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STANDARD ARTICLE

Bronchoalveolar lavage fluid lymphocytosis in 104 dogs (2006-2016)

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Abstract

Background: Bronchoalveolar lavage (BAL) fluid cytology and culture are used to characterize respiratory diseases in dogs. Little is known about disorders associated with increased numbers of lymphocytes in BAL fluid.

Objective: To evaluate duration of clinical signs and detection of specific respiratory diagnoses in dogs with BAL lymphocytosis.

Animals: One-hundred four client-owned dogs evaluated for respiratory signs.

Methods: Medical records of dogs that had >300 cells/ μ L and >20% lymphocytes on a differential cell count of BAL fluid between January 1, 2006, and January 1, 2016, were reviewed retrospectively. Cases were evaluated for the duration of clinical signs and respiratory diagnoses, including aspiration injury, infectious or inflammatory respiratory disease, and airway collapse.

Results: Dogs ranged in age from 0.5 to 16 years (median, 7.9 years) and had a median body weight of 11.4 kg (range, 2.0-42.7 kg). Eosinophilic lung disease was documented in 13 of 104 dogs (Group 1) and airway neutrophilia associated with infectious or inflammatory disease was found in 59 of 104 dogs (Group 2). Lymphocytosis alone in BAL fluid was described in 32 dogs (Group 3). Duration of cough did not differ among groups, but airway collapse was significantly more common in dogs with solitary lymphocytosis than in those with other types of inflammation.

Conclusions and Clinical Importance: Lymphocytosis in BAL fluid is common in dogs and, in many cases, likely represents a common response to airway injury, independent of the type or duration of insult. It is unknown whether airway collapse leads to lymphocytosis or if the inflammatory process causes airway collapse.

KEYWORDS

bronchitis, bronchomalacia, eosinophilic lung disease, infection

1 | INTRODUCTION

Bronchoalveolar lavage (BAL) fluid cytology and culture are used to characterize respiratory diseases in dogs and cats. In particular, variations in differential cell percentages in BAL fluid are used to diagnose

Abbreviations: BAL, bronchoalveolar lavage; TNCC, total nucleated cell count.

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inflammatory diseases such as eosinophilic lung disease and chronic bronchitis, and findings of degenerate neutrophils in BAL cytology along with neutrophils containing bacteria are used to make a diagnosis of pneumonia. Eosinophilic lung disease is a well-defined but poorly understood chronic respiratory disease associated with airway and pulmonary infiltration by eosinophils and consequent bronchiectasis or airway collapse.¹⁻⁴ Chronic bronchitis is typified by increased BAL fluid neutrophils in the absence of demonstrable intracellular bacteria and growth of pathogens in culture.^{5,6} It also can be associated with bronchiectasis or airway collapse.⁴ Therefore, both eosinophilic and neutrophilic inflammatory conditions are sometimes found concurrently with bronchomalacia. With the exception of pulmonary lymphoma,⁷ little is known about the clinical relevance or underlying disease processes associated with an increased percentage of lymphocytes in BAL fluid.

Interpretation of BAL fluid is hampered by variations in the collection and analytical technique used by individual clinicians and laboratories as well as inherent variability in BAL fluid cytology across multiple lung lobes in animals with respiratory disease. Multi-segment lavage resulted in different cytologic interpretations in 1 of 3 of dogs with diffuse disease and in almost half of the population of cats evaluated.^{8,9} Determining a final respiratory diagnosis requires interpretation of cytologic results in conjunction with clinical and radiographic findings.

In our hospital, BAL fluid lymphocytosis has been observed in dogs evaluated for various respiratory diseases, but little information is available in the literature to guide interpretation of this finding. Lymphocytic airway inflammation has been reported in dogs with airway collapse in the presence or absence of left atrial enlargement,^{10,11} although a separate study did not report BAL fluid lymphocytosis in any dog with bronchomalacia.¹² The purpose of our study was to identify dogs with unequivocal BAL fluid lymphocytosis and to determine underlying etiologic causes associated with lymphocytic airway inflammation. Based on our clinical experience, we hypothesized that dogs with BAL fluid lymphocytosis alone would have longer duration of cough and increased detection of bronchomalacia in comparison to dogs with concurrent eosinophilic or neutrophilic airway inflammation.

