Cellular composition of bronchial brushings obtained from healthy dogs and dogs with chronic cough and cytologic composition of bronchoalveolar lavage fluid obtained from dogs with chronic cough

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Objective—To determine whether bronchial brushings from dogs with chronic cough have increased numbers of goblet cells and WBCs, compared with numbers for healthy dogs, or have differing WBC populations, compared with populations in bronchoalveolar lavage (BAL) fluid obtained from dogs with chronic cough.

Animals—9 healthy dogs and 10 dogs with chronic cough.

Procedure—Specimens were collected by use of bronchoscopy. Cellular composition was determined for brushings, and results from dogs with chronic cough were compared with those from healthy dogs. Cellular composition of brushings was compared with composition of BAL obtained from dogs with chronic cough.

Results—Brushings from healthy dogs contained a median of $2.9 \times 10^6$ epithelial cells, comprising 100% epithelial cells (96% ciliated, 3% goblet, and 1% other) and no WBCs. Brushings from dogs with chronic cough had $4.5 \times 10^6$ epithelial cells, comprising 93% epithelial cells (86% ciliated, 2% goblet, and 12% other). Dogs with chronic cough had significantly greater percentages of WBCs (7%) and neutrophils (6%), compared with values for healthy dogs. Five dogs with chronic cough had no neutrophilic inflammation evident in BAL, but 4 of these had evidence of neutrophilic inflammation in brushings.

Conclusions and Clinical Relevance—Neutrophils, but not goblet cells, were increased in brushings from dogs with chronic cough. Analysis of bronchial brushings provides information about airway inflammation that differs from that found by examination of BAL in some dogs with chronic cough and is a more sensitive indicator of airway inflammation than cytologic examination of BAL in these dogs. (Am J Vet Res 2006;67:160–167)

Tracheal washing and BAL are techniques commonly recommended for use in obtaining specimens for the evaluation of respiratory tract disease in dogs. Recommendations for specimen collection by bronchial brushing emphasize its use for the evaluation of focal bronchial lesions. In humans, cytologic evaluation of bronchial brushing of focal lesions is particularly useful for supporting a diagnosis of endobronchial neoplasia. However, evaluation of bronchial brushings may provide useful information regarding diseases associated with diffuse airway inflammation, resulting in information that differs from the information obtained by cytologic evaluation of BAL fluid. Bronchoalveolar lavage fluid represents processes involving the airway lumens, alveoli, and, in some instances, interstitium. Although bronchial brushing may yield a few cells from the airway lumen, brushings are not affected by alveolar or interstitial processes. More importantly, bronchial brushing may allow for the identification of inflammatory cells found solely within the airway mucosa or adherent to the airway epithelium, and bronchial brushing has been recommended for the phenotypic and functional study of epithelial cell populations of the airways.

Several studies in humans have revealed differences in inflammatory or epithelial cell populations in bronchial brushings between healthy people and patients with asthma, cystic fibrosis, and chronic bronchitis associated with smoking. Although the technique of bronchial brushing in dogs has been mentioned in some review articles, the authors are aware of only 1 study in which results of cytologic evaluation of bronchial brushings were reported for dogs with respiratory tract disease. The dogs in that report had eosinophilic bronchopneumopath, but results of cytologic evaluation of brushings and interpretations of results of examination of BAL fluid were combined, and expected results from brushings of unaffected dogs were not provided. Another report provides a general description of brushings obtained from clinically normal Beagles, but specific data are not provided.

Chronic bronchitis, a common cause of chronic cough in dogs, can be a challenging condition for...
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mucus production by use of molecular techniques. A
cytologic evaluation of BAL fluid, but not cytologic eval-

chronic bronchitis (ie, chronic cough).

Materials and Methods

Animals—Nineteen dogs were included in the study (9 healthy dogs and 10 dogs with chronic cough). Healthy dogs were obtained from Laboratory Animal Resources of North Carolina State University and comprised 4 Beagles, 4 mixed-breed dogs, and 1 Labrador Retriever. Exact age of each dog was not known, but none of the dogs was < 2 years old and the oldest dog with a documented age was 5 years old. Eight of the dogs were female (4 sexually intact and 4 of unknown status), and the male was sexually intact. Body weight ranged from 10 to 23 kg (mean ± SD, 16 ± 6 kg; median, 15 kg).

