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# Clinical and Histopathologic Characterization of Canine Chronic Ulcerative Stomatitis

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## Abstract

Canine chronic ulcerative stomatitis, also known as chronic ulcerative paradental stomatitis, is a painful condition of the oral cavity. The purpose of this study was to determine if there are commonalities in clinical and radiographic features among patients, whether the histopathologic evaluation might inform the pathogenesis, and whether the condition appears similar to human oral mucosal diseases. To do this, we prospectively collected clinical, radiographic, and histopathologic data from 20 dogs diagnosed with the disease. Clinical data were based on a clinical disease activity index, oral and periodontal examination parameters, and full-mouth dental radiographs. The histopathological and immunohistochemical data were based on oral mucosal samples obtained from erosive or ulcerated areas. Our findings revealed that canine chronic stomatitis is clinically characterized by painful oral mucosal ulcers of varying size, pattern, appearance, and distribution, most often associated with teeth with early periodontitis. Histologic examination revealed a subepithelial lichenoid band (interface mucositis) where B cells, T cells, and Forkhead-box protein 3 (FoxP3)– and interleukin-17–expressing cells were present. These cells might play a role in the underlying immune response and an immune-mediated pathogenesis is suspected. The clinical and histopathologic features of this chronic inflammatory mucosal disease in dogs resemble those of oral lichen planus in humans.

## Keywords

canine chronic ulcerative stomatitis, FoxP3, histopathology, IL-17, immune-mediated disease, oral cavity, dentistry

Canine chronic ulcerative stomatitis (CCUS)<sup>7,21</sup> is a condition clinically characterized by chronicity and pain associated with focal to diffuse oral ulceration, inflammation, and frequently mucosal necrosis. The ulcers typically occur on the alveolar and buccal mucosal surfaces opposite plaque on the dentition.<sup>21</sup> Ulcers may also be identified on the lateral margin of the tongue, the glossopalatine folds, and the mucocutaneous border of the lips. Maltese, Cavalier King Charles Spaniel, Labrador Retriever, and Greyhound dogs have a higher reported prevalence.<sup>7,21</sup> A more recent publication described maxillary osteomyelitis in 2 unrelated Scottish Terrier dogs with CCUS.<sup>5</sup> The differential diagnosis for CCUS includes lupus erythematosus,<sup>25,42</sup> pemphigus vulgaris,<sup>4,48,52</sup> bullous pemphigoid,<sup>41,47</sup> erythema multiforme,<sup>38,61</sup> epitheliotropic T-cell lymphoma,<sup>16,37,38</sup> and less likely uremic stomatitis.<sup>1,3</sup>

Resolution of some CCUS lesions occurs with extraction of the teeth opposite the ulcers.<sup>31</sup> However, in other cases, ulceration does not improve, and a vicious cycle of ulceration and scarring ensues (J. G. A., personal observation). Considerable morbidity occurs with chronic ulceration, as dogs may refuse to eat, lose weight, and can exhibit chronic oral pain.<sup>7</sup> Administering oral

medication and oral home care is often difficult due to extreme patient discomfort.

A commonly held hypothesis is that in predisposed dogs, the chronic presence of dental plaque results, either directly or through some indirect mechanism, in oral mucosal inflammation and ulceration.<sup>7,21,31,59</sup> The microbiota of canine plaque is complex<sup>10,13</sup> and dissimilar to people.<sup>11,22</sup> Moreover, the oral

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ecosystem is in balance with the host and the environment,<sup>9</sup> and thus the pathogenesis of CCUS has yet to be fully elucidated. Periodontal disease begins with the accumulation of salivary glycoproteins and bacteria (plaque). Subsequent gingival tissue damage and alveolar bone loss are due to inappropriate inflammation and host responses. Periodontal indices and full-mouth dental radiographs inform the stage of periodontal disease in both people and dogs.

The histopathologic analysis included examination of routine hematoxylin and eosin (HE)-stained slides as well as special stains for mast cells<sup>17,18</sup> (toluidine blue) and eosinophils (Luna's stain). Immunohistochemistry was performed to identify B-cell (CD79a+), T-cell (CD3+), and Forkhead-box protein 3 (FoxP3+) cell infiltrates.<sup>19,20</sup> The importance of T-cell diversity in the mucosal immune system of the oral cavity has been documented in humans.<sup>60</sup> A recent review details regulatory T cells and their role in animal diseases.<sup>58</sup> FoxP3 is known to be the key transcription factor controlling T regulatory cell (Treg) development and function.<sup>8</sup> Treg cells are thought to be critical for the regulation of the immune response and T-cell tolerance. Abnormalities in Treg number or function may contribute to the pathogenesis of CCUS as it does in canine inflammatory bowel disease.<sup>26,35</sup>

