal LCs in skin adjacent to the histiocytomas did not express CD1a in the 4 CD1a-negative tumors, which indicated that these dogs did not express the CD1a allotype recognized by CA9.AG5. In tumors that exhibited prominent epidermal invasion, CD1a expression was prominent in both the dermal and epidermal histiocytes (Figure 4). Tumor cells did not express α_D, a novel β_2-integrin that is highly expressed by splenic red pulp macrophages.[29] CD11b was expressed by histiocytes either diffusely or regionally in 63% of cases; a similar distribution of the β_1-integrin (VLA-4) and ICAM-1 was observed within histiocytomas in 91 and 94% of cases, respectively. CD44 was intensely expressed by tumor histiocytes in all instances. Prominent high endothelial venules were visible in the superficial and deep dermis of histiocytomas stained with anti-Thy-1 MAb. Intense endothelial expression of Thy-1 was observed in these high endothelial venules, although tumor histiocytes lacked Thy-1 expression (Figure 5). Likewise, tumor histiocyte expression of CD4 was not observed.

Marked lymphoid infiltration, often accompanied by necrosis of histiocytoma cells, was observed in 52% of CCH lesions. The lymphoid infiltrates were initially heaviest in perivascular locations near the junction of the subcutis and the deep dermis in some tumors (Figure 6). In other tumors, the lymphoid infiltrates were diffuse and radiated from perivascular locations at all levels of the lesion. The infiltrating lymphocytes expressed T cell receptor-$\alpha\beta$ and CD3 and were almost exclusively of the CD8 subset (Figure 6). CD4 lymphocytes and B lymphocytes (CD21$^+$ and CD79a$^+$) were encountered less frequently.

In formalin-fixed, paraffin-embedded sections, histiocytomas could be readily distinguished from cutaneous lymphomas by demonstration of intense CD18 expression by tumor histiocytes (using

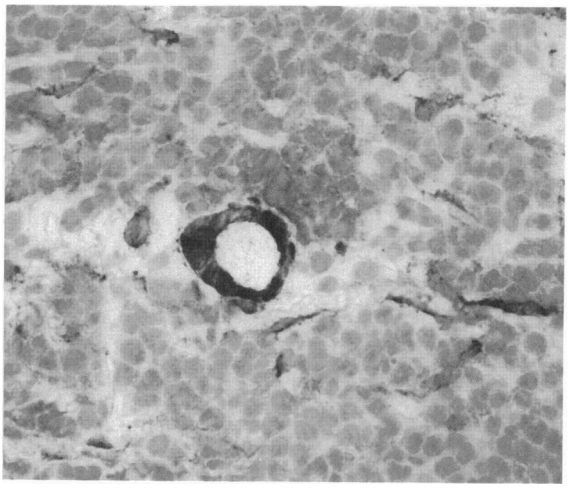

Figure 5. *Thy-1 expression by endothelial cells in dermal high endothelial venules in cutaneous histiocytoma. Several reactive Thy-1$^+$ dermal dendritic cells are interspersed among Thy-1− tumor histiocytes. Immunoperoxidase; magnification, ×340.*

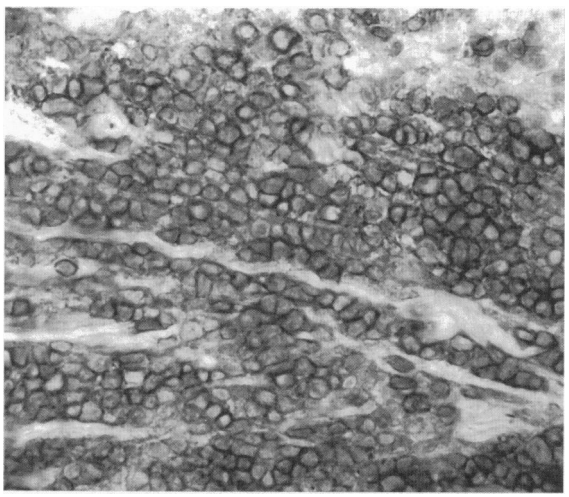

Figure 6. *$CD8^+$ lymphoid infiltrate in regressing cutaneous histiocytoma. Immunoperoxidase; magnification, ×340.*

CA16.3C10) and lack of CD3 and CD79a expression to rule out T cell and B cell proliferative disorders, respectively (data not shown). These three reagents have much utility in routine diagnostic pathology when fresh, unfixed tissue is routinely unavailable.

