Veterinary Dermatology

DOI: 10.1111/j.1365-3164.2009.00833.x

Indolent cutaneous T-cell lymphoma presenting as cutaneous lymphocytosis in dogs

Verena K. Affolter*, Thelma Lee Gross† and Peter F. Moore*

*Department of Pathology, Microbiology, Immunology (VM PMI), University of California Davis, CA 95616, USA
†IDEXX Laboratory, Sacramento and California Dermatopathology Service, Davis, CA 95616, USA
Correspondence: Verena K. Affolter, VM PMI, University California Davis, Davis, CA 95616, USA. E-mail: vkaffolter@ucdavis.edu

Source of Funding
Self funded.
Conflict of Interest
Authors acknowledge no conflict of interest.

Abstract

Cutaneous lymphoproliferative diseases encompass a spectrum of lesions, ranging from self-limiting, reactive infiltrates to high-grade lymphomas. In humans, cutaneous lymphocytosis (CL) refers to self-limiting or slowly progressive monomorphic lymphocytic infiltrates of mostly unknown cause. It morphologically mimics cutaneous lymphoma. CL in cats also is a slowly progressive disease. Immunophenotyping and clonality testing of feline CL support an indolent lymphoma for the majority of cases studied. This study reports CL in dogs. Erythematous, scaly and alopecic macules, patches or plaques were present in eight dogs. Breed predilection was not observed; six of eight dogs were females; and ages ranged from 5 to 14 years. Diffuse monomorphic non-epitheliotropic infiltrates of $CD3^+$ (eight of eight), $CD45^-$ (four of eight) or $CD45^{+/-}$ (four of eight) and $CD45RA^-$ (seven of eight) T lymphocytes were present in the superficial and mid-dermis. Further immunophenotyping of five cases revealed TCR-$\gamma\delta^+$ T cells (one of five) or TCR-$\alpha\beta^+$ (four of five) T cells. TCR-$\alpha\beta^+$ populations were either $CD8^+$ (two of four) or $CD4^-CD8^-$ (2/4). Clonality testing found clonal (seven of eight) or pseudoclonal (one of eight) rearrangement of the TCR-gamma locus of the lesional T cells. Prednisone, prednisolone and methylprednisolone acetate were the most commonly administered drugs. The lesions remained stable for long periods up to 6 years. Five dogs were euthanized due to progression of the skin lesions (three of five), peripheral lymphadenopathy of unknown origin (one of five) or high-grade lymphoma (one of five). One dog was lost for follow-up and two dogs are still alive (17 and 9 months after diagnosis). Canine CL is best considered an initially indolent lymphoma, with slow progression and a potential for progression to high-grade lymphoma.

Accepted 1 July 2009

Introduction

Cutaneous lymphocytosis (CL), cutaneous pseudolymphoma, cutaneous lymphoid hyperplasia, lymphocytoma cutis and lymphoid dyscrasia are terms used to refer to a heterogeneous group of lymphoid proliferations in the skin of humans.[1–10] Typically, CL is considered the result of a persistent antigenic stimulation of either T or B cells. Drug reactions, arthropod-bites, contactants, and infections, such as borreliosis, Molluscum contagiosum, leishmaniosis and herpesvirus infection are commonly listed as underlying aetiologies for CL in humans. In contrast to cutaneous lymphoma, CL may be self-limiting and regress completely.[1–7] Alternatively, the lesions exhibit slow progression, but lack evidence of metastasis in most instances.[1–7] Occasional progression to lymphoma has been described.[3,7]

Differentiation of cutaneous lymphoma from reactive lymphoid proliferations based on morphological features presents a diagnostic dilemma and thus makes accurate prognosis difficult in each case.[1,3,4,9,11,12] Monomorphic lymphocyte populations with an altered immunophenotype suggest the diagnosis of neoplasia rather than a reactive process.[11,12] The presence of clonal lymphocyte expansions further supports a neoplastic process, while polyclonal populations indicate a reactive lymphoid infiltrate. Clonality assessment is achieved by amplification of T cell receptor-gamma (TCRG) gene locus for T cells or immunoglobulin heavy chain (IgH) gene locus for B cells respectively.[11,13–15] Occasionally, polyclonal lymphoid populations may harbour occult monoclonal populations; this has been referred to as clonal cutaneous lymphoid hyperplasia or lymphoid dyscrasia.[3,7] Alternatively, this may represent an emerging lymphoma within a reactive lymphocyte population. Recently it has been proposed that cutaneous lymphoid hyperplasia, clonal cutaneous lymphoid hyperplasia and cutaneous lymphoma present a continuum of lymphoproliferative disease.[3]

A recent study described CL in 23 cats.[16] The cats presented with erythematous plaques and papules characterized by perivascular to diffuse dermal infiltration of well-differentiated $CD3^+$ T cells.[16] Some lesions harboured small aggregates of well-differentiated $CD79^+$ B cells.[16] Most cats had a slow progression of their cutaneous disease. Neither morphological nor immunohistochemical evaluations allowed differentiation between reactive lymphocytosis and lymphoma in these cats.

Subsequently, clonal TCRG rearrangement was identified in the majority of these feline lesions.[17] The lesions were therefore considered to be indolent lymphoma. Ultimately, some cats declined and developed internal lesions, which indicated that feline indolent cutaneous lymphoma – referred to as CL – has the potential to progress to debilitating internal or systemic disease.[17]

The current study reports cutaneous lymphoid infiltrates in eight dogs, which histologically had resemblance to CL in cats and humans. The morphological features, immunophenotypic characteristics, and evaluations of TCRG rearrangements are presented and support indolent cutaneous T-cell lymphoma. Clinically, a slow progression of the cutaneous lesions was noted. However, these indolent clonal proliferations may progress to high-grade lymphoma as shown in one dog included in this study.

Material and methods

Case material

Over a period of 10 years, skin samples from eight dogs with histological lesions characterized by a superficial to mid-dermal band of monomorphic lymphoid infiltrates were evaluated by either the Veterinary Medical Teaching Hospital at UC Davis; IDEXX Laboratory and California Dermatopathology Service (Sacramento, CA, USA); Yager-Best Veterinary Surgical Pathology (Guelph, ON, Canada) or Histopathology Consulting (Clifton Park, NY, USA). For five of eight of the dogs several skin samples were collected at different times.