CD4+ Th17 cells play an important role in host defense against extracellular pathogens by mediating the recruitment of neutrophils and macrophages to infected tissues.<sup>53</sup> Interleukin-17 (IL-17) is a proinflammatory cytokine derived mainly from activated CD4+ Th17 cells and also from innate lymphoid cells, CD8+ T cells, neutrophils, monocytes, macrophages, mast cells, and vascular smooth muscle cells. Because of their potent role in inflammation, Th17 cells are implicated in a variety of autoimmune and immune-mediated diseases of people, including rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis, periodontitis, and oral lichen planus (OLP).<sup>17</sup>

This study prospectively documents the clinical, radiographic, and histopathologic changes, including immunohistochemistry and immunofluorescence, in dogs with CCUS with the goal of informing the pathogenesis. An improved understanding of this idiopathic disease in dogs may facilitate the characterization of a naturally occurring useful animal model for diseases with similar presentation in people, specifically OLP.

## Materials and Methods

### Animals and Clinical Assessment

Twenty dogs with clinically evident ulcerative stomatitis were prospectively enrolled in this study from the clinical caseloads of the first (J.G.A.) and second (S.P.) authors. An Institutional Animal Care and Use Committee protocol (#2013-0106) at Cornell University (CU) was approved for patients enrolled by S.P. Patients enrolled by J.G.A. followed the same procedures with verbal informed consent; diagnostic or treatment procedures were limited to the standard of care for patients enrolled in the study. Inclusion criteria included 1 or more erosions or ulcers in the buccal mucosal tissue opposite teeth

with plaque. Exclusion criteria included cases with known oral neoplasia or immune-mediated disease and the current use of immune-suppressing drug therapy.

A canine ulcerative stomatitis disease activity index (CUSDAI) (Suppl. Table S1) was developed based on a similar scoring system used in feline gingivostomatitis.<sup>33</sup> A CUSDAI score was assigned to each patient at the time of general anesthesia. Use of the CUSDAI allowed for an objective assessment of ulcer number, size, location, characteristics, and owner subjective scoring. The owner subjective score was based on fair attitude (score of 1), poor appetite and lethargy (2), or suffering (3). A pain score of mild (1), moderate (2), or severe (3) was also included. The possible range of the scoring system was 0 to 32. Clinical types of CCUS were noted based on the following criteria: ulceration/erosion (presence of well-defined erythematous ulcers), reticular/lichenoid (presence of lace-like white lesions on the oral mucosa), and ulcers with a pseudomembrane.

Full-mouth radiographs were obtained using computerized dental radiographic systems. Mild, moderate, and severe bone loss was recorded, as well as the pattern of bone loss (horizontal, vertical, or both). A comprehensive oral examination was performed, including periodontal probing using a 6-point sulcular check. Parameters/abnormalities recorded included gingival index,<sup>32</sup> clinical attachment loss (in millimeters), tooth mobility, stage of furcation involvement, loss of crown integrity, crown discoloration, plaque, and calculus index.

A periodontitis score was recorded for each tooth present in each patient based on clinical attachment loss as indicated by clinical and radiographic findings as follows: periodontal disease (PD) 1, no attachment loss; PD2, less than 25% loss of attachment; PD3, 25% to 50% attachment loss; and PD4, greater than 50% attachment loss. A generalized score for the entire mouth was then established based on clinical and radiographic findings: focal, only 1 tooth involved; localized, up to 30% of the teeth involved; and generalized, more than 30% of the teeth involved. The periodontal disease score for ulcers opposite teeth was also recorded.

### Histopathology and Immunohistochemistry

After a physical examination and under general anesthesia, an approximately 10-mm × 10-mm wedge of affected oral mucosa was harvested, fixed in 10% buffered formalin for a minimum of 48 hours, and bisected or trisected, and 5- $\mu$ m sections were routinely prepared. Histologic stains included HE, toluidine blue for mast cells, and Luna's stain for eosinophils. Immunohistochemistry (IHC) was performed on 4-micron thick, formalin-fixed, paraffin-embedded (FFPE) tissue sections for labeling CD3, CD79a, and FoxP3. Sections were deparaffinized, quenched with 0.3% hydrogen peroxide in methanol, and rehydrated in water. Antigen retrieval was performed (Dako Target Retrieval Solution, S1699; Dako, Glostrup, Denmark) for 30 minutes at 95°C, cooled for 20 minutes, rinsed in water, equilibrated with phosphate-buffered saline (PBS, 0.1 M, pH 7.4), and blocked in 10% normal horse serum in PBS for 20 minutes. The primary