Discussion

This investigation has demonstrated that tumor histiocytes in CCH are phenotypically related to LCs by virtue of their expression of a spectrum of CD1 molecules. Tumor histiocyte expression of CD1a, which is normally restricted in its expression to cortical thymocytes and dendritic/APCs in the thymic medulla and cutaneous or mucosal epithelia (LCs), was most supportive of this conclusion. Furthermore, histiocytoma cells have a tropism for skin, particularly for epidermis. This behavior is also consistent with an epidermal LC lineage. In previous investigations of CCH, it was concluded that tumor cells were likely of mononuclear phagocytic lineage but were unlikely to be related to LCs because they lacked Birbeck's granules.[3,4] However, it was later shown that normal canine epidermal LCs lacked Birbeck's granules and did not express broadly distributed antigenic and enzymatic markers commonly associated with LCs in other species, such as S100 and ATPase.[13] However, as we have shown here, canine epidermal LCs resemble their counterparts in humans in terms of their leukocyte antigen expression profile, particularly their abundant expression of CD1a, CD1c, CD11c, and MHC class II molecules.

LCs are members of the dendritic/APC lineage and are located within squamous epithelia such as epidermis and certain mucosal epithelia.[34] Dendritic/APCs originate from bone marrow and occur at low frequency in blood but are widely distributed in lymphoid and nonlymphoid tissues.[8] Mature dendritic/APCs are morphologically, immunophenotypically, and functionally distinct from cells of monocyte/macrophage lineage.[8] However, dendritic/APCs and monocytes may originate from a common bone marrow precursor, and diversion of cells committed to a monocytic lineage to dendritic/APCs has not been excluded.[35,36] The conditions that direct differentiation and growth of dendritic/APCs are poorly understood, although recent evidence implicates a major role for granulocyte/macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF)-α in the differentiation of LCs from $CD34^+$ hemopoietic precursors.[37] The infrequency of dendritic/APC precursors among bone marrow cells implies that dendritic/APCs could potentially mature outside of bone marrow from stimulation of circulating $CD34^+$ hemopoietic precursors by locally derived GM-CSF and TNF-α.[37] In support of this hypothesis, intradermal injection of GM-CSF leads to the selective accumulation of $CD1a^+$ LCs in the dermis of human subjects.[38] Additionally, keratinocytes and skin-seeking lymphocytes can produce GM-CSF and TNF-α, and dermal mast cells have TNF-α in cytoplasmic granules.[39,40] Hence, current opinion favors the derivation of dendritic/APCs from a common hemopoietic precursor and suggests a role for locally derived cytokines in directing the localization and differentiation of specific subsets of dendritic/APCs. The possibility of inter-relationships between sub-lineages of cutaneous dendritic/APCs is apparent. The observed morphological and immunophenotypic differences between dendritic/APCs in the dermis and epidermis (LCs) may be more reflective of local environmental factors than major lineage differences.[12]

