Inflammatory bowel disease is a group of disorders that is characterized by persistent or recurrent gastrointestinal signs and evidence of intestinal inflammation via histologic examination. The disease is a common cause of chronic vomiting and diarrhea in dogs and cats and must be differentiated from other possible causes such as infection, food allergy, neoplasia, exocrine pancreatic insufficiency, and hypoadrenocorticism.1

Objective—To determine whether lymphocyte apoptosis in intestinal mucosae is more common in healthy dogs than dogs with inflammatory bowel disease (IBD) and whether numbers of apoptotic cells increase after successful treatment of affected dogs.

Animals—8 dogs with IBD (IBD dogs) and 8 healthy control dogs.

Procedures—Biopsy specimens of the duodenum and colon were obtained via endoscopy from dogs with IBD before and after 10 weeks of standard treatment and compared with specimens obtained from control dogs. Expression of activated caspase 3 (Casp3), caspase-cleaved fragment p85 from poly-ADP-ribose polymerase (PARP), and B-cell leukemia/lymphoma 2 (Bcl-2) was measured in the duodenal (villous tip and base) and colonic mucosae.

Results—Expression of Casp3 was greater in the duodenal villous tips of control dogs, compared with expression in similar tissues from dogs with IBD before or after treatment. Despite clinical improvement of dogs with IBD, expression of Casp3 did not increase after treatment. Expression of PARP did not differ between groups at any time point. Expression of Bcl-2 was greater at all 3 tissue sites in control dogs, compared with expression at the same sites in dogs with IBD. Furthermore, Bcl-2 expression in duodenal villous tips was higher in dogs with IBD after treatment but was not higher elsewhere. In control dogs, expression patterns for all 3 markers were similar between sites (villous tip > villous base > colon).

Conclusions and Clinical Relevance—Expression of Casp3 in lymphocytes in duodenal villous tips was significantly reduced in dogs with IBD, compared with expression in healthy dogs, but no increase was detected following successful treatment of IBD. Increased expression of Bcl-2 may be a potential marker of the success of treatment. (Am J Vet Res 2008;69:1279–1285)

Glucocorticoids are usually administered as first-line drugs, but the optimal treatment regimen for dogs with IBD is unknown and may include other immunosuppressive agents. Glucocorticoids bind to intracellular and extracellular receptors to exercise various effects. One of these effects in dogs is a reduction of proportions of lymphocytes that express CD3 and CD4.3 In vitro exposure of canine lymphocytes obtained from popliteal lymph nodes to glucocorticoids also increases the rate of apoptosis.3 Most studies of canine IBD have revealed increased proportions of T lymphocytes in the small1,2 and large intestines6,7 of dogs with IBD, compared with proportions in the same tissues of healthy dogs. Investigators in 1 study6 histologically compared lesions before and 10 weeks after standardized treatment of dogs with IBD. Interestingly, despite clinical
improvement, there was no significant improvement in the severity of the lesions. Furthermore, the number of T cells that expressed CD3 did not decrease after treatment.  

In humans, the 2 main types of IBD are Crohn's disease and ulcerative colitis. Abnormalities in innate immunity (eg, increases in expression of toll-like receptors and nucleotide-binding oligomerization domains) are associated with IBD. A role for an abnormality in adaptive immunity may also exist because T cells from humans with IBD are resistant to apoptosis, which leads to increased inflammation in the gut.

Apoptosis is important for regulating T cells. It is a critical step in establishing tolerance and termination of the adaptive immune response. Glucocorticoids induce apoptosis of lymphocytes, and various members of the Bcl-2 family of proteins play a role in glucocorticoid-induced apoptosis in mouse lymphocytes. However, the situation is less clear for various members of the caspase family of proteins. Treatments used to restore clinically normal rates of T-cell apoptosis (eg, antibodies against TNF-α or treatments that inactivate IL-6 receptors) have been successful in humans with IBD.