antibodies were applied without rinsing and incubated for 1 hour. This and all subsequent incubations were performed in a humidity chamber at room temperature. Primary antibodies were as follows: rat anti-CD3 (clones 3–12, Dr P. Moore, UC Davis) at 1:10, mouse anti-CD79a (clone MH57, MCA2538 H; AbD Serotec, Raleigh, NC) at 1:100, and rat anti-FoxP3 (clone FJK-16 s, 14-5773-80; eBioScience, San Diego, CA) at 1:100. The antibody diluent and reagent rinses consisted of PBS–Tween 20 (0.02%). The secondary antibody was applied for 30 minutes (polymer–horseradish peroxidase [HRP] anti-mouse MC541 H or anti-rat BRR4016 H; BioCare Medical, Concord, CA), rinsed, and visualized with NovaRED for peroxidase (SK-4800; Vector, Burlingame, CA) per the manufacturer's instructions; counterstained in Gill's hematoxylin; and air-dried, and coverslips were applied. Nonspecific background was evaluated with duplicate sections receiving diluent in place of the primary antibody. A normal canine lymph node was used as the positive control tissue, which stained appropriately with FoxP3 IHC. As a positive control for IL-17 immunofluorescence staining, a canine reactive mesenteric lymph node biopsy was used.

HE, toluidine blue, Luna's stain, CD3 IHC, CD79a IHC, and FoxP3 IHC slides were examined by a single anatomic pathologist (B.M.). The semiquantitative scoring system was based upon published standards (*Principles for Valid Histopathologic Scoring in Research*).<sup>18</sup> For each submitted tissue sample, the pathologist was blinded to the signalment and clinical severity score. Semiquantitative ordinal scoring systems were developed for degree of overall inflammation, number of mast cells/200× microscopic field, number of eosinophils/200× field, number of FoxP3-expressing cells/200× microscopic field, percent surface area covered by CD3+ or CD79a+ lymphocytes in a 400× microscopic field, and finally, the number of IL-17+ cells/200× microscopic field. In addition, CD3+ and FoxP3+ cells were independently scored for both intraepithelial and subepithelial infiltrates. IL-17+ cells were concurrently evaluated for CD3 expression as well.

In addition to the overall severity of the inflammation, the presence or absence of the following lesional parameters was evaluated for the mucosal epithelium: saw-tooth rete ridge hyperplasia (RR), inflammation that obscures the epithelial-subepithelial interface (O), basal cell vacuolization (V), basal cell apoptosis (A), epithelial cell degeneration/spongiosis (D), epithelial erosion (EE), epithelial ulceration/necrosis (EU), dyskeratosis/hyperkeratosis (DH), pigmentary incontinence (PI), and/or necrosis of individual keratinocytes within the stratum spinosum (KN). Parameters noted in the subepithelium included subepithelial necrosis (N), granulation tissue/neovascularization (GN), lymphoid nodules (LN), perivascular inflammation (PV), suppurative infiltrate (S), and/or pseudo-membrane formation (P).

### Immunofluorescence

Samples were stained exactly as previously described.<sup>28</sup> Briefly, FFPE tissue sections were routinely deparaffinized in

xylene and serial ethanol dilutions, following heat-induced antigen retrieval (antigen retrieval buffer; Dako). Samples were further blocked with normal donkey serum and FcR Blocking Reagent (Miltenyi, San Diego, CA) and incubated overnight with rabbit anti-human CD3 polyclonal antibody (Dako) and goat anti-human IL-17 polyclonal antibody (R&D Systems, Minneapolis, MN). The next day, slides were extensively washed and treated with donkey anti-rabbit Alexa Fluor 488 and donkey anti-goat Alexa Fluor 555. Finally, nuclei were labeled with DAPI. Images were acquired with a super-resolution confocal microscope (Leica, Wetzlar, Germany) and analyzed using available software.

### Hematology, Serum Chemistry, and Serum IL-17

Blood was sampled for complete blood count (CBC), serum chemistry profile, and IL-17 enzyme-linked immunosorbent assay (ELISA). Serum samples, stored at –20°C, were assayed for canine IL-17 using a commercial assay, according to the manufacturer's instructions (Canine IL-17A DuoSet ELISA; R&D Systems).

### Statistical Analysis

The data were independently tested for a possible correlation between the CUSDAI score and each of the semiquantitative leukocyte scores by Spearman's rank correlation coefficient and the Jonckheere-Terpstra test. The Jonckheere-Terpstra test was used to evaluate the quantitative association between pairs of ordinally measured variables. Spearman's rank correlation coefficient ( $\rho$ ) was calculated to quantify the monotonic relationship between ordered variables. The null hypotheses of no association (i.e.,  $\rho = 0$ ) were tested using a level of significance of .05.