2 | MATERIALS AND METHODS

Medical records at the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis, were searched for all dogs that had bronchoscopy performed between January 1, 2006, and January 1, 2016. The endobronchial tree was evaluated in standard fashion, and site(s) for BAL were chosen by the endoscopist based on radiographic and bronchoscopic findings. The endoscope was withdrawn from the airway to irrigate the channel and wipe contaminating material from the exterior of the scope. The endoscope was wedged into the smallest airway possible followed by instillation and aspiration of warm, sterile saline through the biopsy channel of the endoscope. Lavage volume was based on the size of the dog, the outer diameter of the bronchoscope used, and the ability to wedge

the bronchoscope in the airway in a way that provided adequate return. A rough guideline of 1 mL/kg/lavage site as a single aliquot was utilized, and 1 to 4 sites were lavaged as clinically indicated. Lavages from distinct lobar sites were processed within 1 hour of collection and were examined separately for cytologic analysis by board-certified clinical pathologists. Total nucleated cell counts (TNCC) were performed on unfiltered BAL fluid using an automated cell counter (Advia 120; Siemens, Deerfield, Illinois) and reported as TNCC/ μ L. Slides were prepared for cytologic analysis by cytocentrifugation (Cytospin3; ThermoShandon, Pittsburgh, Pennsylvania) followed by Wright-Giemsa staining using an automated cell stainer (Model 7151 Wescor Aerospray Hematology Pro; ELITech Bio-Medical Systems, Logan, Utah). A differential cell count was based on an assessment of 200 cells.

Normal reference values used for canine BAL fluid differential cell counts in our laboratory are approximately 80% macrophages, 7% lymphocytes, 5% neutrophils, 6% eosinophils, 1% mast cells, and 1% epithelial cells in dogs.¹³ Samples with a TNCC <300 cells/ μ L were excluded from analysis to ensure that a representative sample of epithelial lining fluid had been obtained. For the purposes of our study, bronchoalveolar cytology reports were searched for dogs that had a lymphocyte differential count >20% from at least 1 lavage site, and all dogs were included in the study regardless of previous medication administered or coincident diseases other than neoplasia. Cases were further segregated into those that had concurrent airway eosinophilia (Group 1) defined as >14% eosinophils from at least 1 lavage site (representing >90th percentile for this cell type determined in a BAL study of dogs with histologically normal lungs)¹³ and dogs with airway neutrophilia >8% from at least 1 lavage site (Group 2), for comparison with dogs that had airway lymphocytosis in the absence of increases in other inflammatory cell types (Group 3).

A pooled sample of BAL fluid was plated onto 5% sheep blood agar and MacConkey's agar for isolation of aerobic organisms and onto pleuropneumonia-like organism base with thallium acetate (antifungal) and penicillin G (antibacterial) for *Mycoplasma* spp. isolation. At the discretion of the clinician, a pooled sample also was plated onto pre-reduced anaerobic *Brucella* plates (Anaerobe Systems, Morgan Hill, California) for anaerobic culture and onto inhibitory mold agar (Hardy Diagnostics, Santa Maria, California) for fungal culture. Microbial growth was assessed in a semi-quantitative fashion by counting the number of quadrants with growth and reported as 1+, 2+, 3+, or 4+. Standard biochemical methods were used to identify cultured bacteria.

Data abstracted from the medical record included age, breed, and sex, type and duration of clinical signs, and previous antimicrobial or glucocorticoid treatment within the past week. Underlying respiratory disease processes associated with airway lymphocytosis were diagnosed based on bronchoscopic findings and ancillary tests, including radiologic or tomographic findings, airway culture, fecal evaluations, serologic testing, and response to treatment. Dogs with neoplasia were excluded from the study. Specific respiratory diagnoses and the criteria used to assign the diagnosis are listed in Table 1.