Little is known about apoptosis in dogs, and, to the authors' knowledge, no reports on apoptosis of lymphocytes in the intestinal mucosa of dogs with IBD have been published. The purpose of the study reported here was to determine whether resistance of lymphocytes to apoptosis plays a central role in the etiology of canine IBD. Specific markers of apoptosis in mucosal lymphocytes of interest were Casp3 and PARP, a presumed marker of antiapoptosis, Bcl-2, was also measured. Our hypotheses were that apoptosis of lymphocytes in intestinal mucosa is more common in healthy dogs than in dogs with IBD and that successful treatment of IBD increases the rate of apoptosis of lymphocytes.

**Materials and Methods**

**Animals**—Inflammatory bowel disease was diagnosed in 8 dogs (IBD dogs) with chronic enteropathy of > 6 weeks' duration after a full clinical evaluation that excluded other potential causes. The evaluation included a CBC; serum biochemical analysis; urinalysis; parasitologic examination of fecal specimens by use of a flotation technique; bacteriologic culture of feces; examination of fecal specimens by use of a flotation technique; and bacteriologic culture of feces. Examinations and were deemed healthy on the basis of a lack of diarrhea or vomiting and unremarkable results of CBC, serum biochemical analysis, urinalysis, parasitologic examination of fecal specimens by use of a flotation technique, and bacteriologic culture of feces.

**Specimen collection**—Biopsy specimens of mucosa were obtained from the duodenum and colon (in the junction of descending to transverse duodenum and in the middle portion of the descending colon when no obvious lesions were detected) of dogs with IBD before treatment with prednisolone was initiated. Prior to the endoscopy-guided biopsy procedure, dogs were sedated with diazepam and anesthesia was induced with propofol. Endoscopy was performed by use of a videendoscope, and specimens were retrieved with adequate biopsy forceps. At least 10 biopsy specimens/site were collected during each endoscopic procedure and were deemed adequate when ≥ 6 had sufficient material for histologic assessment. Ten weeks after treatment with prednisolone was initiated (approx 2 weeks after treatment ceased), the biopsy procedure was repeated and biopsy specimens were collected from the same areas from which they were obtained before treatment. Biopsy specimens from control dogs were obtained endoscopically (n = 2) or at necropsy (n = 6) at 1 time point. Specimens from all dogs were histologically examined by the same pathologist (VFB).

Severity of the clinical disease at both time points was assessed on the basis of the CIBDAI. A score of 0 to 3 indicated clinically unremarkable disease (class 1), a score of 4 to 5 indicated mild IBD (class 2), a score of 6 to 8 indicated moderate IBD (class 3), and a score of ≥ 9 indicated severe IBD (class 4).

**Histologic evaluation**—For histologic analyses, ≥ 6 biopsy specimens/site were fixed in a buffered 4% formalin solution, embedded in paraffin wax, and routinely processed with H&E stain. All biopsy specimens were evaluated in accordance with published criteria and were classified as having no (class 0), mild (class 1), moderate (class 2), or severe inflammatory changes (class 3). The pathologist (VFB) was unaware of the identities of any specimens.

**Immunohistochemical evaluation**—Slides of formalin-fixed biopsy specimens were incubated overnight at 30°C before paraffin was extracted and the specimens were rehydrated via standard protocols. Activated caspase 3, caspase-cleaved fragment p85 from PARP, and Bcl-2 were retrieved via 3 different protocols optimized for each antigen. Activated caspase 3 was retrieved by soaking slides in 10mM sodium citrate buffer (pH, 6). Slides were warmed in a microwave for 1 minute at full power, warmed for an additional 4 minutes at reduced power to keep the solution hot without boiling, and
then cooled in distilled water for 20 minutes. Retrieval of PARP was achieved by soaking slides in a mixture of nonionic surfactant and PBS solution for 5 minutes at 20°C. Retrieval of Bcl-2 was achieved by soaking slides in a solution of 10mM sodium citrate (pH 6). Slides were autoclaved for 20 minutes at 121°C, cooled for 20 minutes in a bath of distilled water, and rinsed with distilled water for 35 minutes.