The β2-integrins and CD1 molecules are important markers for the identification of specific macrophage and dendritic/APC populations. The β2-integrins CD11b, CD11c, and α_D are differentially expressed by histiocytic populations in normal canine tissues.[16,29] For instance, hepatic Kupffer cells predominantly express CD11b. In spleen, CD11c is abundantly expressed by dendritic/APCs, which include interdigitating dendritic cells in T cell domains and marginal zone dendritic cells. In contrast, splenic red pulp macrophages predominantly express α_D. CD11c is also preferentially expressed by epidermal LCs and perivascular dendritic/APCs (dermal dendrocytes). Hence, expression of CD11c by resident histiocytic populations best correlates with dendritic/APC lineage. Likewise, the expression

of CD1 molecules is also highly regulated in the thymus and peripheral lymphoid organs.[23] CD1 expression is down-regulated as thymocytes become committed to either CD4 or CD8 expression. Hence, CD1 expression maps to the thymic cortex; CD1 expression in the thymic medulla is limited to resident dendritic/APCs. Beyond the thymus, CD1 expression is restricted and correlates with cells involved in antigen presentation. CD1c is expressed by dendritic/APCs and by subpopulations of B cells and monocytes. CD1a in particular is expressed mainly by epidermal and mucosal LCs and is inconsistently expressed by dendritic/APCs within lymphoid tissue. Collectively, the expression of CD1a, CD1c and CD11c reliably identifies epidermal LC in canine skin. In normal canine dermis, the exact lineage relationships of dendritic/APC which express CD1a, CD1b, CD1c, Thy-1 or CD11c are still unsettled, as is the case in humans.[12]

Tumor histiocytes in CCH expressed MHC class II, CD1b, CD1c, and CD11c in all cases. CD1a expression was detected on infiltrating histiocytes in all cases in which the CD1a allotype, detected by MAb CA9.AG5, was present (19 of 23 cases). Therefore, the phenotype of infiltrating histiocytes in cutaneous histiocytoma was identical to the phenotype of normal epidermal LCs with respect to the expression of CD1 and β2-integrins, the most definitive markers for the identification of canine macrophages and dendritic cells.

Up-regulation of the expression of a β1-integrin (VLA-4), CD11b, CD44, and ICAM-1 was observed in a variable proportion of histiocytomas. The expression of CD44, VLA-4, and ICAM-1 were particularly prevalent (90 to 100% of cases). These three molecules are not expressed by canine epidermal LCs. Up-regulation of VLA-4 in CCH may be of significance in determining the migration of cells to the lesion or from the site of the lesion to draining lymph nodes. For instance, up-regulation of CD44, VLA-4, ICAM-1, and MHC class II has been observed on cultured murine LCs and is thought to simulate the functional differentiation of LCs to mature dendritic/APCs that occurs during LC migration to lymph nodes after antigen challenge.[20] The migration of these activated LCs is believed to depend on up-regulation of appropriate cell adhesion molecules. Under these circumstances, up-regulation of VLA-4 is also observed and may play an important role in LC migration across activated endothelium that expresses VCAM-1, one of the ligands of VLA-4, or through connective tissue that contains fibronectin, another ligand for VLA-4.[41]

Investigation of leukocyte cell adhesion molecule expression in human LCH highlighted differences between LCH cells and epidermal LCs, which were suspected to be involved in abnormal homing and differentiation.[18] Specifically, these authors noted consistent, intense expression of CD11c and inconsistent, variable CD11b expression. Intense CD44, CD54 (ICAM-1), and CD58 expression was consistently observed. They concluded that LCH cells express markers expected of epidermal LCs when they are activated and migrate to draining lymph nodes. LCH is currently believed to represent a disorder of immune regulation,[42] although evidence for clonal LC proliferation in LCH has also been reported.[33] The phenotype of the LCH cell resembles that of activated epidermal LCs, which are capable of migratory behavior. This activated phenotype may contribute to the aberrant migration, persistence, and proliferation of abnormal cells in LCH.