TABLE 1 Clinical criteria used to establish individual respiratory diagnoses

Respiratory diagnosis	Criteria
Pneumonia	
Bacterial	Historical and clinical findings in conjunction with compatible radiographic changes and airway sepsis (intracellular bacteria observed cytologically or positive growth of potential pathogens on culture)
Fungal	Positive serology for coccidioidomycosis with compatible clinical and radiographic findings or identification of <i>Pneumocystis canis</i> cysts on BAL cytology or with PCR and sequencing
Foreign body	Bronchoscopic identification of foreign material
Eosinophilic lung disease	BAL eosinophils >14% Negative Baermann fecal examination or failure to respond to fenbendazole (50 mg/kg PO × 10 days) Therapeutic response to steroids
Chronic bronchitis	Cough duration >2 months BAL neutrophils >8% Lack of intracellular bacteria on cytologic assessment or no bacterial growth on culture
Lymphocytic inflammatory airway disease	BAL lymphocytes >20% with eosinophils <14% and neutrophils <8%
Airway collapse Tracheomalacia Tracheobronchomalacia Bronchomalacia	Bronchoscopy demonstrating >50% collapse of the airway lumen
Aspiration injury	History of antibiotic responsive pneumonia, middle or cranioventral pulmonary infiltrates, presence of risk factors for aspiration including laryngeal paresis or paralysis, esophageal disease, brachycephalic obstructive airway syndrome

Abbreviations: BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

2.1 | Statistical analysis

Data were assessed for normality using the D'Agostino & Pearson omnibus normality test. (GraphPad Prism version 5.0f) Normally distributed data are presented as mean ± SD, and statistical evaluation among groups was performed using analysis of variance followed by post hoc comparisons between groups using Tukey's test. Data with a non-Gaussian distribution are presented as median with range. Non-parametric data were assessed using the Mann-Whitney *U* test with Dunn's multiple comparisons for post hoc testing. The effect of age on TNCC and percent lymphocytes was assessed by linear regression.

A chi-square test was used to compare the frequency of airway infection, airway collapse, and aspiration injury among groups. For all analyses, significance was set at $P < .05$.

3 | RESULTS

Between January 1, 2006, and January 1, 2016, bronchoscopy with BAL was performed on 569 dogs. Lymphocytosis >20% was found in BAL fluid from at least 1 lavage site in 123 dogs. In 19 of 123 dogs (15%), TNCC was <300/μL, and those dogs were excluded from further analysis. Dogs with BAL fluid lymphocytosis ranged in age from 0.5 to 16 years (median, 7.9 years) and had a median body weight of 11.4 kg (range, 2.0-42.7 kg). The study population comprised 6 intact female dogs, 45 spayed females, 7 intact males, and 46 neutered male dogs. Cough was the presenting complaint in 91 of 104 dogs (88%) with duration between the onset of this sign and bronchoscopic evaluation ranging from 1 day to 8 years (median, 7 months). The remaining dogs were presented for combinations of exercise intolerance, wheezing, and tachypnea or excessive panting. Antimicrobials had been administered to 21 dogs, and 3 dogs had received glucocorticoids within the week before evaluation. Median BAL fluid lymphocyte percentage for all dogs was 25% (range, 20%-52%), and median TNCC was 900 cells/μL (range, 300-17 660 cells/μL).

Bronchoalveolar lavage fluid lymphocytosis with concurrent eosinophilia >14% was documented in 13 of 104 dogs (12%), assigned to Group 1. Concurrent airway neutrophilia >8% was recorded in 59 of 104 dogs (57%, Group 2), and lymphocytic inflammation alone was present in 32 of 104 dogs (31%) comprising Group 3. Age and sex did not differ among the 3 groups, but body weight was significantly higher in Group 1 dogs (24 kg; range, 5.4-40.4 kg) than in Group 2 (10.7 kg; range, 2-42.7 kg) and Group 3 (9.5 kg; range, 2.3-36.2 kg; $P = .03$; Table 2). Median TNCC/μL and median percentage of lymphocytes did not differ among groups. Total nucleated cell count and percent lymphocytes were not correlated with age ($P = .35$ and $.07$ respectively; data not shown).