### Results

Most dogs (14/20) in the study population were male castrate and older than 9 years. Terrier breeds were overrepresented (6/20); 10 of 20 dogs weighed 10 kg or less, but large dogs (greater than 25 kg) were also represented (3/20). Oral ulcers (4/20), evidence of periodontitis (6/20), halitosis (2/20), and/or the specific diagnosis of CCUS (7/20) were the most common presenting complaints. Common clinical signs included those of periodontal disease (6/20), oral mucosal ulcers (11/20), and oral pain (16/20). Associated findings were mandibular lymphadenopathy (5/20) and osteomyelitis (1/20). Concurrent disease included a cardiac murmur (3/20), cardiac arrhythmia (1/20), and chronic dermatitis/atopy (5/20).

Standard clinical pathology evaluations were generally unremarkable aside from mild elevations in total protein (3/19) and globulins (4/19), leukopenia (2/19), and neutrophilia (2/19). Mild increases in alanine aminotransferase (2/19) and alkaline phosphatase were present in 2 patients, one of which was tapering off prednisone therapy (dog No. 1). In 3 patients with angular cheilitis or mucocutaneous lip ulceration, an



**Figures 1–4.** Chronic canine ulcerative stomatitis. **Figure 1.** Deep ulceration of the buccal mucosa (white arrow) apposed to the teeth. **Figure 2.** Erythematous lesions apposed to an edentulous area (white arrow). **Figure 3.** Ulcerative lesion with formation of a pseudomembrane (white arrow). **Figure 4.** A lesion with white striae apposed to the teeth (white arrow).

antinuclear antibody titer and serum levels for B12 and folate were also determined and were normal.

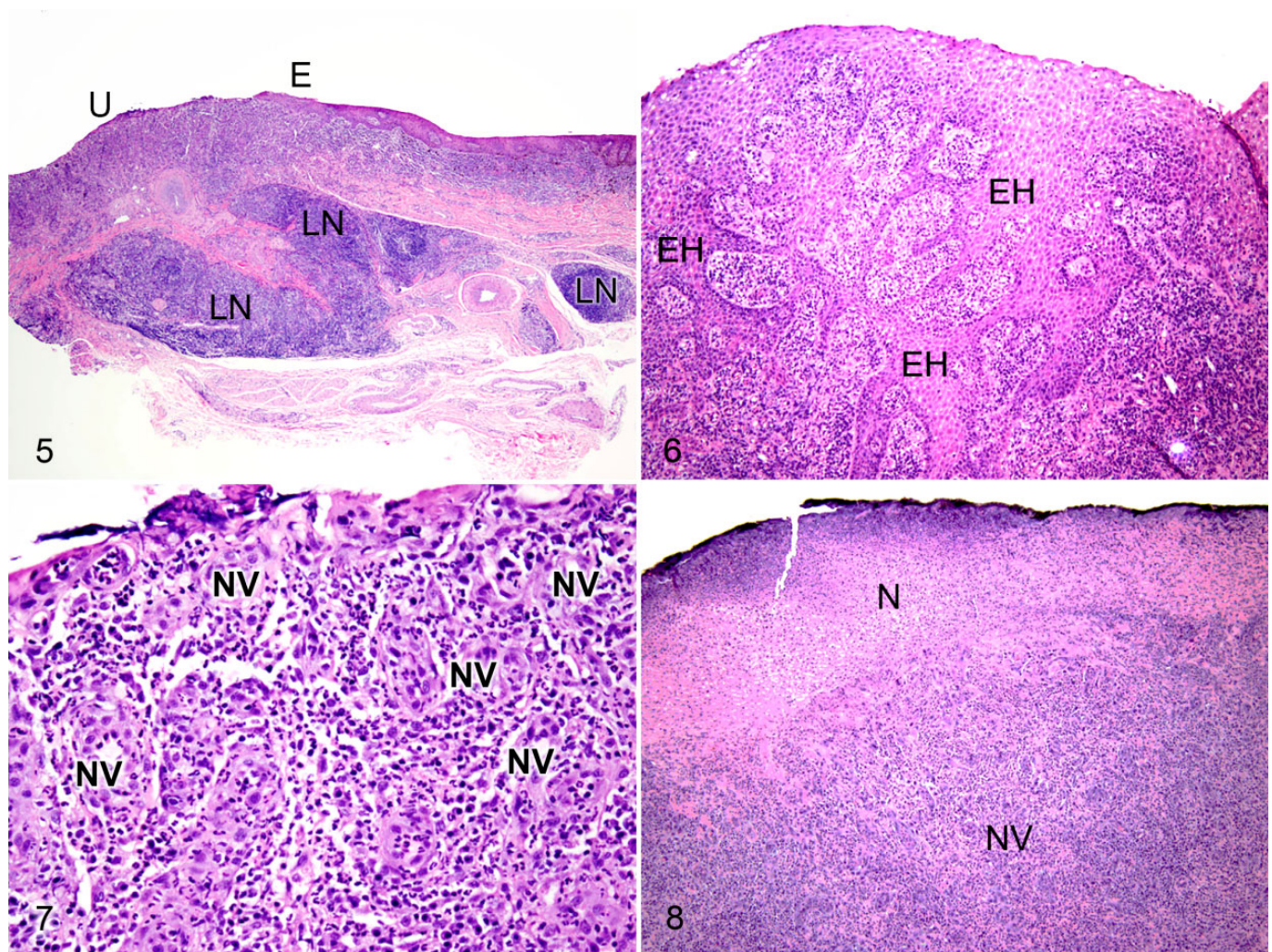
Oro dental findings in the patient population (Suppl. Table S2) included the CUSDAI score and ranged from 7 to 27, with an average score of 16 of 32. Pain scores were mild (4/20), moderate (7/20), and severe (9/20). Owner subjective scoring revealed that 11 of 20 had a fair attitude and were eating, 3 of 20 had poor appetite and lethargy, and 6 of 20 were thought to be suffering.