Tissue handling

Formalin-fixed, paraffin embedded tissues were available from all eight dogs. Paraffin sections were used for histological examination, immunohistochemistry and for DNA extraction for subsequent clonality testing. Additional fresh tissue samples were available from five of eight dogs to perform immunohistochemistry with an expanded panel of antibodies (Table 1). The fresh tissues were bisected; one half was fixed in 4% buffered formalin and embedded in paraffin. The other half was snap-frozen by immersing the sample in methylbutane previously cooled to its freezing point in liquid nitrogen. The frozen tissue samples were stored at –70 °C.

Table 1. Monoclonal (MAB) and polyclonal (PAB) Antibodies used for immunophenotyping in formalin-fixed, paraffin-embedded tissue sections and fresh, snap-frozen tissue sections

Antigen	MAB	Species-specificity	Source	Fresh tissues	Fixed tissues
CD1	CA13.9H11	Canine MAB	LABL†	X	
CD3	CA17.2A12	Canine MAB	LABL	X	
CD3ε	CD3-12	Human*	SE‡	X	X
CD4	CA13.1E4	Canine MAB	LABL	X	
CD8α	CA8.JD3	Canine MAB	LABL	X	
CD8β	CA15.4D2	Canine MAB	LABL	X	
CD11b	Ca16.3E10	Canine MAB	LABL	X	
CD11c	CA11.6A11	Canine MAB	LABL	X	
CD11d	CA11.8H2	Canine MAB	LABL	X	X
CD18	CA1.4E9	Canine MAB	LABL	X	X
CD20	Rabbit polyclonal	human	Labvision§	X	X
CD21	Ca2.1D6	Canine MAB	LABL	X	
CD45	CA12.10C12	Canine MAB	LABL	X	X
CD45R	CA21.4B3 CA4.1D3	Canine MAB	LABL	X	X
CD79a	HM57	Human*	DAKO	X	X
MHC II	CA2.1C12	Canine MAB	LABL	X	
TCR-αβ	CA15.8G7	Canine MAB	LABL	X	
TCR-γδ	CA15.8H1	Canine MAB	LABL	X	

*Cross-reactive with canine tissues; †LABL = Leukocyte Antigen Biology Laboratory, Peter F. Moore, University of California, Davis; ‡SE = Serotec, Oxford, UK; §Labvision: Freemont, CA, USA.

Histological examination and immunohistochemistry

Morphological features were evaluated using 5 μm paraffin sections stained with haematoxylin and eosin (H&E). The antibodies used for immunophenotyping are listed in Table 1. Paraffin sections or cryosections (4 μm) were mounted on superfrost-plus glass sides (Fischer Scientific, Waltham, MA, USA) and air-dried. Paraffin sections were deparaffinized in xylene and hydrated through graded ethanol solutions. Hydrated sections were steamed in 10 mmol/L Citrate buffer, pH6 (DAKO, Carpenteria, CA, USA) at 95 °C for 30 min followed by cooling for 20 min and quenching of endogenous peroxidase in 0.3% hydrogen peroxide in methanol (30 min). Cryosections were directly fixed in acetone for 2 min. Endogenous peroxidase was quenched by immersion of the slides in hydrogen peroxide (0.3%) and sodium azide (0.1%) in phosphate-buffered saline (PBS) for 10 min.

Both cryosections and paraffin sections were subsequently treated identically. Immunohistochemistry was performed as previously described.[18] Amino-9-ethyl-carbazole (AEC) was used as chromagen and the sections were counterstained with haematoxylin (Gill's formula 3; Fischer, Pittsburg, WA, USA).

DNA extraction

Depending on the size of the tissue sample submitted, two or three 25 μm paraffin sections were collected in an Eppendorf tube; knives were changed after each case to avoid DNA cross-contamination. The sections were deparaffinized in xylene and washed twice in 100% Ethanol. Tissue lysis was performed with proteinase K for 16 to 24 h. After complete lysis, genomic DNA was extracted using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The concentration of genomic DNA was measured using an Introspect 2100 pro spectrophotometer UV/Visible spectrophotometer (Amersham Pharmacia Biotech, Uppsala, Sweden).

Polymerase chain reaction

A single primer pair was used for assessment of T-cell clonality, as previously designed and described.[19,20] An approximately 110 bp segment (±20 bp) of the T-cell receptor gamma (TCRG) locus was amplified, using the forward primer in the variable region (5′-3′: TGK TGC AGA ARC TGG AGA AGA K: G and T; R: A and G) and the reverse primer in the joining region (5′-3′: GCA CTG TGC CAG GAC CAA ATA). Two segments of the immunoglobulin heavy chain (IgH) locus were amplified to assess B-cell clonality. One upper (forward) primer (5′-3′: GMC GVT TCA CCA TCT CCA RRG M: A and C; V: A and C and G; R: A and G) was paired with two lower primers (5′-3′: TGA RGA GAC RGT GAC CWG GGT R: A and G; W: A and T and GGA CAC GAA GAS TGA GGT GCC S: C and G) to amplify framework 2 (an approximately 250 bp segment) and framework 3 (an approximately 180 bp segment) of the complementary-determining region of IgH.[20]

Each reaction was performed with 100 ng of genomic DNA. A reaction mixture was used as previously described, with 2-step touch down amplification conditions applied.[20] The polyclonal control was composed of genomic DNA extracted from canine peripheral blood mononuclear cells; DNA extracted from canine lymph nodes with confirmed T-cell lymphoma or B-cell lymphoma was used as clonal control. All PCR reactions were run in duplicates to confirm results and avoid false positive results (referred to as pseudoclonality).

Native samples were used directly for gel electrophoresis. In addition, heteroduplex analysis, previously described to assist separation of true clonal from false-positive results, was performed.[14,21–24] Native PCR products (10 μL) were denatured at 95 °C for 10 min, allowing them to reanneal at 4 °C for 1 h prior to gel electrophoresis. Duplicate PCR samples (10 μL) of each native and heteroduplex sample, including controls (negative, polyclonal and clonal) were analysed.

Analysis of PCR products by gel electrophoresis was performed using pre-cast 10% non-denaturating polyacrylamide Tris-Borate-EDTA (TBE) gels (Criterion Pre-cast gels; Bio-Rad, Hercules, CA, USA) subsequently stained with Gel Star™ nucleic acid stain (Cambrex Bioscience Rockland Inc., Rockland, ME, USA) as previously described.[19,20] Bands and smears were visualized on a UV transilluminator and photographed. Equivalent samples were also evaluated by capillary gel electrophoresis using e-Gene HDA-GT12 (QIAxcel System by Qiagen) and the QIAxcel DNA High Resolution Kit 1200 (Qiagen) per kit instructions.[15] Samples (10 μL) were loaded into the wells of 12 strip, 0.2 mL PCR tubes and placed into the e-Gene HDA-GT12. Electrophoresis was run using the BioCalculator software method 'OL700.5s' and QX Alignment Marker #929527 (15 bp/400 bp) (Qiagen). The results were additionally visualized and analysed using the Biocalculator Software.