Dogs were considered healthy on the basis of assessment of the respiratory tract. Criteria included a medical history of no coughing or nasal discharge for at least the preceding 6 months during at least daily observation, anticipated results for physical examination and thoracic radiography, negative results for a heartworm antigen test, and negative results for fecal examinations to detect pulmonary parasites (floatation, sedimentation, and Baermann examinations). Arterial blood gas measurement was performed in 5 healthy dogs; PaO2 in all dogs was ≥ 80 mm Hg. Additional specimens were obtained from these dogs for use in other studies not reported here.

Dogs with cough were patients at the North Carolina State University Veterinary Teaching Hospital that underwent bronchoscopy for diagnostic purposes. This group comprised 2 Golden Retrievers, 2 mixed-breed dogs, 1 Cocker Spaniel, 1 Samoyed, 1 Standard Poodle, 1 Miniature Poodle, 1 Boxer, and 1 Jack Russell Terrier. Dogs were 1.5 to 12 years old (mean ± SD, 7 ± 4 years; median, 7 years). Seven of the dogs were spayed females, and 3 of the dogs were castrated males. Body weight ranged from 7 to 36 kg (mean, 21 ± 9 kg; median, 21 kg).

These dogs had a history of coughing for 2 or more months and no evidence of a specific underlying cause as determined on the basis of 1 or more diagnostic procedures used to assess the healthy dogs; thoracic radiography was conducted in all dogs with cough. Duration of coughing ranged from 2 to 84 months (mean ± SD, 20 ± 26 months; median, 9 months). Thoracic radiographs were considered unremarkable in 12 dogs, revealed a bronchial pattern in 4 dogs (all with a diffuse distribution [1 mild to normal in severity, 2 mild in severity, and 1 with bronchial mineralization and considered severe]), and revealed a bronchointerstitial pattern in 4 dogs (all with a diffuse distribution [1 mild in severity, 2 considered severe, and 1 not further characterized]). Heartworm antigen tests yielded negative results in all dogs. Five dogs had fecal examinations performed (floatation procedures), and all had negative results for parasites. Of these 3 dogs, 3 also had negative results for parasites when tested by use of sedimentation techniques and 2 had negative results for Baermann examinations. Arterial blood gas analysis was performed in 9 dogs with cough. The PaO2 ranged from 64 to 100 mm Hg (mean, 87 ± 12 mm Hg; median, 88 mm Hg).

Medical records of dogs with cough were reviewed for information on signalment, body weight, duration of coughing, results of the tests conducted, and final clinical diagnoses as determined on the basis of discharge summaries and subsequent communication logs. Results of bronchoscopy and cytologic evaluation of BAL fluid, but not cytologic evaluation of bronchial brushing, were available at the time the clinical diagnoses were made.

All procedures were approved by an institutional animal care and use committee. Signed informed consent was obtained from owners of the dogs with cough.

Bronchoscopy—The anesthetic protocol for all healthy dogs and most of the dogs with cough was medication with glycopyrrolate (0.01 mg/kg, IM) and hydromorphone (0.05 mg/kg, IM) followed by administration of propofol to achieve general anesthesia. Anesthetic protocols for client-owned dogs were adjusted to meet the needs of each patient. Bronchoscopy was performed by use of a 5.0-mm (outer diameter) flexible pediatric bronchoscope.

Uniform terms were used to describe bronchoscopic findings, prior to availability of any cytologic results. Subjectively assessed degrees of hyperemia and edema were used as gross indications of airway inflammation. Hyperemia was classified as normal, mild (light red), moderate (red), or severe (deep red). Edema was classified as normal, mild (blunting of bifurcations), moderate, or severe (airway occlusion). Airway secretions were classified as normal, mildly increased (strands), moderately increased (globs), or severely increased (occluding airways). Other abnormalities were also described.

