Inflammatory bowel disease includes several diseases that are considered idiopathic and are characterized by histologic evidence of chronic inflammatory infiltrate in the lamina propria of the intestinal tract mucosa. Inflammatory bowel disease is associated with persistent or recurrent nonspecific signs such as diarrhea, vomiting, or weight loss. Pathognomonic signs for IBD have not been described. A complete diagnostic evaluation of patients must be performed to rule out any other cause of intestinal inflammation. Typically, medical history and results of a CBC, serum biochemical analyses, analysis of feces, pancreatic function tests, and diagnostic imaging (radiography and ultrasonography) are obtained for patients with IBD-compatible clinical signs. A final diagnosis of IBD is confirmed via histologic examination of intestinal biopsy specimens; however, severe IBD can be difficult to distinguish histologically from intestinal lymphoma. Because animals with IBD or intestinal lymphoma can have similar clinical, laboratory, and ultrasonographic findings, it is difficult to distinguish these diseases clinically. It has also been suggested that IBD, namely lymphocytic-plasmacytic enteritis, could progress to intestinal lymphoma in dogs. In recent decades, several serologic markers have been used to support the diagnosis and classification of chronic IBD in humans. One such indicator is the detection of ANCA in a perinuclear pattern in isolated neutrophils via IIF microscopy. Prevalence of circulating pANCA in humans with ulcerative colitis or Crohn's disease differs (50% to 80% and 10% to 20%, respectively). The presence of these anti-

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**Objective**—To assess and compare the expression of perinuclear antineutrophilic cytoplasmic antibodies (pANCA) in sera obtained from dogs with inflammatory bowel disease (IBD) and dogs with intestinal lymphoma.

**Animals**—104 dogs with IBD and 23 dogs with intestinal lymphoma.

**Procedures**—Each ill dog had persistent gastrointestinal signs (> 3 weeks in duration) and absence of response to diet changes or antimicrobial treatments. Gastrointestinal endoscopy was performed in ill dogs to obtain intestinal biopsy specimens for histologic confirmation of IBD or lymphoma. A serum sample was obtained from each ill dog. Neutrophils were isolated from a blood sample from the healthy dog; neutrophil-bearing slides were incubated with serum from each ill dog and examined for expression of pANCA by use of an indirect immunofluorescence technique. Detection of cells that had a perinuclear fluorescence pattern was considered a positive result.

**Results**—The 2 groups of dogs did not differ with regard to breed and sex but did differ with regard to age. Expression of pANCA was detected in 38 of the 104 (36.5%) dogs with IBD and 4 of the 23 (17.4%) dogs with intestinal lymphoma. Although the frequency of pANCA expression was higher in dogs with IBD, compared with findings in dogs with intestinal lymphoma, the difference was not significant.

**Conclusions and Clinical Relevance**—Results indicated that circulating pANCA are present in some dogs with IBD or intestinal lymphoma. However, pANCA detection does not seem to be useful for distinguishing dogs with IBD from dogs with intestinal lymphoma. (Am J Vet Res 2011;72:1333–1337)
bodies has also been found in dogs with IBD, which illustrates the usefulness of pANCA detection in the diagnosis of this disease.19–21 In dogs with IBD, the sensitivity of the pANCA assay ranges from 36% to 51%.19 Higher specificity (82% to 95%) of this technique in groups of dogs without IBD (ie, healthy dogs, healthy working dogs, dogs with acute gastrointestinal disorders, and dogs with chronic non–IBD-associated gastrointestinal disorders) has been reported. Therefore, pANCA detection has been proposed as a complementary technique to differentiate dogs with IBD from dogs with other chronic gastrointestinal tract disorders.