Clinical follow-up

Referring veterinarians and/or owners were contacted to receive clinical follow-up. A questionnaire was prepared to inquire about development of lesions, response to treatments, additional medical history and survival time of each dog.

Results

Clinical presentation

The clinical data for the eight dogs is listed in Table 2. The group included two Welsh corgis, two golden retrievers, a Chinese crested dog, a Chihuahua, a Shetland sheepdog and a dachshund. There were six female dogs, including three intact and three spayed bitches, and two male castrated dogs. The age of onset ranged from 5 to 14 years, with a mean of 8.26 years. The dogs presented with erythematous, macules and patches with variable degree of scaling and alopecia (Figure 1). In dogs 5 and 6 the lesions were slightly raised, forming erythematous, scaly and crusty plaques (Figure 2). Pruritus was observed in three of eight dogs. The lesions were seen in the axillae, inguinal region, thorax, abdomen, flank, thighs, legs, head and neck (Table 2). The location of the lesions was not listed for dog 5. A bilateral distribution of some lesions was documented in three of eight dogs. Lymphadenopathy was not noted at first presentation to the referring veterinarians in any of the dogs. Dog 4 had borderline low T4 levels and received thyroid replacement therapy and dog 7 had been treated with prednisolone for allergic skin disease. The remaining dogs did not have any skin lesions prior to presentation of the current problem.

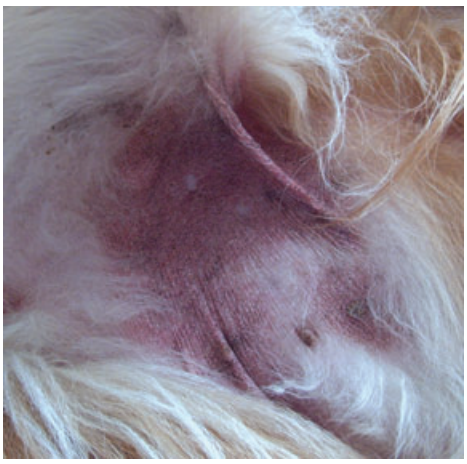

Figure 1. Skin, dog 7: Erythematous and alopecic skin in the axilla.

Histological examination

All samples revealed consistent morphological features characterized by a variably dense band of lymphocytes occupying the upper dermis, often extending into the

Table 2. Summary of clinical data and follow-up on the eight dogs with cutaneous lymphocytosis

No.	Breed	Sex	Age* (year)	Location of lesions	Skin lesions	Therapy	Follow-up
1	Welsh Corgi	f	8	Axillae bilateral, ventral abdomen, left forelimb	NI	Prednisone	Stable for 2 years *Euthanasia*
2	Welsh Corgi	fs	14	Head, neck thorax bilateral	Erythroderma scaling patchy	Prednisone	Waxing and waning – 6 months *Euthanasia*
3	Chinese Crested dog	f	8	Axillae, inguinal, thighs	Alopecia erythema patchy	NI	Stable 2 years; Extending slowly – 4 years: ear, back, neck After 6 years: lymphadenopathy due to high grade lymphoma *Euthanasia*
4	Shetland sheepdog	fs	10	Thorax bilateral	Scaling alopecia	NI	After 1 year: lymphadenopathy of unknown origin *Euthanasia*
5	Golden retriever	fs	8	NI	Erythema scaling plaques pruritus	Methylprednisone acetate; essential fatty acids; dexamethasone	Stable for 2 years *No follow-up*
6	Chihuahua	f	5	Flank, ventral abdomen	Plaques scaling pruritus	NI	After 1 year: spreading to vulva, ventral neck, chest *Euthanasia*
7	Golden retriever	mc	8	Feet, axilla	Erythema pruritus	Prednisone	17 months: waxing and waning *Still alive*
8	Dachshund	mc	5	Flank	Patch	Topical tacrolimus prednisone	9 months: slightly extending *Still alive*

*Age of onset; F, female; fs, female spayed; m, male; mc, male castrated; NI, no information.

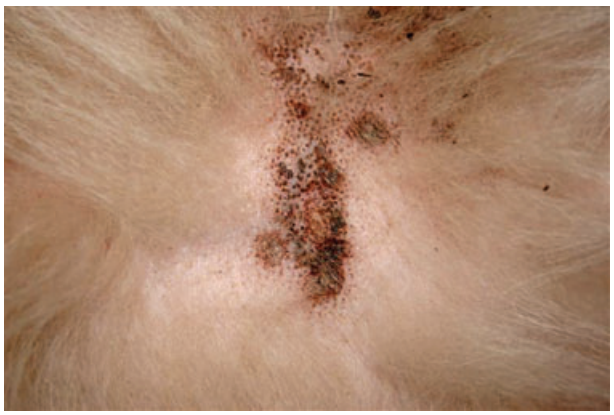

Figure 2. Skin, dog 5: Irregular erythematous crusting plaque; location of lesion not known.

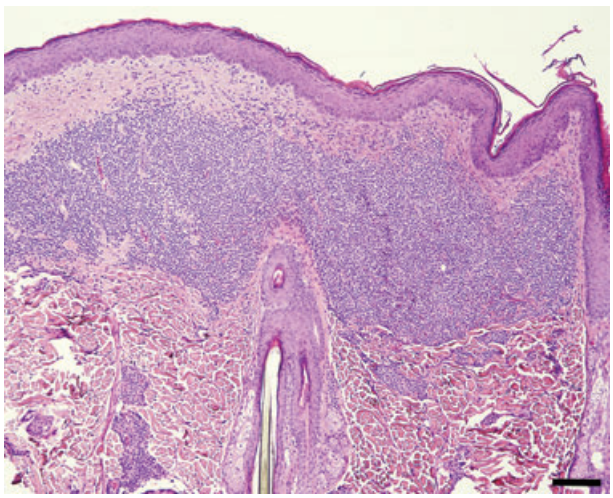

Figure 3. Skin, dog 5: The lymphocytic infiltrate is forming a band in the superficial and mid dermis. A Grenz zone separates the infiltrate from the epidermis. Intraepithelial infiltrates are not present. H&E stain; Bar = 300 μm.