In Group 1 dogs ($n = 13$), median BAL fluid eosinophilia was 30% (range, 15%-49%) with TNCC ranging from 400 to 5400/μL (median, 1030/μL). Lymphocytes in BAL fluid ranged from 20% to 36% (median, 25%). Concurrent BAL fluid neutrophilia >8% was present in 6/13 Group 1 dogs. Microbial cultures identified 1-3 colonies of bacteria in 4/13 dogs, in combination with rare *Aspergillus* colonies in 1 dog. No dog was treated with antimicrobial agents, and all dogs in Group 1 responded to treatment for eosinophilic lung disease. Aspiration injury was not suspected in any of the dogs in Group 1, but airway collapse was identified bronchoscopically in 2 of 13 dogs.

In Group 2 dogs ($n = 59$), BAL fluid neutrophilia ranged from 2% to 59% (median, 12%) and median TNCC in BAL fluid was 940 cells/μL (range, 300-17,660 cells/μL). Lymphocyte percentages in BAL fluid from Group 2 dogs ranged from 20% to 52% (median, 26%). Infectious lower respiratory tract disease was documented in 14 dogs as a result of bacterial infection ($n = 9$), coccidioidomycosis ($n = 2$), *Pneumocystis canis* ($n = 1$), and foreign body pneumonia ($n = 2$). *Bordetella*

TABLE 2 Demographic and bronchoalveolar lavage data in dogs with BAL fluid lymphocytosis that had concurrent BAL fluid eosinophilia (Group 1), neutrophilia (Group 2), and lymphocytosis alone (Group 3)

	Group 1 (n = 13)	Group 2 (n = 59)	Group 3 (n = 32)	P
Age (years)	7 (1-13)	8 (0.7-14)	7 (0.5-16)	.81
Weight (kg)	24 (5.4-40.4)*	10.7 (2.0-42.7)	9.5 (2.3-36.2)	.03
Male/female	4/9	29/30	13/19	NA
TNCC/ μ L	1060 (400-5400)	940 (300-17 660)	680 (320-2300)	.09
BAL fluid % lymphocytes	26 (20-36)	26 (20-52)	24 (20-43)	.25
BAL fluid % neutrophils	9.2 \pm 7.1	12 (2-59)	5 (1-8)	NA
BAL fluid % eosinophils	30 (15-49)	3 (0-14)	3 (0-14)	NA

Abbreviations: BAL, bronchoalveolar lavage; TNCC, total nucleated cell count.

*Indicates that the table entry is significantly different from others within the same row.

TABLE 3 Clinical features and diagnoses in dogs with BAL fluid lymphocytosis associated with eosinophilia (Group 1), neutrophilia (Group 2), and lymphocytosis alone (Group 3)

	Group 1 (n = 13)	Group 2 (n = 59)	Group 3 (n = 32)	P
Cough	11/13	50/59	30/32	NA
Cough duration before bronchoscopy (months)	9 (1-24)	4.5 (0.03-96)	9 (0.1-60)	.28
Antimicrobials within 1 week of bronchoscopy	1/13	14/59	6/32	.41
Glucocorticoids within 1 week of bronchoscopy	0/13	0/13	3/21	NA
Infection	0/13	14/59*	2/32	.02
Airway collapse	2/13	21/59	19/32*	.006
Aspiration injury	0/13	9/59	8/32	.56

Abbreviations: BAL, bronchoalveolar lavage; NA, not available because conditions for chi-square analysis have not been met (values <1).

*Indicates that the table entry is significantly different from others within the same row.

bronchiseptica was isolated from 4 of 9 dogs with lower respiratory tract infection in this group, as the sole pathogen in 2 dogs and in combination with mixed growth in 2 dogs. All dogs with bordetellosis were <1 year of age. Interstitial pneumonia and thromboembolic disease were suspected in 1 dog each. Chronic bronchitis with or without airway collapse was diagnosed in 23 dogs, and airway collapse alone was noted in 8 dogs. In total, airway collapse was documented bronchoscopically in 21 of 59 dogs. Aspiration injury was suspected in 9 dogs, and a diagnosis was undetermined in 3 dogs presented for exercise intolerance.