The adhesion molecule expression pattern in CCH is quite similar to human LCH. Similar pathogenetic mechanisms, which dictate abnormal differentiation, homing, and migration may be operative in cutaneous histiocytoma, although the lesions in the majority of cases of cutaneous histiocytoma are usually more limited in extent and remain localized to the skin. However, we are aware of four instances of confirmed migration of tumor histiocytes from the skin to draining lymph nodes. Two of these cases were presented here, and two cases were communicated to us by Liz Wilson. An additional two cases developed lymphadenopathy in association with cutaneous histiocytoma, although migration of tumor histiocytes was not confirmed by histopathology. Draining lymph nodes are not routinely sampled in cases of CCH, because a favorable prognosis is anticipated. Therefore, the true incidence of migration of CCH to lymph nodes may in fact be higher. In most instances, spontaneous regression occurred, even despite obliteration of draining lymph nodes. The only exception was a dog that had multiple skin tumors and developed massive lymphadenopathy. Euthanasia was performed at the request of the owner. No evidence of spread beyond the involved lymph node was found at necropsy. Hence, CCH is a self-limited form of LCH that remains largely localized to the skin, despite the up-regulated expression of adhesion molecules capable of conferring aberrant migratory capability. Localized histiocytomas and migratory histiocytomas did not differ with respect to the expression of these molecules. Other factors must also influence the migratory potential of tumor histiocytes in this disease.

The factors that determine the onset of regression in CCH are unknown. Evidence of regression is usually observed in lesions that have been present for only a few weeks, although regression can be delayed for many months in problem cases. Regardless, regression is mediated by $CD8^+$ $\alpha\beta$ T cells; only scant numbers of $CD4^+$ T cells are observed in CCH lesions. By implication, the stringent co-stimulatory requirements of $CD8^+$ cytotoxic effector T cells must have been met for them to mediate regression.[43] This could occur in several ways. Migration of tumor histiocytes to draining lymph nodes could activate resident dendritic/APCs and $CD4^+$ T cells, which would assist in $CD8^+$ cytotoxic T cell activation via exogenous interleukin-2. Alternatively, tumor histiocytes may undergo local differentiation and up-regulate co-stimulatory molecules of the B7 family and thereby directly activate $CD8^+$ cytotoxic T cells and mediate their own destruction.[43] Studies are currently underway to test the latter hypothesis.

A major point of departure between human LCH and CCH is the lack of expression of CD4 by the cells of histiocytoma. Up-regulation of CD4 expression is consistently observed in human LCH and distinguishes LCH cells from resident epidermal LCs, which do not express significant amounts of CD4.[28,33] Preliminary observations indicate that histiocytic expression of CD1a, -b, and -c and CD4 is observed in other, more generalized histiocytic diseases of canine skin (Affolter and Moore, unpublished observations), such as systemic histiocytosis, which mostly afflicts Bernese Mountain dogs,[44] and cutaneous histiocytosis.[45] These diseases are LCHs that occur in the context of disordered immune regulation and are responsive to immunosuppressive therapy with steroids (cutaneous histiocytosis) or with cyclosporin A (systemic histiocytosis; Affolter and Moore, unpublished observations). It is possible that CD4 expression in this context may be a key feature that indicates an activated cell phenotype associated with more aggressive behavior.

The availability of MAbs specific for molecules that determine canine leukocyte differentiation, migration, and adhesion will enable further investigation of cell lineage and pathogenetic mechanisms in cutaneous lymphoid and histiocytic neoplasia in dogs. The distinction of epidermally invasive CCH from epidermotropic lymphoma (mycosis fungoides)[46] is readily achievable by the application of these markers. Also, the stage is set for a re-evaluation of the classification and pathogenesis of the spectrum of canine histiocytic proliferative disorders.[44,45,47] Here, we have provided evidence that CCH is a localized form of LCH in which regression is expected to occur. Other forms of canine LCH, which encompass more aggressive clinical and pathological disorders, exemplified by systemic histiocytosis and malignant histiocytosis,[44,47] have been more recently identified (Affolter and Moore, unpublished observations). The high frequency of these diseases within the general canine population (histiocytoma) or in genetically defined segments of the canine population (malignant and systemic histiocytosis) offers the potential that the dog may contribute interesting model systems to further the understanding of LC proliferative disorders of diverse etiology.

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