In human medicine, the presence of ANCA in patients with neoplastic diseases such as bronchoalveolar carcinoma, adenocarcinoma of the lungs, or several types of lymphoma or leukemia has been previously described.22–27 Codevelopment of these antibodies and a neoplastic disease is rare, and the link between the 2 processes is not well defined. The prevalence of ANCA in humans with neoplastic disease ranges from 2% to 12%, although ANCA have not been extensively evaluated in patients with neoplastic disease and serial studies25–27 are limited. It has been suggested that the presence of these antibodies could be related to vasculitis that can develop as an autoimmune paraneoplastic process.23–28 However, the mechanisms of development of neoplasia-associated vasculitis are complex and the paraneoplastic characteristic of the vasculitis is not constant.26

The purpose of the study reported here was to assess and compare the expression of circulating pANCA in dogs with IBD and dogs with intestinal lymphoma. The hypothesis was that pANCA detection could be used to differentiate dogs with IBD from dogs with another chronic gastrointestinal tract disorder.

**Materials and Methods**

**Animals**—One hundred twenty-seven dogs were included in the study. Inflammatory bowel disease was diagnosed in 104 dogs, and intestinal lymphoma was diagnosed in 23 dogs. Owners of each dog signed a letter of consent allowing use of their dog for scientific research purposes. All experimental procedures were approved by the Ethical Committee of the Complutense University of Madrid Veterinary Medical Teaching Hospital.

In both groups of dogs, the same diagnostic protocol was performed. The minimal diagnostic evaluation included a complete anamnesis, CBC, and serum biochemical analyses; once-daily fecal examination for cestodes, nematodes, and protozoa (via direct smear evaluation and Telemann and merthiolate-iodine-formalin techniques) for 3 consecutive days; and assessment of serum trypsin-like immunoreactivity for pancreatic function. In addition to a lack of abnormalities detected via the thorough diagnostic evaluation, the study dogs had persistent gastrointestinal signs (> 3 weeks in duration) and lack of response to changes in diet and to antimicrobial treatments. Each dog underwent gastrointestinal tract endoscopy during which an intestinal biopsy specimen was collected. Diagnosis of IBD or lymphoma was confirmed on the basis of the results of histologic examination of the intestinal biopsy specimen.

Two milliliters of blood was obtained from a peripheral vein of each dog included in the study. The sample was placed in a tube without anticoagulant. Each blood sample was centrifuged at 450 × g for 20 minutes; serum was removed and stored at −80°C until use.

**Detection of pANCA**—Based on the technique previously described by AllenSpach et al,9 IIF was used to detect pANCA in canine neutrophils. Neutrophils were isolated from a 20-mL sample of blood (collected with heparin) obtained from a university-owned healthy donor dog by density gradient centrifugation with a sodium diatrizoate (13.8% [wt/vol]) and dextan 500 (8% [wt/vol]) isolation solution.2 Briefly, the isolation solution was overlaid with the heparinized blood sample and centrifuged at 450 × g for 30 minutes at room temperature (approx 22°C). The neutrophil phase was washed twice with saline (0.45% NaCl) solution via centrifugation at 450 × g for 10 minutes at 22°C. The pellet was resuspended in PBS solution. The neutrophils were added to cytocentrifuge chambers and centrifuged at 200 × g for 10 minutes. The neutrophil content of the solution was assessed microscopically by use of May-Grünwald-Giemsa stain. The solution was apportioned onto slides that were air-dried and fixed in 96% ethanol at 4°C for 5 minutes. Slides were kept frozen at −20°C until use.

The serum sample from each of the study dogs was diluted (1:10 dilution) in PBS solution (pH, 7.2). The IIF technique included 2 incubations in a humid chamber at 22°C. First, neutrophil-bearing slides were incubated sequentially with the serum samples (1:10 dilution) for 1 hour; then, slides were incubated with a secondary fluorescein isothiocyanate–labeled sheep anti-canine IgGα (1:50 dilution) for 1 hour. Each slide underwent 2 washes (5 minutes each) in PBS solution (pH, 7.2). After the last wash, slides were left to dry in the dark. The dried slides were mounted with buffered glycerine and observed under a fluorescence microscope by 1 observer (AS). Each serum sample was examined in duplicate. Only sera that produced a perinuclear fluorescence pattern were considered positive for pANCA.