Lymphocytosis in BAL fluid >20% in the absence of other inflammation was documented in 32 of 104 dogs (31%) in Group 3. Age of affected dogs ranged from 0.5 to 16 years (median, 7 years) and median body weight was 9.5 kg (range, 2.3-36.2 kg), with 6 dogs <5 kg, 12 dogs 5-10 kg, 5 dogs 10-20 kg, and 9 dogs >20 kg. Cough was the presenting complaint in 30 of 32 dogs with duration ranging from 1 day to 5 years (median, 9 months) from the onset of clinical signs to bronchoscopic evaluation. Reverse sneezing and exercise intolerance were reported in the remaining 2 dogs. Median lymphocyte percentage was 24% (range, 20%-43%) and TNCC ranged from 320 to 2300 cells/ μ L (median, 680 cells/ μ L). Total nucleated cell count per microliter and the percentage of lymphocytes did not differ from that found in Group 1 and Group 2 dogs ($P = .09$ and $.18$, respectively). No intracellular bacteria were observed cytologically in any dog, but *Bordetella bronchiseptica* infection was confirmed in 2 dogs, both of which were <1 year of age.

Cough was the most prominent clinical finding in our study, and the duration of cough did not differ among groups. (Table 3) Airway collapse was identified in 19 of 32 (59%) of Group 3 dogs and was significantly more common than in Group 1 (2/13, 15%) and Group 2 dogs (21/59, 36%; $P = .006$). Aspiration injury was diagnosed clinically in a total of 17 dogs in Groups 2 and 3. Eight of these dogs, 2 of which also had bronchomalacia, were in Group 3, and the proportion of dogs diagnosed with aspiration injury did not differ from that in Group 2 dogs ($P = .27$). The number of dogs treated with antimicrobials or glucocorticoids did not differ among groups.

In 6 dogs from Group 3, a specific disease process accompanying airway lymphocytosis was not identified. These dogs ranged in age from 1 to 11.5 years (median, 3.2 years) and 5 of 6 dogs presented for cough, ranging in duration from 9 months to 5 years (median, 12 months). One dog presented for exercise intolerance and a specific etiology was not determined. In the 5 dogs with cough, 1 had primary ciliary dyskinesia diagnosed by scintigraphy and 3 dogs had concurrent inflammatory rhinitis. The TNCC in BAL fluid ranged from 440 to 1660/ μ L (median, 820/ μ L) with 20%-33% lymphocytes (median, 26%).

4 | DISCUSSION

Bronchoalveolar lavage fluid lymphocytosis >20% of the differential cell count was a common finding in dogs evaluated for respiratory disease during this 10-year period, affecting approximately 20% of dogs

undergoing bronchoscopy. We chose a cutoff of >20% lymphocytes to create an unequivocal definition of an abnormal increase. Although our laboratory uses <7% BAL fluid lymphocytes to define normal cytology,¹³⁻¹⁵ others have suggested that up to 14% lymphocytes can be identified in BAL fluid from normal dogs.¹⁶ A more strict definition of BAL fluid lymphocytosis might have required BAL fluid lymphocytosis from >1 lavage site. However, given the paucity of information on BAL fluid lymphocytosis, our study was designed to evaluate the largest cohort of dogs possible. Therefore, unequivocal BAL fluid lymphocytosis in at least 1 lavage sample was the selected criterion.

Our study identified a variety of disease processes associated with BAL fluid lymphocytosis in dogs, suggesting that lymphocytes could play a key role in the response to airway injury, regardless of the etiology of disease. In people, increased BAL fluid lymphocytes can be observed in asthma, sarcoidosis, infectious diseases such as human immunodeficiency virus and tuberculosis, lung neoplasia, bronchiolitis, and hypersensitivity lung diseases associated with eosinophilia.¹⁷ In horses, BAL fluid lymphocytosis also can be associated with various respiratory disease processes,¹⁸ likely because lymphocytes in the surface epithelium are involved in both protective immunity against infectious agents and immune responses to inhaled antigens. An unrecognized virus, pathogen, or irritant could have been responsible for BAL fluid lymphocytosis in some dogs examined here. The BAL fluid was markedly hypercellular in most cases in our study, which implies a concurrent increase in total macrophage numbers in airway fluid as well as lymphocytes. Macrophages also are responsible for identification and removal of intracellular pathogens and particulate antigens.¹⁹