Clinical evaluation included a variable number and size of ulcers. The number of ulcers per case was less than 4 (3/20), 4 to 6 (5/20), more than 6 (6/20), and diffuse or generalized in 6 of 20. The size of ulcers was less than 8 mm in 8 of 20 cases and greater than 8 mm in 12 of 20 (including some large areas that were 24 to 36 mm or coalescing). Sixty percent of cases had more than 6 ulcers that were larger than 8 mm. Bilaterally symmetrical disease was almost exclusively seen. In all cases, teeth with plaque were present opposite the ulcers; however, in 8 of 20, there were ulcers opposite edentulous areas. The most common locations were the buccal or alveolar mucosa

opposite dentate areas (20/20); lesions were seen on the glosopalatine arch in 4 of 20 and various anatomic locations on the tongue in 5 of 20 (Figs. 1–4). The ulcers were usually erythematous and flat, although 3 of 20 had slightly raised margins or a small central area that was circular and raised. Pseudomembranous change was also identified in 10 of 20, and fine white reticulation (striae) was present in 5 of 20 (Suppl. Table S3).

Teeth associated with ulcers generally had heavy plaque and variable degrees of calculus and periodontitis. Overall periodontal disease scores revealed 8 of 20 with stage 2, 6 of 20 with stage 3, and 6 of 20 with stage 4. The overall extent of periodontitis was focal (3/20), localized (11/20), or generalized (5/20). The periodontal disease score of teeth contacting ulcers was as follows: PD2 (7/20), PD2–3 (1/20), PD3 (4/20), PD2–4 (4/20), PD3–4 (1/20), and PD4 (3/20).

Radiographically, all of the dogs' teeth that were associated with ulcers had mild, moderate, or severe alveolar bone loss that followed a horizontal, vertical, or combined pattern, with or without furcation involvement of multirooted teeth.



**Figures 5–8.** Chronic canine ulcerative stomatitis. Hematoxylin and eosin. **Figure 5.** Ulcerative stomatitis characterized by erosion (E), ulceration (U), subepithelial lichenoid band, and lymphoid nodules (LN) in the deep submucosa. **Figure 6.** Epithelial hyperplasia (EH) in stomatitis lesion. **Figure 7.** Neovascularization (NV) in the inflamed subepithelial propria-submucosa in an area of ulceration. **Figure 8.** Superficial necrosis (N) with neovascularization (NV) of the deeper lamina propria.