**Statistical analysis**—A Student t test for independent samples was used to analyze the differences between dogs with IBD and dogs with intestinal lymphoma in relation to age. Data were analyzed statistically by use of a χ² test to evaluate differences in sex, breed, and pANCA expression between the IBD group and the intestinal lymphoma group. When appropriate, a Yates correction was conducted. Relative risk and odds ratio of the expression of pANCA were calculated to compare the 2 groups of dogs. Significance was set at P < 0.05. Measures of diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value
with 93% confidence intervals) were calculated for the group of dogs with IBD versus the group of dogs with intestinal lymphoma.

**Results**

Perinuclear antineutrophilic cytoplasmic antibody detection was performed in 127 dogs. The IBD group included 104 dogs, of which 36 were female and 68 were male. The ages of these dogs ranged from 1 to 14 years (mean ± SEM age, 5.21 ± 2.98 years). Breeds in this group included mixed (n = 29), German Shepherd Dog (11), Yorkshire Terrier (8), West Highland White Terrier (7), Boxer (5), Poodle (4), Cocker Spaniel (4), Catalan Sheepdog (4), Belgian Shepherd (3), Miniature Schnauzer (3), Chinese Shar-Pei (3), Collie (2), Rottweiler (2), Siberian Husky (2), Lhasa Apso (2), and 1 dog from each of the following breeds: Akita Inu, Alaskan Malamute, Beagle, Bouvier des Flandres, Brittany, English Shepherd Dog, Samoyed, Dachshund, French Bulldog, English Bulldog, Fox Terrier, Greyhound, Golden Retriever, Great Pyrenees, and Irish Setter.

The intestinal lymphoma included 23 dogs, of which 10 were female and 13 were male. The ages of these dogs ranged from 5 to 12 years (mean age, 8.65 ± 2.19 years). Breeds in this group included mixed (n = 7), Cocker Spaniel (3), Catalan Sheepdog (2), Siberian Husky (2), German Shepherd Dog (2), Yorkshire Terrier (2), and 1 dog from each of the following breeds: Pug, Fox Terrier, Rottweiler, French Bulldog, and Miniature Schnauzer.

When sex and breed were analyzed, no significant (P = 0.726 and 0.885 for sex and breed, respectively) differences were found between dogs with IBD and dogs with intestinal lymphoma. However, the mean age of dogs with intestinal lymphoma was significantly (P < 0.001) greater than that of dogs with IBD.

Of the 104 dogs with IBD, 38 (36.5%) expressed pANCA and 66 (63.5%) did not (Figure 1). Of the 23 dogs with intestinal lymphoma, 4 (17.4%) expressed pANCA and 19 (82.6%) did not. There was no significant difference in pANCA expression between dogs with IBD and dogs with intestinal lymphoma. Calculation of the relative risk and odds ratio of pANCA expression in the group of dogs with IBD, compared with the group of dogs with intestinal lymphoma, were not significant (2.1 [95% confidence interval, 0.832 to 5.305] and 2.73 [95% confidence interval, 0.866 to 8.637], respectively). The sensitivity of pANCA detection for differentiating dogs with IBD from dogs with intestinal lymphoma was 0.37 (95% confidence interval, 0.27 to 0.46). The specificity of pANCA detection for differentiating dogs with IBD from dogs with intestinal lymphoma was 0.83 (95% confidence interval, 0.67 to 0.98); the positive predictive value was 0.9 (95% confidence interval, 0.82 to 0.99), and negative predictive value was 0.22 (95% confidence interval, 0.13 to 0.31).

**Discussion**

Diagnosis of IBD or neoplasia of the digestive tract in dogs is difficult and necessitates full diagnostic evaluation that includes collection of an intestinal biopsy specimen for histologic examination. The study of this report was performed to ascertain whether pANCA expression in dogs with IBD is different from that in dogs with intestinal lymphoma. Furthermore, the intent was to evaluate the usefulness of pANCA screening as a tool for differential diagnosis of IBD from intestinal lymphoma in dogs because of the well-known difficulty in differentiating these 2 diseases on the basis of clinical and histopathologic findings. With regard to the use of the pANCA assay for differentiation of dogs with IBD from dogs with intestinal lymphoma, sensitivity was 0.37 and specificity was 0.83. These findings were in agreement with previously reported data.19,21