Specimen collection—All specimens were collected through the biopsy channel of the bronchoscope. Brushings were obtained first, followed immediately by airway washings. Brushings were performed as part of another study in which airway epithelial cells were retrieved for the measurement of mucus production by use of molecular techniques. A 3.0-mm sheathed cytology brush was passed through the biopsy channel of the bronchoscope. The brush was extended past the end of the bronchoscope and out of the sheath, rubbed gently back and forth across the mucosal surface approximately 5 times, and pulled back into the sheath and removed from the bronchoscope. The brush was then reextented out of the sheath, briskly agitated in 2 to 10 mL of sterile cell culture media, and rinsed in saline solution. The procedure was repeated 8 to 10 times/dog by use of the same brush, with the material from each brushing pooled in the same vial of media. In each dog, half of the brushings were collected from the distal portion of
the trachea and half were collected in the main stem and lobar bronchi. Grossly excoriated areas or sites of visible secretions were avoided during specimen collection.

Airway washings were performed by lodging the bronchoscope in a bronchus. Sterile saline solution was instilled by syringe and immediately retrieved by applying suction with the same syringe. In the healthy dogs and in some dogs with cough, a single, low-volume bolus (15 to 25 mL) was instilled to maximize sample collection of bronchial secretions to meet the requirements of another study. For the purposes of the study reported here, this low-volume technique was considered a BW. In all dogs with cough, BAL was performed by use of more traditional volumes of 40 to 75 mL of sterile saline solution, which was divided into 2 or more boluses. None of the healthy dogs underwent BAL. Specimens were obtained by use of BW from 2 lobes of each healthy dog. Specimens were obtained by use of BAL or BW/BAL from 1 to 4 lobes of dogs with cough.

Processing of specimens—Total epithelial cell counts were performed on bronchial brushings to determine epithelial cell yield, and total nucleated cell concentrations were determined for BW and BAL fluid. Counts were performed by use of a hemacytometer. Bronchial brushing specimens were drawn into and ejected from a pipette tip several times to disrupt rafts and clumps of cells. Trypan blue dye exclusion was used to determine viability of airway epithelium obtained by brushing. Slides of bronchial brushings and BAL and BW fluid were prepared by use of a cytocentrifuge technique (18 \( \times \) g for 3 minutes for brushing specimens and 64 \( \times \) g for 5 minutes for BAL and BW specimens) and stained with Wright-Giemsa for determination of differential cell counts.

A single investigator (ECH) evaluated all slides to ensure consistency in comparisons. Slides were randomly numbered to prevent bias in interpretation. A total of 300 cells was counted on each slide. For bronchial brushings, clumps and rafts of epithelial cells were excluded because of the difficulty in determining cell types, and unidentifiable cells (eg, as a result of disruption) were also excluded. Differential cell counts were reported as percentage of total nucleated cells. Differential cell counts were conducted for epithelial cells and WBCs; additional differential cell counts were obtained for ciliated epithelial cells, goblet cells, basal epithelial cells, neutrophils, eosinophils, basophils, mononuclear cells, and mast cells. Degree of RBC contamination was assessed by use of a scale (rare, none to a few RBCs detected per high-power field; moderate, 5 to 25 RBCs/200 \( \times \) field; marked, > 25 RBCs/400 \( \times \) field).

Definitions of inflammatory response—Criteria were used for describing an inflammatory response in BAL and BW fluid. It was considered inflammation when the percentage of neutrophils was \( \geq 12\% \) of nucleated cells or the percentage of eosinophils was \( \geq 14\% \) of nucleated cells.\(^1\)\(^2\) These values represented the 90th percentile for BAL fluid collected from 30 histologically normal lung lobes of 5 dogs by use of the same BAL technique described here. To reflect how such results would be interpreted clinically, it was considered inflammation when the percentage of neutrophils or eosinophils from any lobe in a specific dog met these criteria.