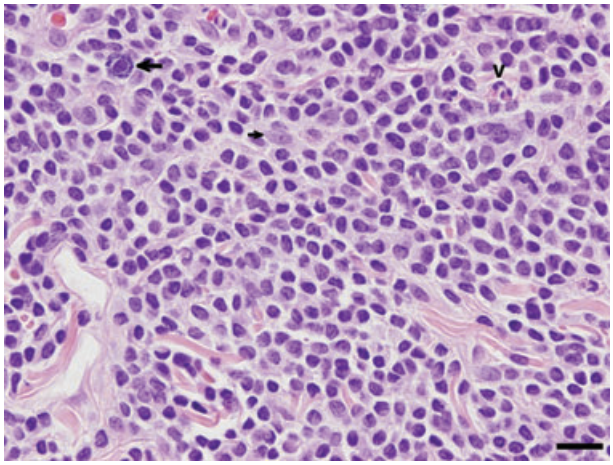

Figure 4. Skin, dog 7: The diffuse lymphocytic infiltrate displays minimal anisocytosis and anisokaryosis and is characterized by round to oval nuclei with fine, stippled chromatin, often inconspicuous nucleoli, occasional nuclear clefting and a small to moderate amount of pale pink cytoplasm. Mitotic figures are not evident. Few histiocytes (small arrow), rare mast cells (large arrow) and eosinophils (v) are present. H&E stain; Bar = 30 μm.

mid-dermis and rarely into the lower dermis (Figure 3). The immediate subepidermal dermis was variably involved. The dermal infiltrate either extended up to the basement membrane zone or was separated from the overlying epidermis by a distinct Grenz zone. Occasionally, the cellular infiltrate surrounded hair follicles. With the exception of small areas of mild exocytosis, intraepidermal or follicular infiltration was not observed. The lymphocytes had round to oval nuclei with fine, stippled chromatin, often inconspicuous nucleoli, occasional clefting and a small to moderate amount of pale cytoplasm (Figure 4). Minimal anisocytosis and anisokaryosis were observed. Mitotic figures were not evident. Few histiocytes, occasional mast cells and rare eosinophils were associated with the lymphoid infiltrate. Several dogs (five of eight) were rebiopsied at different times. The morphological features of the subsequent skin biopsies revealed identical features. There was no evidence of increased pleomophism or mitoses in the subsequent samples submitted.

Immunophenotyping

Paraffin sections were used in all eight dogs and fresh, snap-frozen tissues were used for additional immunohistochemistry in five of eight dogs. The consistent expression of the β2 integrin β-chain CD18 confirmed the leukocytic origin of the diffuse dermal infiltrate in all dogs. Additional detailed results of immunohistochemistry are summarized in Table 3. The common leukocyte antigen CD45 was not detected (three of eight dogs; Figure 5), weakly positive in a subpopulation of T cells I (four of eight dogs) and strongly expressed in dog 6 only. CD45RA, an alternative spliced isoform of CD45 expressed by naive cells, was not expressed in six of eight dogs. The cells consistently expressed CD3, indicative of T lymphocytes (Figure 6). Further immunopheno-

Table 3. Immunophenotype of the dermal round cell infiltrate and evaluation of T cell receptor-gamma (TCRG) rearrangement by PCR

	Breed	Immunophenotype paraffin sections + frozen sections	Clonality TCRG
1	Welsh Corgi	$CD45^{-}$ $CD45R^{-}$ $CD3^{+}$ $TCR\alpha\beta^{+}$ $CD4^{-}$ $CD8^{\pm}$	Clonal
2	Welsh Corgi	$CD45^{-}$ $CD45R^{-}$ $CD3^{+}$	Pseudoconal
3	Chinese Crested dog	$CD45^{-}$ $CD45R^{-}$ $CD3^{+}$	Clonal bi-allelic
4	Shetland sheepdog	$CD45^{\pm}$ $CD45R^{-}$ $CD3^{+}$ $TCR\text{-}\gamma\delta^{+}$ $CD4^{-}$ $CD8^{-}$	Clonal bi-allelic
5	Golden retriever	$CD45^{\pm}$ $CD45R^{-/+}CD3\varepsilon^{+}TCR\alpha\beta^{+}$ $CD4^{-}$ $CD8^{-}$	Clonal
6	Chihuahua	$CD45^{+}$ $CD45R^{+}$ $CD3^{+}$ $TCR\alpha\beta^{+}$ $CD4^{-}$ $CD8^{-}$	Clonal
7	Golden retriever	$CD45^{\pm}$ $CD45R^{-}$ $CD3^{+}$	Clonal
8	Dachshund	$CD45^{\pm}$ $CD45R^{-}$ $CD3^{+}$ $TCR\alpha\beta^{+}$ $CD4^{-}$ $CD8^{+}$	Clonal

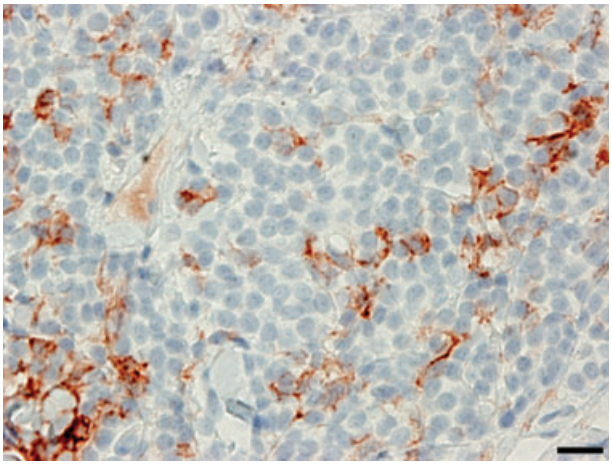

Figure 5. Skin, dog 5: The expression of CD45 is limited to a few dispersed reactive cells. The diffuse round cell infiltrate lacks expression of this common leukocyte antigen. Immunohistochemistry; chromogen: AEC; Bar = 30 μm.