In the present study, no significant differences were found between the group of dogs with IBD and the group of dogs with intestinal lymphoma with respect to sex or breed. Sex predisposition for either IBD or intestinal lymphoma has not been described, although it has been suggested that lymphoma is more common in males.2 With respect to breed, the German Shepherd Dog, Chinese Shar-Pei, Rottweiler, Basenji, and Soft Coated Wheaten Terrier seem to be predisposed to development of IBD.1,2,5

In general, IBD develops in adult dogs of middle and advanced age, and intestinal lymphoma most often develops in older dogs, typically those > 7 years of age.1,2 This age difference was reflected in the 2 groups of dogs included in the present study, in that the mean age of dogs with IBD was significantly lower than that of dogs with intestinal lymphoma.

In the present study, the frequency of pANCA expression was fairly high both in dogs with IBD (36.5%) and in dogs with lymphoma (17.4%): pANCA expression did not differ significantly between the 2 groups. Even taking into account the possible limited power of the data, these values indicate that pANCA are pres...
ent in some dogs with IBD, as has been confirmed by other investigators, and in some dogs with intestinal lymphoma. The frequency of pANCA expression in either group in the present study was higher than the reported frequency in healthy dogs. The pathogenesis of IBD in dogs involves changes in permeability of the intestinal mucosa together with local production of proinflammatory cytokines. It has been suggested that canine antibodies in general cross-react with many different antigens, as do their human counterparts, contributing to an autoimmune reaction that perpetuates the chronic inflammation associated with IBD in dogs. With regard to the presence of pANCA in some dogs with lymphoma, the antibodies could be a result of concurrent IBD in those dogs. Infiltrations of lymphocytes and plasma cells resembling those associated with mild lymphocytic-plasmacytic enteritis have been detected in samples of intestinal segments adjacent to lymphoma-affected intestinal segments. However, given the paucity of information available in the veterinary medical literature, this clinical situation does not seem to be common. Because of that, pANCA in dogs with intestinal lymphoma appears to be directly related to the neoplastic condition. Expression of ANCA in association with several neoplastic processes in humans has been described. Several hypotheses have been developed to explain the codevelopment of pANCA and neoplastic disease. Antineutrophilic cytoplasmic antibodies are typically associated with small-vessel vasculitides, although they are also found in other conditions. The nature of the link between systemic ANCA-associated vasculitis and neoplastic disease is unknown. The mechanisms of the vasculitis associated with malignancy are complex, and that vascular disorder has been considered as a true paraneoplastic syndrome. Vasculitis secondary to neoplastic diseases in dogs has also been described. However, to date, the presence of ANCA in dogs with vascular disorders has not been reported, to our knowledge. The pathogenic mechanisms of vasculitis in humans and dogs are considered to be similar, a fact that could support the expression of ANCA in both dogs and humans.

On the other hand, several studies have identified pANCA in humans with hematopoietic neoplasms such as leukemia and lymphoma. The ANCA probably develop secondary to immune dysregulation and the lymphoproliferative characteristic of those hematologic disorders and are not necessarily associated with the presence of vasculitis. This fact could also explain the higher frequency of pANCA expression in dogs with intestinal lymphoma in the present study, compared with findings in healthy dogs. Therefore, derived from an aforementioned hypothesis, a combination of an intestinal inflammatory infiltrate, a possible vasculitis, and any lymphoproliferative condition could be the cause of pANCA expression in dogs with lymphoma in the present study.

Results of the study reported here suggested that pANCA may be present in some dogs with IBD or intestinal lymphoma. However, pANCA detection does not seem to be very useful to distinguish dogs with IBD from dogs with intestinal lymphoma. Studies to evaluate the presence of vasculitis and the intensity of inflammatory infiltrate in the intestinal wall of dogs with IBD or intestinal lymphoma are required to confirm these results and to clarify the role of pANCA in the pathogenesis of IBD or intestinal lymphoma in dogs.

References