After antigens were retrieved, the biopsy specimens were rinsed and activity of endogenous peroxidase was blocked in a solution of methanol with 3% peroxidase for 5 minutes. The specimens were then rinsed and blocked in PBS solution containing 5% goat serum for 20 minutes. Separate slides were incubated with each of the following antibodies: anti-Casp3 rabbit polyclonal antibody, diluted 1:125 in PBS solution containing 5% goat serum for 75 minutes at 20°C; anti-PARP p85-fragment rabbit polyclonal antibody, diluted 1:50 in PBS solution containing 5% goat serum, and incubated overnight at 4°C in a humid chamber; and anti-Bcl-2 mouse monoclonal antibody, diluted 1:500 in PBS solution with 5% goat serum, and incubated overnight at 4°C in a humid chamber. Slides were subsequently rinsed with water.

Primary antibodies were detected via a streptavidin-biotin peroxidase technique by use of a commercial kit, according to the instructions of the manufacturer. Briefly, the primary antibody was first detected by incubation of slides with biotinylated goat anti-mouse and anti-rabbit immunoglobulins for 10 minutes at 20°C. Streptavidin conjugated to horseradish peroxidase was added, and slides were incubated for 10 minutes at 20°C. Finally, a chromogen (hydrogen peroxidase and 3-amino-9-ethylcarbazole) was added, and slides were incubated for a maximum of 10 minutes at 20°C. Slides were rinsed and mounted with mounting medium. Specimens of histologically normal canine lymph node were used as positive control samples for Casp3 and PARP assays, and liver biopsy specimens obtained from a dog with hepatic lymphoma were used for the Bcl-2 assay. Neurons from histologically normal brain tissue were used as negative control samples for the immunolabeling procedures because healthy neurons do not undergo apoptosis.

Lymphocyte counts—Slides were coded and examined via microscope, and digital photomicrographs of areas of interest were obtained by the same investigator (JRD). Antibody-labeled lymphocytes were counted as described elsewhere. Briefly, labeled lymphocytes were counted per 10,000 μm² of lamina propria in which epithelial and vascular structures had been excluded. From each duodenal sample, 5 areas from the tips of villi and 5 areas from the bases of villi were selected randomly within all villi that were visible on each slide, whereas 5 areas from the colon were evaluated by means of the same technique. Care was taken to count only stained cells with lymphoid morphology. Counts of labeled lymphocytes were interpreted as the degree of expression of the 3 markers.

Statistical analysis—Response to treatment was defined as a reduction in the CIBDAI score after treatment, whereas failure to respond was defined as a lack of reduction in the same score. All calculations were performed with commercially available statistical software. All data were normally distributed and are therefore reported as mean ± SD. Comparison of results between healthy dogs and dogs with IBD was performed via 1-way ANOVA. Comparison of results within groups of dogs was performed via repeated-measures ANOVA, and the Tukey-Kramer multiple-comparison test was used to compare results between tissue sites. Correlations between degree of expression of each marker (Casp3, PARP, and Bcl-2), histologic class, and CIBDAI score were assessed via Spearman rank correlations. Values of P < 0.05 were considered significant.

Results

Animals—Median CIBDAI score for the 8 IBD dogs was 8.5 (range, 4 to 9) before treatment with prednisolone began and 3.0 (range, 0 to 6) after treatment was discontinued (10 weeks after the 8-week treatment began). This reduction in CIBDAI score was significant (P < 0.001). All control dogs had a CIBDAI score of 0, which was significantly (P < 0.001) different from scores for IBD dogs before and after treatment. Before treatment, 1 dog was classified as having mild IBD, 3 dogs were classified as having moderate IBD, and 4 dogs were classified as having severe clinical IBD. After treatment, 4 dogs were classified as having unremarkable disease, 3 dogs were classified as having mild IBD, and 1 dog was classified as having moderate IBD. The CIBDAI scores of all dogs decreased after treatment.

Histologic analysis—No abnormalities were detected in the specimens of intestinal mucosa obtained from control dogs. Mild to severe infiltration of the mucosal lamina propria was detected in all IBD dogs; cells predominantly consisted of lymphocytes and plasma cells, with or without eosinophils. Mean histopathologic class of disease detected among duodenal biopsy specimens was 2.2 for IBD dogs before treatment was initiated and 2.2 for IBD dogs after treatment was concluded. Mean histopathologic class of disease detected among colonic biopsy specimens was 2.3 for IBD dogs before treatment was initiated and 2.0 for IBD dogs after treatment was concluded. Histopathologic scores were not significantly different between time points at either location. The mean histopathologic score for specimens from control dogs was 0 (no signs of inflammation), which was significantly (P < 0.001) less than scores for IBD dogs at either stage of treatment.