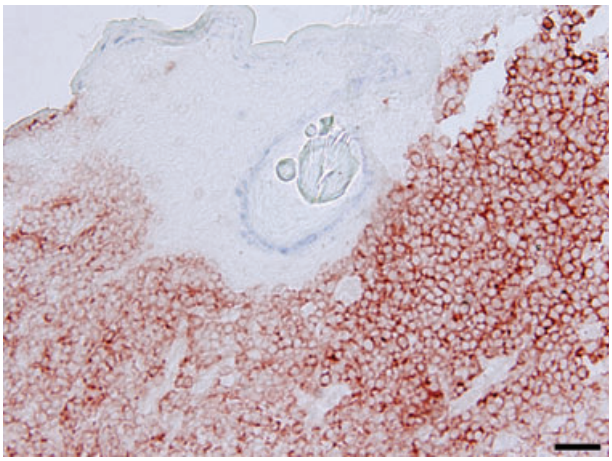

Figure 6. Skin, dog 6: The diffuse lymphocytic infiltrate is composed of $CD3^+$ T cells. Immunohistochemistry; chromagen: AEC; Bar = 60 μm.

typing on frozen tissues in five of eight dogs, indicated that CL is more commonly composed of TCR-$\alpha\beta^+$ T-cell population (four of five dogs; Figure 7); TCR-$\gamma\delta^+$ T cells were documented in only one of five dogs. TCR-$\alpha\beta^+$ T cells co-expressed CD8 (two of four dogs) or they lacked expression of CD4 and CD8, which is referred to as double negative T cells (two of four dogs).

Occasional, dispersed small $CD20^+$ and $CD79a^+$ B lymphocytes were noted within the diffuse T-cell infiltrate (four of eight dogs). A dispersed reactive TCR$\alpha\beta^+$ T-cell infiltrate was observed in dog 4, in which the proliferative cells expressed TCR$\gamma\delta$. Some of the reactive T cells co-expressed CD4, others were positive for CD8.

Evaluation for clonality

The clonality results are summarized in Table 3. TCRG rearrangement was evaluated in duplicate native and heteroduplex samples. The lesional T cells were characterized by a clonal TCRG rearrangement in seven of eight dogs, with a bi-allelic rearrangement in two of these dogs.

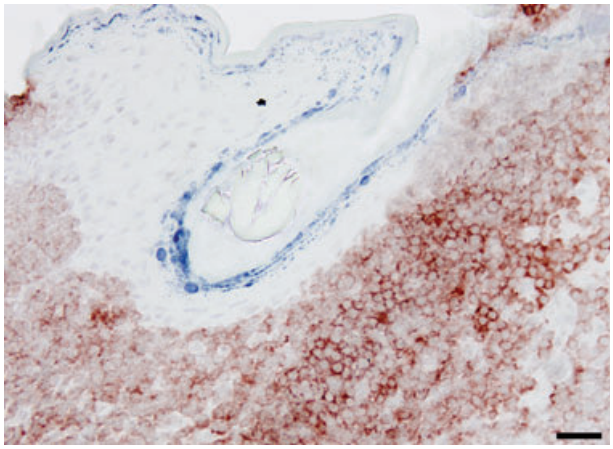

Figure 7. Skin, dog 6: The T-cell infiltrate expresses TCR-αβ. Immunohistochemistry; chromagen: AEC; Bar = 60 μm.

Pseudoclonality was observed in one of eight dog, characterized by shifting of the band in duplicate samples. Clonal rearrangement of IgH was not detected.

Clinical follow-up

Clinical follow-up data are summarized in Table 2. Prednisone treatment was initiated in four to eight dogs, accompanied by topical tacrolimus in one dog. Alternatively, dexamethasone, essential fatty acids and methylprednisone acetate were administered in one dog. None of the lesions regressed completely with long-term steroid therapy. Dog 7 and 8 were still alive 17 and 9 months after diagnosis respectively; both showed partial response to prednisone. No information about treatment was available in three dogs.

The skin lesions remained stable, slightly waxed and waned or slowly progressed over a period of 6 months to 2 years in four of eight dogs. Three of these dogs were euthanized upon the owners' request, because skin lesions were worsening towards the end of that period. Necropsies were not performed for these dogs. The fourth dog was lost to follow-up after 2 years.

Eventually two of eight dogs developed peripheral lymphadenopathy (Table 2). After the lesions remained quiescent for 2 years in dog 3, they started to progress over the next 4 years. Fine needle aspirate revealed high-grade lymphoma of the enlarged lymph nodes and the dogs was euthanized due to poor prognosis. Dog 4 presented with lymphadenopathy 1 year after development of the skin lesions. The owner declined further diagnostic procedures and the dog was euthanized upon the owner's request. No necropsies were performed on these two dogs.

Discussion

Recently the term cutaneous lymphocytosis (CL) has been introduced into the veterinary literature to describe solitary or multifocal skin lesions in cats, characterized by diffuse well-differentiated lymphocytic dermal infiltrates.[16] CL has best been characterized in humans. It encompasses a heterogeneous group of lymphocytic proliferations considered by most investigators to be a

reaction pattern that reflects a localized immunological response to a range of stimuli including drugs, arthropod-bites, contactants or infectious diseases such as borreliosis, leishmaniosis or Molluscum contagiosum.[1,2,8,10,25] However, because a direct association with antigen stimulation cannot be substantiated in many cases, the lesions are often referred to as idiopathic CL. In addition to histopathological features, immunopheontyping of the lesions as well as clonality testing may assist to separate reactive from neoplastic infiltrates.[11,12,14]

In contrast to cutaneous lymphoma, CL in humans may be self-limiting and regress completely.[1–7] This behaviour has been noted with polyclonal and only occasionally with clonal expansion of lymphocytes in the skin. In most instances, CL lesions exhibit slow progression, but lack evidence of metastasis.[1–7] However, progression to lymphoma has been described.[3,7]

Canine CL is an uncommon disease. Its clinical course is characterized by slow progression, which is strikingly different from cutaneous epitheliotropic and non-epitheliotropic lymphoma.[26,27] Based on the small number of dogs included in this study, no breed predilection was observed. Similar to the study of CL in cats, female dogs were overrepresented in this small group.[16,17] Middle aged to older dogs (mean 8.26 years**)** presented with patches of erythroderma, associated with scaling and alopecia or occasionally plaques. Nodules or papules, a common feature in human and feline CL, were not seen in any of the dogs.[1,4,16] Moreover, pruritus was uncommon, while many cats exhibited excoriations due to pruritus.[16] Most dogs had multiple lesions, occasionally in bilateral, but not symmetrical distribution. While humans may have solitary or multiple lesions, cats more often develop a solitary, although often locally expansive lesion.[1,4,16] At the time of diagnosis peripheral lymphadenopathy was not present in these dogs, as for CL in cats and humans. Except for one dog with allergic skin disease, the dogs (seven of eight) did not exhibit skin lesions prior to the development of CL.