Twelve percent of dogs with BAL fluid lymphocytosis had concurrent eosinophilia >14%. Increased lymphocytes are expected in dogs with eosinophilic lung disease, because cytokines produced by lymphocytes play a role in triggering IgE production and attracting eosinophils in Th2 hypersensitivity type disease. In humans with hypersensitivity pneumonitis, BAL fluid lymphocytosis was always present in the acute phase of disease (first 3-8 weeks)²⁰ although not always later in the course of disease. In a separate study of eosinophilic lung disease in 86 dogs, encompassing the same time frame as our study, BAL fluid lymphocytosis >20% was present in only 5 of 86 dogs.²¹ It is possible that lymphocytes are present only in the early stage of hypersensitivity-type lung disease in dogs and decrease with chronicity, although this possibility was not examined specifically here. Interestingly, in humans, hypersensitivity pneumonitis can result in BAL fluid lymphocytosis approaching 80% of the differential cytology, yet BAL fluid eosinophilia might be found in only half of these individuals.²⁰ This possibility has not been examined in dogs. In normal dogs, the CD4:CD8 ratio of airway T lymphocytes is 1.3,¹⁶ which is similar to that found in normal humans. Eosinophilic lung disease in dogs is associated with a dramatic increase in the BAL fluid lymphocyte CD4:CD8 ratio because of a large increase in CD4 cells,² consistent with the observation of concurrent increases in both BAL fluid eosinophils and lymphocytes in dogs with eosinophilic airway disease. T-cell subtypes alterations in BAL fluid were not examined here and have not been evaluated in other respiratory diseases of dogs.

Concurrent neutrophilic inflammation was the most common finding in dogs examined here, with bacterial infection identified in 9 of 59 dogs. In a study of bacterial pneumonia in dogs, BAL fluid lymphocytes approached 20% of the differential cell count.²² In experimental studies of humans, lipopolysaccharide inhalation caused a 100-fold increase in airway neutrophils but also increased lymphocytes 3-fold.²³ Therefore, although bacterial airway diseases are anticipated to result in neutrophilic inflammation, it is not unexpected to observe concurrent lymphocytosis in some instances. Th17 T-cells are a subset of CD4+ T helper cells, and a Th17 response can recruit neutrophils as a result of IL17 induction of IL-8 production.²⁴ Th17 cytokines can play a protective role against pathogens but also can induce pathology. Interleukin 17 recruits neutrophils in response to *Klebsiella* challenge²⁵ and *Streptococcus* infection²⁶ as well as fungal invasion. Th17 cytokines might serve as a bridge between innate and adaptive immune responses in the host defense against a variety of extracellular pathogens at mucosal surfaces.²⁷ However, many Group 2 dogs that had both neutrophilia and lymphocytosis in BAL fluid did not have a demonstrable infectious component of disease. This observation might suggest a host-driven Th17-dominant immune response to a variety of antigens, as has been suggested in both allergic skin disease and allergic lung disease in people, including asthma.²⁸ The discovery of new cytokines and T-cell subsets that play a role in shaping inflammatory responses has resulted in evolution of the classical Th-1/Th-2 paradigm. Interleukin 17 clearly plays a role in the induction and maintenance of hypersensitivity responses long considered as solely Th-1/Th-2-mediated disorders.²⁸

Aspiration injury was found in dogs with both neutrophilic and lymphocytic inflammation as well as solely lymphocytic inflammation in BAL fluid. Aspiration injury is thought to trigger a Th17 response as part of the immune response to inhalation of pathogens or irritant material,²⁹ and microaspiration has been proposed to play an important role in a number of respiratory diseases in dogs.^{30,31} In children, both strongly and weakly acidic chemical insults resulted in increased BAL fluid neutrophils and increased lymphocytes, indicating a non-specific response to airway injury.³² It is unclear why some dogs in our study developed mixed inflammation rather than purely lymphocytic inflammation, but it might reflect a disease process more similar to aspiration pneumonitis in some dogs versus the neutrophilic response of aspiration pneumonia in other dogs.