Degree of expression of markers of apoptosis—Expression of Casp3 in the duodenal villous tip was greater in control dogs versus IBD dogs before treatment with prednisolone (P = 0.001) and in control dogs versus IBD dogs after treatment (P < 0.001; Table 1). The difference between values for IBD dogs before and after treatment was not significant (P = 0.64). In the duodenal villous base, expression of Casp3 did not differ significantly in control dogs versus IBD dogs before treatment (P = 0.13), control dogs versus IBD dogs after treatment (P = 0.06), and IBD dogs before versus after treatment (P = 0.64). In specimens of colonic mucosa,
expression of Casp3 was not significantly different between control dogs versus IBD dogs before treatment (P = 0.14), control dogs versus IBD dogs after treatment (P = 0.27), and IBD dogs before versus after treatment (P = 0.87).

Expression of PARP in the duodenal villous tip was not significantly different in control dogs versus IBD dogs before treatment (P = 0.32), control dogs versus IBD dogs after treatment (P = 0.34), and IBD dogs before versus after treatment (P = 0.82). In the duodenal villous base, PARP expression did not differ significantly in control dogs versus IBD dogs before treatment (P = 0.22), control dogs versus IBD dogs after treatment (P = 0.14), and IBD dogs before versus after treatment (P = 0.86). In specimens of colonic mucosa, expression of PARP was not significantly different in control dogs versus IBD dogs before treatment (P = 0.56), control dogs versus IBD dogs after treatment (P = 0.27), and IBD dogs before versus after treatment (P = 0.08).

Expression of Bcl-2 in the duodenal villous tip was significantly greater in control dogs versus IBD dogs before treatment (P < 0.001), in control dogs versus IBD dogs after treatment (P < 0.001), and in IBD dogs before versus after treatment (P = 0.047). In the duodenal villous base, expression of Bcl-2 was greater in control dogs versus IBD dogs before treatment (P < 0.001) and control dogs versus IBD dogs after treatment (P = 0.006); however, expression of Bcl-2 in the duodenal villous base of IBD dogs before treatment did not differ significantly (P = 0.16) from that of IBD dogs after treatment. In specimens of colonic mucosa, expression of Bcl-2 was significantly greater in control dogs versus IBD dogs before treatment (P = 0.002) and in control dogs versus IBD dogs after treatment (P = 0.005), but values for IBD dogs before and after treatment were similar (P = 0.94).

The same expression pattern was detected for all 3 markers in tissues from control dogs, with highest numbers of labeled lymphocytes detected in the duodenal villous tip, then the duodenal villous base, and finally the colonic mucosa. A similar pattern was detected for expression of Bcl-2 in tissues from IBD dogs before and after treatment with prednisolone. In tissues from control dogs, the only significant (P < 0.001) difference in the expression pattern between localizations was for degree of expression of Bcl-2, whereas degrees of expression of Casp3 (P = 0.052) and PARP (P = 0.071) only approached significance. In tissues obtained from IBD dogs before and after treatment, none of the markers were expressed to significantly different degrees between tissue sites.

Table 1—Mean ± SD number of lymphocytes/10,000 µm² that stained for 2 markers of apoptosis (Casp3 and PARP) and 1 marker of antiproteinosis (Bcl-2) as measured via immunohistochemical evaluation of biopsy specimens obtained from the duodenum and colon of 8 dogs with IBD before and after treatment with prednisolone* and 8 healthy control dogs.