Canine CL is characterized by a fairly discrete superficial and mid-dermal band of $CD3^+$ T lymphocytes. This is compatible with one pattern seen with human CL of T-cell origin as well as with feline CL.[1,4,11,16] In contrast to feline CL, the T-cell infiltrate in the dogs of this study rarely extended into the deeper dermis.[16] None of the dogs presented with a nodular lymphoid infiltrate, a second pattern observed in human CL.[1,2,4,11] This may explain why canine CL is mostly presenting as patches or flat plaques. Neither diffuse B-cell proliferations, occasionally seen in human CL, nor epitheliotropism, occasionally observed in feline CL, was present in the biopsies examined.[1,4,9,16] The lymphoid infiltrates were monomorphic and not centred on a particular structure within the dermis, which is an unusual pattern for an inflammatory disease. Therefore, non-epitheliotropic T-cell lymphoma was initially considered as a differential diagnosis. Admixed dispersed reactive B cells were seen in very low numbers in half of the cases. Except for one dog with a small B-cell aggregate, densely packed aggregates of reactive $CD79^+$, $CD20^+$ B cells, a common feature in feline CL, was not evident in the samples evaluated from these dogs.

The dermal infiltrate in canine CL was consistently composed of $CD3^+$ T cells. It is not unusual for neoplastic cells to express an aberrant immunophenotype, a feature reported in canine cutaneous lymphoma as well.[11,12,27,28] With the exception of dog 6, a partial or complete lack of expression of the common leukocyte antigen CD45 and variable expression of the spliced variant CD45RA was documented.[29] Further immunophenotyping on frozen tissue revealed $CD8^+$ TCR-$\alpha\beta^+$ T cells in two of five dogs, a T-cell population encountered with both reactive and neoplastic dermal lymphocytic infiltrates. However, $CD4^-CD8^-$, TCR-$\alpha\beta$ T cells, as seen in two of five dogs, are rarely encountered in reactive T-cell infiltrates. Moreover, diffuse TCR-$\gamma\delta^+$ T-cell infiltrates (one of five dogs) are considered unlikely to be associated with inflammatory lesions in the dermis. These immunophentypically unusual T-cell populations are more consistent with a neoplastic cell population.

Clonality assessment by amplification of TCRG gene locus for T cells or the immunoglobulin heavy chain (IgH) gene locus for B cells respectively has been successfully used to differentiate between polyclonal and clonal lymphocyte populations.[11,13–15] While polyclonal rearrangement indicates a reactive process, clonal populations are most consistent with neoplasia. Clonality testing identified a clonal rearrangement of TCRG locus of the lesional T-cell population in seven of eight dogs, which supports the diagnosis of a T-cell neoplasia. The presence of an identical clonal band in duplicate native samples as well as heteroduplex samples ruled out false positive results.[14,21,24] Pseudoclonal TCRG locus rearrangement – characterized by shifting of the clonal band in duplicate native and heteroduplex samples – was identified in dog 2.

It should be emphasized that clonality testing should never be used as an isolated test to differentiate between neoplastic and reactive lymphoid infiltrates. On occasion, clonal expansions have been identified in reactive self-limiting lymphoid processes in humans.[7] Moreover, the sensitivity of clonality testing is about 80% for TCRG and pseudoclonal rearrangements have been observed in the context of T-cell lymphomas.[30,31] It is therefore crucial to correlate clonality results, with immunophenotype and morphological features to avoid misinterpretations. The T-cell populations in all seven dogs with clonal TCRG rearrangements were characterized by an unusual immunophenotype; six of seven had complete or partial lack of CD45 expression and the lesions in dog 6 were composed of double negative TCR-$\alpha\beta$ T cells. The pseudoclonal TCRG rearrangement in context of lack of expression of the common leukocyte antigen CD45 in dog 2 was considered most supportive of a neoplastic population as well.

Canine CL is a slowly progressive disease and regression was not observed. This reflects the situation in CL in cats and is in contrast with some cases of CL in humans.[1,2,4,7,11,16,17] However, clonality testing has not been performed in all human cases; hence, direct comparison is not always applicable. No significant response to therapy was observed in any of the dogs. At best, a waxing and waning of the lesions was observed. However, most canine lesions remained fairly stable over a period of 1 to 2 years or as long as 6 years in one dog.

In fact, owners elected euthanasia in four of eight dogs, because of financial constraints and/or because no regression or responsive to therapy was observed. Two dogs are still alive at the time of writing.

Cutaneous lymphocytosis in dogs has the potential to progress to a high-grade lymphoma. This was evident in dog 6. After a 2-year period of stable lesions, the skin lesions slowly progressed over a period of another 4 years. At that time fine needle aspirates from enlarged peripheral lymph nodes indicated high-grade lymphoma in peripheral lymph nodes. Unfortunately, a necropsy was declined and there is no information about possible involvement of additional organ systems. In dog 4 a possible progression to involvement of lymph nodes was suspected as peripheral lymphadenopathy was observed. Unfortunately, the owner elected euthanasia without further evaluations and post mortem examination was declined.

The aetiology of canine CL remains unknown. CL in humans is typically considered the result of a persistent antigenic stimulation. In most cats with CL no initiating factor could be identified, but drug reactions have been suggested in a few cats.[16,17] CL of the hind leg has been observed in a cat in association with an underlying vaccine reaction (personal observation). With exception of dog 7, which was treated for allergic skin disease, the clinical history did not indicate evidence of previous localized antigen stimulations in seven of eight dogs. Moreover, the skin lesions did not occur in areas of vaccinations sites or previous injection sites.

The current study reports cutaneous lymphoid infiltrates in eight dogs, which histologically had resemblance to CL in cats and humans. Regression of the lesions was not observed and the immunophenotypic characteristics and clonality testing suggested T-cell neoplasia. However, the prolonged quiescent stage and slow progression are not typical for canine cutaneous lymphoma. Therefore, based on this small number of cases, CL in dogs is best considered a form of indolent lymphoma, which over time may progress to high-grade lymphoma.

Acknowledgement

Our gratitude goes to the clinicians and pathologists who provided us with history, biopsies and clinical follow-up of the cases included.