We are not aware of any published criteria on which to base a definition of an inflammatory response in bronchial brushings. For the purposes of the study reported here, neutrophilic and eosinophilic inflammation in bronchial brushings were defined as values \( \geq 90\% \) for the percentage of neutrophils or eosinophils from the 10 healthy dogs, which was consistent with the definition used for cytologic evaluation of BAL fluid. However, the resulting values were subjectively too low for clinical relevance (0.6% of nucleated cells for neutrophils and eosinophils); thus, the definition to indicate inflammation was changed to any value greater than the highest percentage of neutrophils or eosinophils obtained from any healthy dog. Therefore, neutrophilic and eosinophilic inflammations were defined as > 1% neutrophils and > 1% eosinophils in bronchial brushings, respectively.

Statistical analysis—Descriptive statistics were reported as the median and range (minimum and maximum values). Nonparametric analyses were performed because of the small data sets and because several data sets were not distributed normally. Comparisons of results between groups were performed by use of the Mann-Whitney rank sum test for unpaired data and signed rank test for paired data. To evaluate correlations, the Spearman rank correlation was performed. Values of \( P < 0.05 \) were considered significant. Commercially available software \(^1\) was used for analyses. For dogs in which > 1 BW or BAL procedure was performed, differential cell counts from each lobe were used to calculate a mean value before performance of statistical descriptions or analyses.

Results

Results for bronchial brushing and BW in healthy dogs—Total epithelial cells, epithelial cell viability, and cellular composition of bronchial brushings were recorded (Table 1). Nearly all of the cells were epithelial. Most cells were ciliated columnar epithelium (median, 96% of nucleated cells), with the remainder being predominantly goblet cells (median, 3%). There were few basal cells, but the decision to avoid clumps and rafts of cells combined with the inability to confidently identify these epithelial cells separately from clumps also contributed to the lower number. White blood cell counts ranged from 0% to 2% of all nucleated cells. Red blood cell contamination was considered rare in 5 dogs, moderate in 2, and marked in 2. There was no obvious relationship between percentage of neutrophils and RBC contamination, with all brushings having only 0% to 1% neutrophils.

In all healthy dogs, BW was performed in 2 lobes (25 mL/lobe). Recovery volume was recorded for 5 of the dogs, resulting in a median recovery of 50% (range, 40% to 72%). Most of the cells were mononuclear (Table 2). One dog had neutrophilic inflammation, 2 had eosinophilic inflammation, and 1 had both neutrophilic and eosinophilic inflammation. However,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy dogs</th>
<th>Dogs with chronic cough</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells ( \times 10^9 )</td>
<td>2.9 (2.2–13.0)</td>
<td>4.5 (1.7–5.9)</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>87 (77–90)</td>
<td>88 (80–98)</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>100 (98–100)</td>
<td>92 (15–100)</td>
</tr>
<tr>
<td>Ciliated epithelial cells (%)</td>
<td>96 (91–99)</td>
<td>86 (14–100)</td>
</tr>
<tr>
<td>Goblet cells (%)</td>
<td>3 (1–7)</td>
<td>2 (0–16)</td>
</tr>
<tr>
<td>WBCs (%)</td>
<td>0 (0–2)</td>
<td>7 (0–85)</td>
</tr>
<tr>
<td>Mononuclear cells (%)</td>
<td>0 (0–2)</td>
<td>0 (0–7)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>0 (0–1)</td>
<td>6 (0–65)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0 (0–1)</td>
<td>1 (0–7)</td>
</tr>
<tr>
<td>Mast cells (%)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
</tr>
</tbody>
</table>

Percentages reported represent the percentage of nucleated cells. \( * \) Values differ significantly \( P < 0.001 \) and \( \dagger P = 0.005 \); Mann-Whitney rank sum test) between healthy dogs and dogs with chronic cough.

### Table 1—Median (range) values for cytologic examination of bronchial brushings obtained from 9 healthy dogs and 10 dogs with chronic cough.
neutrophils and eosinophils were ≤ 16% of nucleated cells in all lobes.