<table>
<thead>
<tr>
<th>Group, by marker</th>
<th>Duodenal villous tip</th>
<th>Duodenal villous base</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casp3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control dogs</td>
<td>5.5 ± 2.5f</td>
<td>3.3 ± 1.9</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>IBD dogs before treatment</td>
<td>1.5 ± 0.9</td>
<td>2.1 ± 1.4</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>IBD dogs after treatment</td>
<td>1.4 ± 0.7</td>
<td>1.9 ± 1.2</td>
<td>2.2 ± 1.8</td>
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<tr>
<td>PARP</td>
<td></td>
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<tr>
<td>Control dogs</td>
<td>8.2 ± 3.0</td>
<td>7.8 ± 3.6</td>
<td>6.4 ± 3.1</td>
</tr>
<tr>
<td>IBD dogs before treatment</td>
<td>9.3 ± 2.3</td>
<td>9.9 ± 3.4</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>IBD dogs after treatment</td>
<td>9.4 ± 3.6</td>
<td>10.0 ± 2.9</td>
<td>7.7 ± 2.5</td>
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<tr>
<td>Bcl-2</td>
<td></td>
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<tr>
<td>Control dogs</td>
<td>35.0 ± 10.9f</td>
<td>23.1 ± 3.7f</td>
<td>13.8 ± 2.8f</td>
</tr>
<tr>
<td>IBD dogs before treatment</td>
<td>8.6 ± 6.7</td>
<td>8.3 ± 4.4</td>
<td>6.5 ± 2.2</td>
</tr>
<tr>
<td>IBD dogs after treatment</td>
<td>14.5 ± 7.6f</td>
<td>13.0 ± 6.8</td>
<td>6.7 ± 3.2</td>
</tr>
</tbody>
</table>

*Biopsy specimens were obtained, IBD dogs were treated with prednisolone (started at 1 mg/kg, PO, q 12 h and tapered down over 8 weeks), and posttreatment biopsy specimens were collected 2 weeks after treatment completion. ¶Within the marker category, values differed significantly (P < 0.01) between control dogs and dogs with IBD before and after treatment. ¶¶Within the marker category, values differed significantly (P < 0.05) before versus after treatment of IBD dogs.

Correlations between marker expression, histopathologic grades, and CIBDIAI scores—When results for the 3 groups of dogs were combined, a slight correlation between degree of expression of Casp3 and Bcl-2 (r = 0.37; P = 0.002) and a moderate negative correlation between degree of expression of Casp3 and histopathologic class of inflammation (r = –0.57; P < 0.001) were detected. Degree of Bcl-2 expression was moderately and negatively correlated with histopathologic class (r = –0.37; P < 0.001) and CIBDIAI score (r = –0.58; P < 0.001). Histopathologic class of inflammation and CIBDIAI scores were moderately correlated (r = 0.63; P < 0.001). In tissues obtained from IBD dogs before treatment, degree of PARP expression and CIBDIAI score were moderately and negatively correlated (r = –0.60; P = 0.006), whereas in tissues from IBD dogs obtained after treatment, CIBDIAI score and histopathologic class were moderately and positively correlated (r = 0.64; P = 0.001).

When results for the different tissue sites were evaluated separately, degrees of expression of Casp3 and Bcl-2 in the duodenum were slightly (villous base, r = 0.38; P = 0.050) to moderately (villous tip, r = 0.67; P < 0.001) correlated. Expression of Bcl-2 was slightly to moderately and negatively correlated with histopathologic class at all 3 sites (duodenal villous base, r = –0.47 [P < 0.001]; duodenal villous tip, r = –0.69 [P < 0.001]; and colonic mucosa, r = –0.47 [P = 0.027]). Finally, CIBDIAI scores and histopathologic class were moderately correlated in duodenal tissues (r = 0.64; P < 0.001) and colonic tissues (r = 0.60; P = 0.031).

Discussion

Two markers of apoptosis were chosen for the study reported here: Casp3 and PARP. Activated caspase 3 is an effector caspase that represents a point of no return in most cell death cascades, whereas PARP is a substrate of Casp3 that is involved in DNA repair. Immunohistochemical detection of these markers was selected instead of a TUNEL assay, which is a less specific method designed to detect DNA fragmentation that takes place after apoptosis and other types of cell injury, including necrosis. Because of the poorer specificity of the TUNEL method, results of that assay must be assessed in cells with typical apoptotic morphologic changes or in conjunction with other markers specific for apoptosis (e.g., Casp3). Furthermore, Casp3 is activated early in the apoptotic cascade and therefore pre-
cedes PARP degradation or DNA fragmentation. Hence, cells that are lacking the morphologic changes typical of apoptosis but that are already committed to apoptosis can be identified earlier via detection of Casp3. On the other hand, Bcl-2 is an antiapoptotic member of the Bcl-2 family.22 This protein was included as a marker in our study to determine whether upregulation of Bcl-2 expression was associated with resistance of lymphocytes to apoptosis in dogs with IBD.