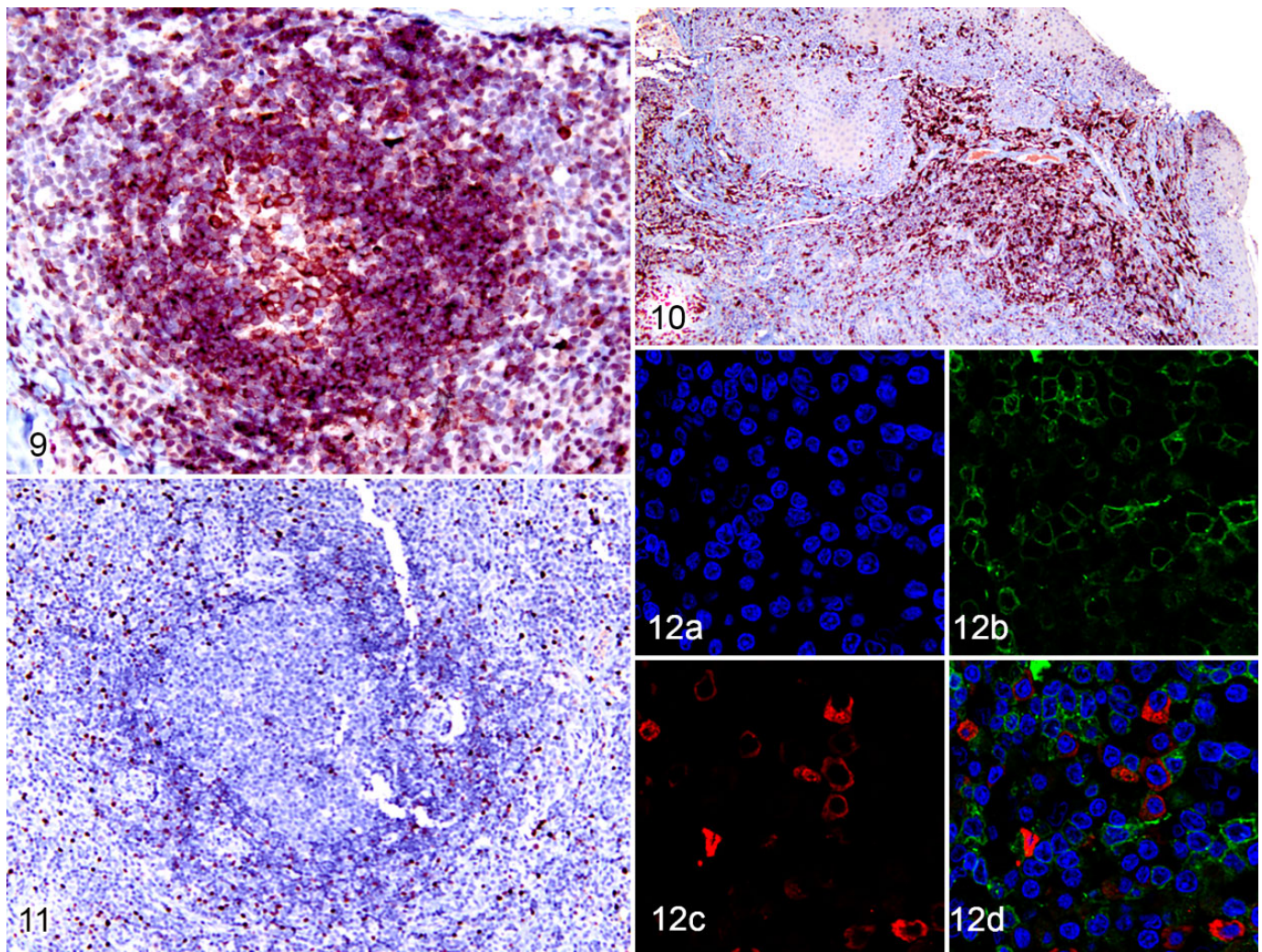
Radiographic findings paralleled clinical periodontitis scores. There were no radiographic findings that were unique to or pathognomonic for the disease process.

Buccal mucosal biopsies were collected in all patients and were characterized as buccal mucosal stomatitis with a variably pronounced subepithelial lichenoid plasmacytic-lymphocytic infiltrate with vascular proliferation and epithelial degeneration (Figs. 5–8). Histologically, the 4 cases with the most severe oral inflammation also had more epithelial changes in all categories. Saw tooth rete ridge hyperplasia was present in 13 of 20 cases. Inflammation obscured the epithelial-subepithelial interface in 11 of 20. Basal cell vacuolization was present in 9 of 20 cases and was uncommon in the less inflamed regions. Eleven of 20 cases revealed basal cell apoptosis, 5 of which also had concurrent vacuolization. Epithelial cell degeneration/spongiosis was a common feature in 13 of 20. Epithelial erosion and ulceration paralleled each other in 14 cases. Less commonly

observed features were individual keratinocyte necrosis in the stratum spinosum (3/20), pigment incontinence (2/20), and dyskeratosis in 1 case.

In the subepithelium, a subepithelial lichenoid plasmacytic-lymphocytic infiltrate was common to all cases. Granulomatous change, perivascular inflammation, and suppurative inflammation were common in 17 of 20 cases. Necrosis in the subepithelium was seen in 12 of 20 and pseudomembrane formation in 10 of 20. Lymphoid nodules in the subepithelium were uncommon (2/20). Subepithelial mast cells were abundant and were identified in most of the lesions while eosinophils were rarely identified.