Results for bronchial brushing in dogs with cough—Total epithelial cells, epithelial cell viability, and cellular composition of bronchial brushings were recorded (Table 1). The percentage of epithelial cells was significantly (P < 0.001) less and the percentage of WBCs significantly (P < 0.001) greater than those for healthy dogs. For the WBCs, only the percentage of neutrophils was significantly (P = 0.005) different between healthy dogs and those with cough. There was no difference for total epithelial cells or cell viability between dogs with cough and healthy dogs. Similar to results for the healthy dogs, few basal cells were found. Dogs with cough did not have significantly greater percentages of goblet cells than healthy dogs, whether considered as a percentage of total nucleated cells or of total epithelial cells. However, 3 dogs with cough had more goblet cells than were found in healthy dogs, whether goblet cells were considered as a percentage of total nucleated cells (maximum in healthy dogs was 7%, whereas it was 9%, 11%, and 16% in the 3 dogs with cough) or as a percentage of epithelial cells (maximum in healthy dogs was 7%, whereas it was 10%, 16%, and 17% in the 3 dogs with cough).

Red blood cell contamination was considered to be rare in 7 dogs, moderate in 1, and marked in 2. There was no obvious relationship between the percentage of neutrophils and RBC contamination. The brushings with rare RBCs had a median of 7% neutrophils (range, 0% to 65%), the brushing with moderate RBCs had 2% neutrophils, and the 2 brushings with marked RBCs had 4% and 13% neutrophils, respectively. The peripheral neutrophil concentrations in the 2 dogs with marked RBCs were within the reference range (8,200 and 7,537 RBCs/µL, respectively).

Comparison of results for bronchial brushing and BAL fluid in dogs with cough—All 9 dogs with cough underwent BAL (Table 2). Three lobes were lavaged in 1 dog, 2 lobes were lavaged in 4 dogs, and 1 lobe was lavaged in 5 dogs. Median instilled volume was 50 mL (range, 45 to 75 mL), and median recovery was 43% (range, 22% to 66%). The percentage of eosinophils was significantly (P = 0.004) higher in BAL fluid than in bronchial brushings. No difference was seen for neutrophils. No correlation was found for percentage of neutrophils or eosinophils (Spearman rank correlation coefficient) between bronchial brushings and BAL fluid (Figures 1 and 2).

Inflammatory responses identified by cytologic evaluation of BAL fluid and bronchial brushings were compared to determine whether these techniques provided information that differed for specific dogs. Although an increased percentage of neutrophils or eosinophils for any lobe was sufficient for a dog to be considered as having inflammatory BAL fluid, in only 1 dog did this scrutiny of separate lobes result in an interpretation of inflammatory response that differed from the interpretation that would have resulted had the mean count from BAL fluid obtained from all lobes been used. In that dog, the eosinophils from 2 separate lobes were 7% and 19% of nucleated cells, resulting in a mean of 13%.

Neutrophilic inflammation was detected in BAL fluid obtained from 5 dogs. Cytologic evaluation of bronchial brushings failed to reveal neutrophilic inflammation in 1 of these dogs (neutrophils in bronchial brushings represented 0%, 4%, 10%, and 65% of nucleated cells, respectively). Neutrophilic inflammation was not detected in BAL fluid obtained from 5 dogs. Neutrophils in BAL fluid obtained from all lobes in 3 dogs comprised ≤ 8% of nucleated cells. Cytologic evaluation of bronchial brushings revealed neutrophilic inflammation in 4 of these dogs (neutrophils in bronchial brushings represented 0%, 2%, 7%, 13%, and 16% of nucleated cells, respectively).

Eosinophilic inflammation was detected in BAL fluid obtained from 4 dogs, 2 of which also had neutrophilic inflammation. Cytologic evaluation of bronchial brushings failed to document eosinophilic inflammation in 1 of these dogs (eosinophils in bronchial brushings represented 0%, 3%, 9%, and 17% of nucleated cells). Eosinophilic inflammation was not detected in BAL fluid obtained from 6 dogs. None of these dogs had eosinophilic inflammation in bronchial brushings (eosinophils in bronchial brushings represented 0% of nucleated cells in 3 dogs and 1% of nucleated cells in the other 3 dogs).