References

1. Ploysangam T, Breneman DL, Mutasim DF. Cutaneous pseudolymphomas. Journal of the American Academy of Dermatology 1998; 38: 877–95.
2. Gilliam AC, Wood GS. Cutaneous lymphoid hyperplasias. Seminars in Cutaneous Medicine and Surgery 2000; 19: 133–41.
3. Nihal M, Mikkola D, Horvath N *et al.* Cutaneous lymphoid hyperplasia: a lymphoproliferative continuum with lymphomatous potential. Human Pathology 2003; 34: 617–22.
4. Rijlaarsdam JU, Willemze R. Cutaneous pseudolymphomas: classification and differential diagnosis. Seminars in Dermatology 1994; 13: 187–96.
5. Kazakov DV, Burg G, Dummer R *et al.* Cutaneous lymphomas and pseudolymphomas: newly described entities. Recent Results in Cancer Research 2002; 160: 283–93.
6. Albrecht J, Fine LA, Piette W. Drug-associated lymphoma and pseudolymphoma: recognition and management. Dermatologic Clinics 2007; 25: 233–44.
7. Magro CM, Crowson AN, Kovatich AJ *et al.* Drug-induced reversible lymphoid dyscrasia: a clonal lymphomatoid dermatitis of memory and activated T cells. Human Pathology 2003; 34: 119–29.
8. Del Boz Gonzalez J, Sanz A, Martin T *et al.* Cutaneous pseudolymphoma associated with molluscum contagiosum: a case report. International Journal of Dermatology 2008; 47: 502–4.
9. Cerroni L, Kerl H. Diagnostic immunohistology: cutaneous lymphomas and pseudolymphomas. Seminars in Cutaneous Medicine and Surgery 1999; 18: 64–70.
10. Flaig MJ, Rupec RA. Cutaneous pseudolymphoma in association with Leishmania donovani. British Journal of Dermatology 2007; 157: 1042–3.
11. Bakels V, van Oostveen JW, van der Putte SC *et al.* Immunophenotyping and gene rearrangement analysis provide additional criteria to differentiate between cutaneous T-cell lymphomas and pseudo-T-cell lymphomas. American Journal of Pathology 1997; 150: 1941–9.
12. Florell SR, Cessna M, Lundell RB *et al.* Usefulness (or lack thereof) of immunophenotyping in atypical cutaneous T-cell infiltrates. American Journal of Clinical Pathology 2006; 125: 727–36.
13. Flaig MJ, Schuhmann K, Sander CA. Impact of molecular analysis in the diagnosis of cutaneous lymphoid infiltrates. Seminars in Cutaneous Medicine and Surgery 2000; 19: 87–90.
14. Cherny S, Mraz S, Su L *et al.* Heteroduplex analysis of T-cell receptor gamma gene rearrangement as an adjuvant diagnostic tool in skin biopsies for erythroderma. Journal of Cutaneous Pathology 2001; 28: 351–5.
15. Lukowsky A, Richter S, Dijkstal K *et al.* A T-cell receptor gamma polymerase chain reaction assay using capillary electrophoresis for the diagnosis of cutaneous T-cell lymphomas. Diagnostic Molecular Pathology 2002; 11: 59–66.
16. Gilbert S, Affolter VK, Gross TL. Clinical, morphological and immunohistochemical characterization of cutaneous lymphocytosis in 23 cats. Veterinary Dermatology 2004; 15: 3–12.
17. Gilbert S, Affolter VK, Schmidt P *et al.* Clonality studies of feline cutaneous lymphocytosis. Veterinary Dermatology 2004; 15 (supplement 1): 24 (Abstract).
18. Affolter VK, Moore PF. Localized and disseminated histiocytic sarcoma of dendritic cell origin in the dog. Veterinary Pathology 2002; 39: 74–83.
19. Vernau W, Moore PF. An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. Veterinary Immunology and Immunopathology 1999; 69: 145–64.
20. Valli VE, Vernau W, de Lorimier LP *et al.* Canine indolent nodular lymphoma. Veterinary Pathology 2006; 43: 241–56.
21. Langerak AW, Szczepanski T, van der Burg M *et al.* Heteroduplex PCR analysis of rearranged T cell receptor genes for clonality assessment in suspect T cell proliferations. Leukemia 1997; 11: 2192–9.
22. Chen M, Deng A, Crowson AN *et al.* Assessment of T-cell clonality via T-cell receptor-gamma rearrangements in cutaneous T-cell-dominant infiltrates using polymerase chain reaction and single-stranded DNA conformational polymorphism assay. Applied Immunohistochemistry & Molecular Morphology 2004; 12: 373–9.
23. Martinelli G, Farabegoli P, Testoni N *et al.* Detection of clonality by heteroduplex analysis of amplified junctional region of T-cell receptor gamma in patients with T-cell acute lymphoblastic leukemias. Haematologica 1997; 82: 161–5.
24. Cook JE, Beverley PC. Analysis of lymphocyte diversity in the elderly: heteroduplex analysis and alternative techniques. Experimental Gerontology 2001; 36: 583–9.
25. Magro CM, Crowson AN. Drugs with antihistaminic properties as a cause of atypical cutaneous lymphoid hyperplasia. Journal of the American Academy of Dermatology 1995; 32: 419–28.

26. Moore PF, Olivry T. Cutaneous lymphomas in companion animals. Clinics in Dermatology 1994; 12: 499–505.
27. Moore PF, Olivry T, Naydan D. Canine cutaneous epitheliotropic lymphoma (mycosis fungoides) is a proliferative disorder of CD8 + T cells. American Journal of Pathology 1994; 144: 421–9.
28. Moore PF, Affolter VK, Olivry T *et al.* The use of immunological reagents in defining the pathogenesis of canine skin diseases involving proliferation of leukocytes. In: Kwochka KW, Willemse T, von Tscharner C, eds. Advances in Veterinary Dermatology, Vol. 3. Oxford, UK, Butterworth-Heinemann, 1998: 77–94.
29. Hedlund G, Hansson J, Ericsson PO *et al.* Expression of CD11a and CD45R isoforms defines distinct subsets of CD8 + TCR alpha beta and TCR gamma delta CTL in vivo. Immunological Reviews 1995; 146: 82–94.
30. Sandberg Y, Heule F, Lam K *et al.* Molecular immunoglobulin/T- cell receptor clonality analysis in cutaneous lymphoproliferations. Experience with the BIOMED-2 standardized polymerase chain reaction protocol. Haematologica 2003; 88: 659–70.
31. Muche JM, Sterry W, Gellrich S *et al.* Peripheral blood T-cell clonality in mycosis fungoides and non-lymphoma controls. Diagnostic Molecular Pathology 2003; 12: 142–50.