Large numbers of CD3+ T cells infiltrated the subepithelium while smaller numbers penetrated the overlying mucosal epithelium. CD79a+ B cells were as common as CD3+ T cells in the subepithelium. The majority of patients (17/20) displayed FoxP3 staining of leukocytes in both the epithelium and subepithelium. IL-17+ cells were present in all cases



**Figures 9–11.** Chronic canine ulcerative stomatitis. Immunohistochemistry. **Figure 9.** Nodule of CD79a-positive cells (B cells). **Figure 10.** Immunohistochemistry for CD3. **Figure 11.** Immunolabeling of FoxP3 in the subepithelial lichenoid band. **Figure 12.** Chronic canine ulcerative stomatitis. Immunofluorescence. (a) DAPI nuclear stain (blue). (b) CD3 (green). (c) Interleukin-17 (IL-17) (red). (d) Merged image. IL-17-expressing cells are CD3 negative.

evaluated and absent in control tissues. However, the majority of IL-17+ cells were T cells that did not label for CD3 (Figs. 9–12). Histopathologic and immunohistochemical analysis of leukocyte infiltrates is presented in Supplemental Table S4. Additional detailed results are available in Supplemental Table S5.

Clinically normal oral mucosa revealed no histologic evidence of inflammation, with only rare mast cells and CD3+ T cells that were primarily present at the epithelium-subepithelium interface. Cells immunohistochemically positive for CD79a+ (B cells), FoxP3, and IL-17 were not identified in the normal oral mucosa.

No statistically significant correlation was identified between the CUSDAI score and any of the semiquantitative histologic leukocyte scores. All of the canine serum samples demonstrated undetectable levels of IL-17. The kit's stated limit of detection was 62.5 pg/ml.

## Discussion

As characterized in this report, canine ulcerative stomatitis most commonly affected neutered male dogs and animals less than 10 kg and terrier breeds. As has been previously reported, clinical pathology parameters have not been particularly helpful in determining the pathogenesis.<sup>7</sup> Systemic disease was uncommon in the study population, indicating that this was not a likely risk factor for development of ulcerative stomatitis. Intercurrent cutaneous lesions in 5 patients may suggest different subtypes of the canine disease, as documented in lichen planus of humans. Clinically, oral manifestations included several patterns—ulcers, erosions, white striae (lichenoid), and erosions with pseudomembranous change—whereas the historical literature refers only to ulcers opposite teeth with plaque.<sup>7,21,31</sup> A stomatitis disease activity index (CCUSDAI) objectively measured the clinical disease severity and response to treatment. However, there was no statistically significant

correlation between the scoring system and histologic or immunohistochemical parameters evaluated microscopically. Results of full-mouth dental radiographs, although important in confirming the presence and pattern of alveolar bone loss, did not reveal characteristics unique to this disease. Periodontal disease assessment revealed that localized stage 2 periodontitis (less than 25% loss of periodontal attachment) was most common.

CCUS is a histopathologically distinct disease process featuring a dense lichenoid lymphocytic-plasmacytic infiltrate at the interface between the mucosal epithelium and subepithelial connective tissue represented by CD79a+ B cells, plasma cells, CD3+ T cells, and Treg cells (interface mucositis). The histological features and phenotypes of inflammatory cells in the CCUS lesion substantially overlap with the human OLP lesion (basal cell vacuolization and apoptosis, lichenoid infiltrate of CD3+ T cells at the epithelial-subepithelial interface and in chronicity, saw-tooth rete ridge hyperplasia). The principal difference between CCUS and OLP appears to be the presence of large numbers of B cells in CCUS. These pathologic changes may result in a weakening of the epithelial-subepithelial interface, predisposing to lesion ulceration.

T cells, present in both the epithelium and subepithelium, are known to play an important role in mucosal immunity and tolerance,<sup>39,63</sup> and when alterations in number or function occur, severe oral mucosal disease may ensue. Tissue destruction in periodontitis is known to be associated with a decrease in local regulatory processes, including a decrease of FoxP3-positive regulatory lymphocytes.<sup>19</sup> FoxP3 expression was identified in the mucosa in the majority of our cases; however, in 6 patients, there was an absence of FoxP3+ regulatory T cells. Further studies are needed to determine if this absence may imply decreased immune inhibitory function.<sup>19</sup>