Comparison of results for bronchial brushing and BW in dogs with cough—Four dogs with cough underwent a BW. Fluid was instilled in 2 lobes in 2 dogs and 1 lobe in 2 dogs. A bolus of 25 mL was used, except in 1 dog in which only 15 mL was instilled. The median recovery was 44% (range, 40% to 56%).

### Table 2—Median (range) values for cytologic examination of fluids obtained by use of BW and BAL in healthy dogs and dogs with chronic cough.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy dogs</th>
<th>Dogs with chronic cough</th>
<th>Dogs with chronic cough</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dogs</td>
<td>9</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Total nucleated cell count (No./µL)</td>
<td>56 (25–143)</td>
<td>94 (23–163)</td>
<td>233 (50–3,030)</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>5 (0–25)</td>
<td>4 (0–28)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>WBCs (%)</td>
<td>94 (0–97)</td>
<td>96 (72–100)</td>
<td>99 (97–100)</td>
</tr>
<tr>
<td>Mononuclear cells (%)</td>
<td>81 (67–89)</td>
<td>70 (55–86)</td>
<td>71 (28–95)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>7 (1–14)</td>
<td>12 (6–38)</td>
<td>13 (3–43)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>6 (1–11)</td>
<td>4 (2–5)</td>
<td>12 (1–65)</td>
</tr>
<tr>
<td>Mast cells (%)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
</tr>
</tbody>
</table>

Percentages reported represent the percentage of nucleated cells.
For the 4 dogs, 2 had neutrophilic inflammation in the BW. These 2 dogs also had neutrophilic inflammation on the basis of cytologic evaluation of fluid obtained during a BAL. Both of these dogs had neutrophilic inflammation in bronchial brushings (neutrophils, 4% and 10% of nucleated cells, respectively). Neutrophilic inflammation was not detected in BW fluid in 2 dogs, both of which had neutrophilic inflammation on the basis of cytologic evaluation of fluid obtained during a BAL. Neutrophilic inflammation was found in bronchial brushings from these 2 dogs (neutrophils, 4% and 65% of nucleated cells, respectively).

Eosinophilic inflammation was not detected in BW fluid obtained from any of the 4 dogs, although 2 dogs had eosinophilic inflammation on the basis of cytologic evaluation of fluid obtained during BAL. Eosinophilic inflammation was detected in 1 of these dogs in bronchial brushings (eosinophils, 0%, 0%, 1%, and 17% of nucleated cells, respectively).

Comparison of results for bronchial brushing and endoscopic findings in dogs with cough—We did not detect an apparent association between results for the bronchoscopic assessment of airway secretions and percentage of goblet cells in dogs with cough. Airway secretions were severely increased in 1 dog and moderately increased in another dog, both of which had only 1% goblet cells. Airway secretions in the remaining dogs were normal to mildly increased, with goblet cells ranging from 0% to 16% of nucleated cells.

We did not detect an obvious association between results of bronchoscopic assessment of hyperemia and percentage of WBCs, although the 1 dog with severe hyperemia had the most WBCs (85% of nucleated cells). Hyperemia was assessed as normal in 2 dogs that had 1% and 12% WBCs, respectively; mild in 5 dogs with WBCs ranging from 0% to 32% of nucleated cells; and moderate in 2 dogs that had 5% and 8% WBCs, respectively. Furthermore, there was not an obvious association between results of bronchoscopic assessment of edema and WBCs, although the 1 dog with severe edema also had the most WBCs (85% of nucleated cells). Edema was assessed as normal in 4 dogs with WBCs ranging from 0% to 12% of nucleated cells, mild in 3 dogs with WBCs ranging from 4% to 32% of nucleated cells, and moderate in 2 dogs with WBCs representing 8% and 14% of nucleated cells.