In the group of dogs examined here, a relatively large number (31%, Group 3) had isolated BAL fluid lymphocytosis. Airway collapse was common in this group of dogs and was found more often in Group 3 dogs than in dogs with other types of airway inflammation. It is possible that cartilage degeneration associated with airway collapse results in release of antigenic material, triggering lymphocytic inflammation. In dogs with intervertebral disk herniation, leakage of antigens from the nucleus pulposus is proposed to be the etiology of cerebrospinal fluid lymphocytic inflammation that is common in this disease.³³ In dogs with left atrial enlargement associated with myxomatous mitral valve disease and concurrent bronchomalacia, BAL fluid lymphocytosis was present in 4 of 10 cases.¹¹ However, in a guinea pig model of airway collapse induced by negative pressure applied to the

airway, the inflammatory response was neutrophilic and macrophagic.³⁴ In dogs with bronchomalacia, it is unknown whether collapse leads to inflammation or inflammation perpetuates collapse. As stated previously, BAL fluid was markedly hypercellular in most instances in our study, which implies a concurrent increase in total macrophage numbers in lymphocytic airway fluid. Increased BAL fluid lymphocytes and macrophages might represent a predominantly Th-1 type response to viral pathogens or local or inhaled antigens, with sequelae that include bronchomalacia and airway collapse.

Interestingly, 6 dogs with BAL fluid lymphocytosis in our study were diagnosed with *Bordetella* infection, 4 of which also had concurrent airway neutrophilia. All 6 dogs were <1 year of age. Although concurrent viral infection might explain lymphocytosis in these dogs, it has not been found previously in dogs with bordetellosis.²² Another explanation for the BAL fluid lymphocytosis in these 6 young dogs is physiologic lymphocytosis. No specific data are available on normal values for BAL fluid differential cytology of dogs <1 year of age. In healthy Beagle dogs, age affects BAL fluid lymphocyte percentages with younger (<5 years) and older (>8 years) animals having 5%-6% BAL fluid lymphocytes in comparison to 1% lymphocytes in dogs 5-8 years of age.¹⁵ Curiously, BAL fluid lymphocyte percentages increase with age in healthy humans and horses,^{18,35} with markedly increased BAL fluid lymphocytes in adult horses (46%) compared to foals (3%-5%).¹⁸ We found no effect of age on BAL fluid lymphocytes, but young dogs have higher blood lymphocyte counts than do older dogs. These varied findings suggest the need to define age-specific normal values and perhaps to investigate potential differences in dog breeds or type.

We hypothesized that dogs with BAL fluid lymphocytosis alone would have both longer duration of cough and increased detection of airway collapse in comparison to dog with concurrent eosinophilic or neutrophilic airway inflammation. Although the latter was true, duration of cough did not differ among groups of dogs with various types of airway inflammation. Our original hypothesis was partly based on the notion that acute inflammation often is associated with influx of neutrophils, whereas chronic inflammation more often is associated with lymphoplasmacytic infiltrates. In retrospect, this hypothesis was clearly naive. Lymphocytosis in BAL fluid can occur with acute airway inflammation,²⁰ and lymphocytes play a pivotal role in airway inflammatory responses in all phases and at all stages.

Prior administration of antimicrobial agents did not have an effect on BAL fluid lymphocytosis detected here because equal numbers of dogs in each group had received these drugs in the week before bronchoscopy. A relatively low number of dogs received glucocorticoids in the week before bronchoscopy, which also did not appear to play a role in the cytologic findings identified here. Interestingly, BAL fluid lymphocytosis was identified in 3 of 21 dogs despite corticosteroid use.

The retrospective nature of our study is a limitation to interpretation of results. None of the dogs had BAL fluid submitted for viral screening or assessment of novel pathogens that might have contributed to lymphocytosis. Our clinical pathology laboratory routinely performs differential cytology on a count of 200 cells; however, 1 study indicated that this methodology does not provide reproducible results

between observers for lymphocyte identification.³⁶ Finally, we elected to include all cases that had at least 1 BAL site with >20% lymphocytes. A prospective study should aim to collect 3 lavages sites to be able to confirm increased lymphocyte percentages in a majority of samples, and the differential cell count should be performed on 500 cells.

We have characterized BAL fluid lymphocytosis in a large cohort of dogs with respiratory disease and determined some disease associations. Further research is required, including defining a discrete population of affected dogs and determining the lymphocyte subsets and cytokine profiles associated with both BAL fluid lymphocytosis alone and BAL fluid lymphocytosis with concurrent eosinophilia and neutrophilia.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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