Degree of expression of Casp3 by lymphocytes was significantly increased in the tips of duodenal villi of control dogs, compared with expression at the same location in duodenal tissues from IBD dogs, but there were no differences in expression of Casp3 at the other sites (ie, colonic mucosa and bases of duodenal villi). In addition, degree of PARP expression did not differ significantly between control dogs and IBD dogs. These findings support the hypothesis that lymphocyte apoptosis in the gastrointestinal mucosa is higher in healthy dogs than in dogs with IBD, at least in the duodenal villous tip. Degree of expression of Casp3 probably cannot be used to differentiate clinically normal dogs from dogs with IBD because numbers of lymphocytes that expressed Casp3 in both groups of dogs overlapped somewhat in our study. Furthermore, the clinical relevance of a few more Casp3-positive cells in 10,000 μm² is questionable. Nevertheless, results support the supposition that the density of apoptotic mucosal lymphocytes is higher in the tips of duodenal villi in healthy dogs, compared with the same value in dogs with IBD, even though results do not support establishment of a cutoff value for differentiating between healthy dogs and dogs with IBD.

To quantify the proportion of lymphocytes that are apoptotic in dogs with IBD and confirm the results of the present study, additional studies that involve double labeling of lymphocytes for CD3 (and other lymphocyte markers) and Casp3 or PARP are warranted. Another study in which investigators used the same population of IBD dogs revealed that total numbers of CD3-positive lymphocytes were not significantly different in specimens of duodenal and colonic mucosa obtained before and after treatment with prednisolone, according to the same protocol as in this study. In our study, degrees of expression of Casp3 and PARP did not differ significantly in IBD dogs before versus after treatment with prednisolone at all 3 tissue sites. Therefore, we did not find support for our second hypothesis that successful treatment of dogs with IBD increases the rate of apoptosis in lymphocytes.

Because the role of PARP in the apoptosis cascade is downstream from that of Casp3, we expected the number of PARP-positive lymphocytes to also be significantly increased in the duodenal villous tip in control dogs, compared with IBD dogs before and after treatment, but this increase was not detected. Apoptosis can result in inactivation of PARP by Casp3,20 and both proteins have a different half-life.25 If Casp3 was expressed for a longer period than PARP, then it is possible that by counting lymphocytes at a moment in time, degree of expression of only Casp3 and not PARP may appear increased in control dogs, compared with degree of expression in IBD dogs. The efficacy of antigen recovery between methods used in the immunohistochemical assays may also have differed. The discrepancy between results for the 2 markers may also be explained by different lymphocytes expressing Casp3 or PARP, and double labeling for Casp3 and PARP would allow this hypothesis to be tested. Another possible explanation is the low number of dogs with IBD included in our study.

Results for degrees of Casp3 and PARP expression in lymphocytes of the dogs with IBD in the present study do not support the hypothesis that the rate of apoptosis in mucosal lymphocytes increases after successful treatment. The lack of an increase corresponds to the lack of significant changes in the degree of inflammation of tissues after treatment with prednisolone ceased. All dogs were reexamined 10 weeks after treatment was initiated. Nevertheless, it is possible that this period of treatment was not long enough to detect changes in the rate of lymphocyte apoptosis despite the clinical improvement. Interestingly, the degree of expression of PARP and CIBDAI score were moderately, negatively correlated in IBD dogs before treatment, whereas there was no correlation in IBD dogs after treatment or control dogs at the same time point. This finding supports the hypothesis of resistance to apoptosis in the pathogenesis of IBD in dogs.