Résumé Les dermatoses lymphoprolifératives regroupent un large éventail de lésions, allant des infiltrats réactionnels auto-traumatiques aux lymphomes de haut grade. Chez l'homme, la lymphocytose cutanée (CL) correspond à des infiltrats lymphocytaires monomorphes auto-limités ou de lente évolution majoritairement de cause inconnue. Morphologiquement elle mime le lymphome cutané. La lymphocytose cutanée féline est également une maladie d'évolution lente. L'immunophénotypage et les tests de clonalité indiquent un lymphome indolent dans la majorité des cas étudiés. Cette étude rapporte des cas de CL chez des chiens. Macules, taches ou plaques érythémateuses, squameuses et alopéciques étaient présentes chez huit chiens. Aucune prédisposition raciale n'a été observée ; 6/8 chiens étaient des femelles et l'âge variait de 5 à 14 ans. Des infiltrats diffus monomorphes non-épithéliotropes de lymphocytes T $CD3^+$ (8/8), $CD45^-$ (4/8) ou $CD45^{+/-}$ (4/8) et $CD45RA^-$ (7/8) étaient présents dans le derme superficiel et moyen. De plus, l'immunophénotypage de cinq cas a montré des cellules T TCR-$\gamma\delta^+$ (1/5) ou TCR-$\alpha\beta^+$ (4/5). Les populations TCR-$\alpha\beta^+$ étaient soit $CD8^+$ (2/4), soit $CD4^-CD8^-$ (2/4). Les tests de clonalité ont révélé un réarrangement clonal (7/8) ou pseudoclonal (1/8) de la région TCR-gamma des cellules T lésionnelles. La prednisone, la prednisolone et la methylprednisolone étaient les médicaments les plus fréquemment administrés. Les lésions restaient stables sur de longues périodes allant jusqu'à 6 ans. Cinq chiens ont été euthanasiés en raison de la progression de leurs lésions cutanées (3/5), d'une lymphadénopathie périphérique d'origine inconnue (1/5) ou d'un lymphome de haut grade (1/5). Un chien a été perdu de vue et deux sont toujours vivants (17 et 9 mois après le diagnostic). La CL canine est considérée comme étant initialement un lymphome indolent d'évolution lente et pouvant évoluer vers un lymphome de haut grade.

Resumen Las enfermadades linfoproliferativas cutáneas abarcan un espectro de lesiones, que van desde procesos autolimitantes, infiltrados reactivos a linfomas de alto grado. En humanos la linfocitosis cutanea (CL) se refiere a un proceso autolimitante o de progresión lenta con infiltrado monomórfico de linfocitos que se produce por causas desconocidas. Morfológicamente se asemeja a linfoma cutáneo. CL en gatos es también una enfermedad de desarrollo lento. Las pruebas de inmunofenotipado y clonalidad de CL felinas indican que es una forma de linfoma indolente en la mayoría de los casos estudiados. Este estudio presenta CL en perros. Ocho perros se presentaron con maculas, pápulas o placas eritematosas, con descamación y alopecia. No se observó una prediposición de raza; 6/8 perros fueron hembras; y las edades oscilaron entre 5 y 14 años. Se presentaron infiltrados difusos monomórficos no-epiteliotrópicos de linfocitos T $CD3^+$ (8/8), $CD45^-$ (4/8) o $CD45^{+/-}$ (4/8) y $CD45RA^-$ (7/8) en la dermis superficial y media. Un fenotipado más extenso en cinco casos indicó la presencia de linfocitos T TCR-$\gamma\delta^+$ (1/5) o TCR-$\alpha\beta^+$ (4/5). Las poblaciones TCR-$\alpha\beta^+$ fueron $CD8^+$ (2/4) o $CD4^-CD8^-$ (2/4). La prueba de clonalidad encontró reorganización clonal (7/8) o pseudoclonal (1/8) del locus TCR-gamma en los linfocitos T. Los fármacos mas comúnmente administrados fueron prednisona, prednisolona y acetato de metilprednisolona. Las lesiones permanecieron estables durante periodos largos de tiempo, hasta 6 años. Cinco perros fueron sacrificados debido a progresión de las lesiones de la piel (3/5), linfadenopatía periférica (1/5) o linfoma de alto grado (1/5). Un perro se perdió para el seguimiento y dos perros continúan vivos (17 y 9 meses tras el diagnóstico). La CL canina se considera un estadío inicial de linfoma indolente con progresión lenta y con posibilidad de progresión a linfoma de alto grado.

Zusammenfassung Kutane lymphoproliferative Krankheiten beinhalten ein ganzes Spektrum an Läsionen, von selbst-limitierenden, reaktiven Infiltraten bis zu hochgradigen Lymphomen. Beim Menschen steht die kutane Lymphozytose (CL) für selbst-limitierende und langsam progressive monomorphe Lymphozyteninfiltrate von weitgehend unbekannter Ursache. Morphologisch ähneln sie stark dem kutanen Lymphom. Auch bei Katzen ist die CL eine langsam progressive Erkrankung. Immunphänotypisierung und Klonalitätstests von felinen CL weisen in der Mehrheit der untersuchten Fälle auf ein indolentes Lymphom hin. Diese Studie beschreibt CL bei Hunden. Bei acht Hunden bestanden erythematöse, schuppige und haarlose kleine und große Maculae oder Plaques. Eine Rassenprädisposition wurde nicht festgestellt; 6/8 Hunden waren weiblich; und das Alter reichte von 5 bis 14 Jahren. Diffuse monomorphe nicht-epitheliotrope Infiltrate von $CD3^+$ (8/8), $CD45^-$ (4/8) oder CD $45^{+/-}$ (4/8) und $CD45RA^-$ (7/8) T Lymphozyten waren in der oberflächlichen und mittleren Dermis zu finden. Eine weitere Immunphänotypisierung zeigte in fünf Fällen TCR-$\gamma\delta^+$ T-Zellen (1/5) oder TCR-$\alpha\beta^+$ (4/5) T-Zellen. TCR-$\alpha\beta^+$ Populationen waren entweder $CD8^+$ (2/4) oder $CD4^-CD8^-$ (2/4). Klonalitätstests zeigten klonale (7/8) oder pseudoklonale (1/8) Reorganisation des

TCR-gamma Lokus der läsionalen T-Zellen. Prednison, Prednisolon und Methylprednisolonacetat waren die am häufigsten verabreichten Medikamente. Die Veränderungen blieben für lange Phasen von bis zu 6 Jahren stabil. Fünf Hunde wurden aufgrund der Progression der Hautläsionen (3⁄5), aufgrund peripherer Lymphadeopathie unbekannter Ursache (1⁄5) oder wegen hochgradigem Lymphom euthanasiert. Ein Hund konnte nicht weiter verfolgt werden, zwei Hunde sind noch immer am Leben (17 und 9 Monate nach der Diagnose). Die canine CL kann am besten als anfänglich indolentes Lymphom, mit einer langsamen Progression und der Möglichkeit sich zum hochgradigen Lymphom zu entwickeln, betrachtet werden.