Comparison of results for bronchial brushing with clinical diagnosis—Final clinical diagnoses related to cough obtained from the medical records included chronic bronchitis for 9 dogs, and a definitive diagnosis was not determined for 1 dog. Of the 9 dogs with chronic bronchitis, 1 also had recurrent pneumonia, 1 also had tracheitis, and 1 dog also had tracheal collapse and left atrial enlargement. One of the dogs was considered to have eosinophilic chronic bronchitis. Eight of the 9 dogs with a clinical diagnosis that included chronic bronchitis had neutrophils, eosinophils, or both that represented ≥3% of nucleated cells in bronchial brushings, whereas the remaining dog had unremarkable results for cytologic evaluation of bronchial brushings (100% ciliated columnar epithelial cells). The 1 dog with cough in which a definitive diagnosis was not determined also had unremarkable results for cytologic evaluation of bronchial brushings (97% ciliated columnar epithelial cells, 2% goblet cells, and 1% WBCs).

Discussion

Obtaining a diagnosis for dogs with chronic cough can be difficult. The diagnosis of chronic bronchitis is made when there is a lack of other specific diseases and is thus a diagnosis of exclusion.24 However, it may be difficult to conclusively determine the contribution of other potential disorders, such as gastroesophageal reflux or left atrial enlargement, to chronic coughing. In the study reported here, we examined the role that determination of cellular composition of bronchial brushings may provide as objective evidence for bronchial inflammation in dogs with chronic cough.
Analysis of results of the study reported here indicated that dogs with chronic cough had increased percentages of WBCs and neutrophils in bronchial brushings, compared with results for clinically normal dogs. Furthermore, most dogs with cough that did not have neutrophilic inflammation in BAL fluid did have neutrophilic inflammation in bronchial brushings. Therefore, in some dogs with cough, cytologic evaluation of bronchial brushings is a more sensitive indicator of bronchial inflammation than is cytologic evaluation of BAL fluid. Mucosal infiltration of neutrophils is consistent with histologic findings in dogs with naturally developing chronic bronchitis, although neutrophils in bronchial brushings were not increased in several studies in which investigators compared people with chronic bronchitis and healthy people.

We expected to find an increased number of goblet cells in bronchial brushings obtained from dogs with chronic cough, compared with results for healthy dogs. This finding would have provided additional information beyond that obtained by cytologic evaluation of BAL fluid because epithelial cells are found in low numbers in airway washings. Increased numbers of goblet cells in specimens have been reported in dogs with naturally developing chronic bronchitis and in some dogs with experimentally induced bronchitis. A study in which investigators evaluated bronchial brushings obtained from people with chronic bronchitis found an increased percentage of goblet cells (mean, 20%), compared with results for healthy nonsmokers (mean, 9%). Although there was no significant difference in percentages of goblet cells between healthy dogs and dogs with cough, 3 of the dogs with cough did have more goblet cells than the highest value obtained for healthy dogs. These findings could have reflected differences in disease pathogenesis among dogs or a lack of a sufficient number of subjects to detect a difference between the populations. Biopsy specimens were not available to confirm goblet cell hyperplasia for any of the dogs.

The failure to find increased numbers of goblet cells in bronchial brushings obtained from dogs with cough, compared with results for healthy dogs, could simply indicate that goblet cells are not increased in bronchial brushings from dogs with chronic bronchitis for the methods used in our study. In a study in humans, investigators did not find a difference in number of goblet cells in bronchial brushings between people with chronic bronchitis and healthy people. In 2 studies of dogs with experimentally induced chronic bronchitis caused by exposure to sulfur dioxide, the number of goblet cells was not uniformly increased as determined on the basis of histologic examinations. In one of those studies, the number of goblet cells was decreased. In the other study, goblet cells could not be identified in the proximal portion of the trachea and lobar bronchi, were decreased in number in the proximal segmental bronchi, and were considerably increased in number in the distal segmental bronchi and bronchioles (distal to the site of brushing in the study reported here). On the basis of some pathologic descriptions, distribution of goblet cell hyperplasia can be patchy and located primarily in epithelial folds in dogs with bronchitis.