The third marker examined in the present study was Bcl-2. This protein is a member of the Bcl-2 family,26 which can be divided in 2 categories of >30 members: the antiapoptotic (Bcl-2-like) and the proapoptotic group (Bcl-2–associated X protein–like and Bcl-2 homology 3). A significantly higher degree of Bcl-2 expression among lymphocytes in intestinal tissues at all 3 sites in control dogs versus IBD dogs was an unexpected result, assuming that Bcl-2 has antiapoptotic properties in dogs as well. In another study,23 an increase in the degree of expression of Bcl-2 by cells from the feline T-cell leukemia cell line was detected after apoptosis was successfully induced with doxorubicin, prednisolone, or vincristine. Furthermore, increased expression of B-cell leukemia XL, another member of the Bcl-2–like family, was detected after apoptosis was induced. The findings of that and the present study may indicate that Bcl-2 is proapoptotic in dogs and cats rather than antiapoptotic, as it is in mice and humans.24 This hypothesis is further supported by the slight positive correlation between degrees of expression of Bcl-2 and proapoptotic Casp3 and the moderate negative correlations between degree of expression of Bcl-2 and histopathologic class or CIBDAI score. Another possible explanation for this unexpected higher degree of expression of Bcl-2 in mucosal lymphocytes of control dogs is the existence of another upregulated proapoptotic factor (eg, Bcl-2–interacting mediator of cell death) that induces a counter-regulatory up-regulation of antiapoptotic Bcl-2. Another member of the Bcl-2 family, B-cell leukemia/lymoma 3, reportedly increases survivability of activated T cells.26 It would be interesting to determine whether B-cell leukemia/lymoma 3 or antiapoptotic myeloid cell leukemia-1 is expressed in lymphocytes in canine IBD because these proteins are good enhancers of lymphocytic resistance to apoptosis.
Expression of Casp3 and PARP in mucosal lymphocytes was not upregulated after successful treatment of dogs with IBD, but surprisingly, IBD dogs had a significantly higher degree of Bcl-2 expression in the duodenum after treatment versus before treatment. This increase in the degree of expression of Bcl-2 after treatment, which brought values in IBD dogs closer to values of control dogs, combined with the successful treatment of most dogs with IBD in our study, raises exciting possibilities for monitoring the progress of dogs with IBD. Although upregulation of the expression of Bcl-2 appears not to have been the mechanism of resistance to apoptosis in the IBD dogs in our study, increases in the expression of Bcl-2 in mucosal lymphocytes may be useful as an early marker of the success of treatment.

The same pattern of expression of all 3 markers was detected in control dogs, and a similar pattern of Bcl-2 expression was detected in IBD dogs before and after treatment with prednisolone. More marker-positive lymphocytes were detected in tips of duodenal villi than in bases of duodenal villi, and more marker-positive lymphocytes were detected in duodenal villous bases than in colonic mucosa. This pattern may reflect a difference in lymphocyte numbers attributable to more interactions with antigens in the villous tip or a difference in the immune response within various regions of the intestine.

The present study had some limitations. Although recommendations usually state that dogs with food-responsive conditions should be fed an appropriate formulation for at least 4 to 6 weeks, the elimination diet trial was limited to 14 days out of concern for owner compliance. Therefore, some dogs may have been misclassified as having IBD instead of a food-responsive condition, even though in our experience, most food-responsive dogs with diarrhea have signs of improvement within 2 weeks. The control dog group was restricted to Beagles that were younger than most dogs with IBD. Control dogs were clinically normal, with no evidence of disease detected during histologic examination of intestinal biopsy specimens. Beagles are not representative of all dog breeds; however, for ethical and legal reasons, they are the only breed of dog that can be used as control dogs for the type of study reported here. We did not assess colocalization of Casp3, PARP, and Bcl-2, which may have elucidated the potential interaction of Casp3 with PARP and the potential colocalization of Casp3 and Bcl-2. Furthermore, only 8 dogs with IBD were evaluated in our study. The limited sample size was partially attributable to the extensive clinical evaluation of these dogs, which included 2 endoscopies, and to the predominance of food-responsive diarrhea versus IBD in our population of dogs with chronic enteropathies. Nevertheless, several significant differences were detected among and between groups of dogs, despite the low number of dogs used.

Our study revealed decreased numbers of apoptotic lymphocytes in the duodenum of IBD dogs, compared with numbers in healthy control dogs. Good outcome with dietary changes and administration of glucocorticoids does not result in an increase of apoptosis in lymphocytes. Finally, upregulation of Bcl-2 is not a mechanism of resistance to apoptosis in IBD dogs, but increase in the expression of Bcl-2 as an early marker of therapy success could potentially be used to monitor dogs under therapy.

References