The oral mucosa is a cytokine-rich environment in both health and disease.<sup>29</sup> Many cell types, including epithelial cells, T cells, macrophages, dendritic cells, and mast cells, are capable of producing a wide array of cytokines, which then direct Th cell responses. This report documents the presence of the cytokine IL-17 in both the epithelium and subepithelium of the oral mucosa of dogs with stomatitis, although the majority of IL-17-expressing cells were CD3 negative (ie, IL-17 producing non-T cells). The phenotype of the IL-17-producing cells was not determined but may be innate lymphoid cells,<sup>2</sup> mast cells, or dendritic/histiocytic cells. Mast cells were generally located in areas away from (deep to) the intense lichenoid/interface inflammation, and their role in CCUS lesion pathogenesis is therefore unclear.<sup>15,27,50</sup> Eosinophils were essentially absent from the examined tissue sections; therefore, an allergic response appears to be unlikely. The finding of IL-17-producing non-T cells in tissue sections is consistent with prior reports of chronic idiopathic inflammatory lesions in dogs, where most of the IL-17+ cells were CD3 negative.<sup>28,43</sup> CD3+/IL17+ double-positive cells were present in less than 10% of CCUS tissue. Inflamed intestinal tract has also shown a low number of double-positive cells.<sup>23</sup> IL-17 secreted by immune cells in the mucosa is sensed by epithelial cells and stromal cells, leading to increased expression of various

chemotactic signals (such as CXCL8, MCP-1) and ongoing recruitment of neutrophils, monocytes, and T cells into the oral mucosa. The exact origin and defined role of IL-17 cells in CCUS lesions remain to be defined.

In the human literature, Tregs are the subset of T helper cells important in immune regulation and prevention of autoimmune diseases.<sup>36</sup>

Aberrancies in Treg numbers and function have been associated with autoimmune disease in both humans and mice. Similarly, a recent report documents decreases in FoxP3 numbers in canine inflammatory bowel disease.<sup>26,35</sup> It is suggested that decreases in Treg numbers may disrupt mucosal tolerance and lead to chronic inflammatory conditions such as inflammatory bowel disease and CCUS. Further studies are required to strengthen this speculation.

The interplay between proinflammatory IL-17/Th17 and anti-inflammatory Tregs in orchestrating various types of inflammation and autoimmune diseases is an active area of research. IL-17-producing cells have recently been found in tissues affected by chronic idiopathic inflammation in dogs, including inflammatory bowel disease, chronic gingivitis, chronic rhinitis, and chronic dermatoses.<sup>28</sup> Our data further indicate the presence of constitutively IL-17-producing non-T cells in CCUS lesions and suggest that these cells may participate in its pathogenesis.

The clinical and histopathologic findings in CCUS have similarities with human OLP, a chronic immune-mediated, mucocutaneous disease that affects the oral mucosa as well as the skin.<sup>23,40,57</sup> The disease most commonly affects middle-aged women<sup>20,57</sup> rather than males, as in the CCUS population. Six clinical types of OLP are reticular, erosive, ulcerative, atrophic, plaque-like, papular, and bullous.<sup>20,24</sup> Histologic characteristics of OLP in people, similar to CCUS in dogs, include a dense subepithelial lymphocytic infiltrate (lichenoid band), lymphocytic invasion of epithelium, saw-tooth appearance of rete ridges,<sup>12</sup> and hydropic degeneration of basal keratinocytes.<sup>6,20</sup> OLP is thought to result from immune responses in the skin or mucosa,<sup>34,46,55</sup> in which autoreactive CD8+ T cells trigger basal cell apoptosis.<sup>14,49,54</sup> Langerhans cells and macrophages are also involved. It is known that innate immune recognition of cells undergoing apoptosis, as a direct result of infection, is a physiological stimulus for Th17 cell differentiation.<sup>56</sup> Mast cells, also thought to play a role in pathogenesis,<sup>15,27,50,62</sup> are found in higher numbers in the deeper stromal tissue (as found in our dog study population) and often co-localize with IL-17+ cells.<sup>15</sup> CD4+CD25+ Foxp3+ regulatory T cells have been shown to be associated with OLP,<sup>30,44,51</sup> as has dysregulation of Th17 cells<sup>45,51</sup> and the presence of CD3-IL-17+ cells.<sup>15</sup> Furthermore, the disturbed balance between Foxp3+ cells and IL-17 cells in OLP suggests altered local immune regulation.<sup>15</sup>

## Conclusions

Canine chronic ulcerative stomatitis is a poorly understood oral mucosal disorder that is associated with significant morbidity.



Our data define a distinct clinical presentation of erosion to deep ulceration of the mucosa opposite plaque-retentive surfaces. Specific histopathologic morphology features an interface mucositis described as a dense lichenoid lymphocytic-plasmacytic infiltrate at the interface between the oral epithelium and subepithelial connective tissue represented by CD79a+ B cells, plasma cells, CD3+ T cells, and Treg cells. The histomorphology of human OLP and CCUS shares several key features, which suggest that CCUS may serve as a valuable naturally occurring disease model. Our data indicate tissue infiltration by FoxP3+ cells (ie, Treg cells) and constitutively IL-17-producing non-T cells. Further investigation of the role that these specialized cells may play in CCUS pathogenesis is needed.

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