Collection of bronchial brushings increases the cost and duration of bronchoscopy; thus, it is not without consequence. The brushings collected in the study reported here were used for the measurement of specific mucin mRNA, and large cell numbers were required. When only cytologic analysis is desired, sufficient cells would likely be collected from a single brushing. In a study of humans with cystic fibrosis, a single brushing yielded 1.7 X 10^5 cells. Bronchial brushing is tolerated well in humans, including those with asthma, cystic fibrosis, and chronic bronchitis. Rather than suspending cells in fluid and then preparing slides by use of cytocentrifugation, cells can simply be smeared directly from the brush onto a glass slide because differential cell counts do not differ significantly between the methods.

The small number of subjects and lack of a criterion-referenced standard (such as histologic examination) for defining the airway inflammatory response and providing a definitive diagnosis limit the conclusions that can be drawn from the study reported here. For our study, neutrophils or eosinophils comprising > 1% of nucleated cells were considered indicative of inflammation because none of the healthy dogs had higher counts. It is quite possible that greater variability among healthy dogs would have been seen had samples been obtained from a larger population. We recommend including clinical judgment when interpreting the inflammatory response for bronchial brushings obtained from a specific patient, which includes putting greater importance on higher numbers of inflammatory cells.

Another confounding factor for the study reported here was the wide variability in cell populations in BAL fluid obtained from apparently healthy dogs. For this study, the criteria used to describe the inflammatory response in BAL fluid were determined by use of results for a small number of dogs confirmed to be healthy on the basis of histologic evaluation of the lungs. Had lower values been used to determine neutrophilic inflammation, the findings of this study would not have changed. Neutrophils were ≤ 8% of nucleated cells in BAL fluid obtained from every lobe lavaged of the 5 dogs with cough identified as not having neutrophilic inflammation in BAL fluid. Two of the dogs considered to be healthy had > 12% neutrophils in BW fluid. Reference values were not available for performing a single, relatively small-volume lavage in dogs; thus, criteria for cytologic evaluation of BAL fluid were used to describe BW results in dogs with cough. However, it is not surprising that the number of neutrophils would be higher in BW fluid, compared with values expected for BAL fluid obtained from healthy dogs. In humans, fluid recovered after instillation of the first bolus during BAL has an increase in the numbers of neutrophils, compared with values for subsequent boluses of BAL fluid. The same result has been found in dogs and cats. Healthy dogs in the study reported here did not have pathologic confirmation of their status.

To our knowledge, the study reported here is the first to provide a detailed description of the cellular composition of bronchial brushings obtained from...
healthy dogs and dogs with chronic cough. A general
description of brushings obtained from clinically
normal Beagles is provided elsewhere. Findings for
that study were similar to those for our study, with the
predominant cell being implicated epithelial cells and inflam-
matory cells of any kind being relatively sparse when
there was a lack of hemorrhage at the site of brushing. Expected findings from bronchial brushings in healthy
dogs have been described in review articles but
without reference to original data. A single clinical study was
conducted in which investigators reported
the cellular composition of bronchial brushings
obtained from dogs with eosinophilic bronchopeu-
mopathy. The authors of that study concluded that
cytologic evaluation of bronchial brushings provided
useful results about bronchial processes. Those authors assigned grades for inflammation in
bronchial brushings and BAL preparations on the basis
of the percentage of eosinophils found in microscopic
fields, with specific targeting to have maximal numbers
of eosinophils (0, no eosinophils; 1, 1% to 20% eosinophils; 2, 20% to 50% eosinophils; and 3, > 50% eosinophils). Unfortunately, details regarding collection or processing techniques were not provided, results for bronchial brushings and BAL fluid were combined, and expected results for healthy dogs were not provided for comparison. In the study reported here, none of the healthy dogs had > 1% eosinophils, and none of the dogs with cough had > 17% eosinophils in bronchial brushings.

The study reported here indicates that cytologic evaluation of bronchial brushings provides additional objective information regarding airway inflammation beyond that provided by cytologic evaluation of BAL fluid in some dogs with chronic cough and that large numbers of viable cells can reliably be obtained from healthy dogs and dogs with cough. Analysis of results for cytologic evaluation of bronchial brushings can contribute to a diagnosis of chronic bronchitis in dogs with cough and may provide useful information in studies of dogs with airway inflammation.

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