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SPONTANEOUSLY ARISING DISEASE

Immunohistochemical and Morphometric Analysis of Intestinal Full-thickness Biopsy Samples from Cats with Lymphoplasmacytic Inflammatory Bowel Disease

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Summary

The distribution and numbers of CD3^{+} T lymphocytes, immunoglobulin^{+} plasma cells and calprotectin (L1)[+] macrophages was analyzed in full-thickness, formalin-fixed biopsy samples from the small intestine (duodenum, jejunum and ileum) and from the colon from nine cats with clinical signs of inflammatory bowel disease (IBD). All animals had lymphoplasmacytic enteritis or lymphoplasmacytic enterocolitis. Equivalent samples from the same intestinal regions from 12 healthy pet cats served as controls. Labelled cells in the lamina propria were counted by computer-aided morphometry. The different cell types were similarly distributed in both groups, but there were differences in their numbers. There were more CD3^{+} T cells in the duodenum and jejunum of cats with IBD; however, the difference was only significant for the duodenum. There were significantly more IgA^{+} cells in the duodenal crypt region. There were significantly more IgG^{+} cells in the lower jejunal crypt region. Plasma cells expressing IgM were decreased in cats with IBD, but the difference was not significant. L1[+] macrophages were significantly decreased in the lower crypt area of the colon in cats with IBD and there was a trend to decreased L1^{+} cells in the upper crypt area of the duodenum and jejunum. Comparison of the results of this study with previous findings on endoscopically-obtained duodenal biopsy samples from cats with IBD revealed some differences. These discrepancies might relate to differences between control cat populations, types of biopsy samples, methodological factors such as different counting techniques and the activity of the disease at the time of sampling.

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Keywords: cat; immunohistochemistry; inflammatory bowel disease

Introduction

Canine and feline inflammatory bowel disease (IBD) is a complex of idiopathic chronic inflammatory gastrointestinal disorders that are immunologically mediated (Jergens, 2012; Jergens and Simpson, 2012). The diagnosis of IBD is based on ruling out other causes of chronic vomiting and/or diarrhoea and by histopathological confirmation of intestinal inflammation (Jergens *et al.*, 1992; Allenspach *et al.*, 2007).

As in dogs, the most frequently reported form of feline IBD is lymphoplasmacytic enteritis (LPE) (Dennis *et al.*, 1992; Jergens *et al.*, 1992). Several studies of the subsets of immune cells in dogs with LPE revealed increased numbers of lamina propria CD3^{+} T lymphocytes, IgA^{+} and IgG^{+} plasma cells and macrophages (Stonehewer *et al.*, 1998; Jergens *et al.*, 1999; German *et al.*, 2001), although in one investigation similar numbers of CD3^{+} T cells were found before and after clinical remission (Schreiner *et al.*, 2008).

So far, only one systematic and detailed report describes the numbers and distribution of immune cell subsets in the duodenal mucosa of cats with IBD

(Waly *et al.*, 2004). In that study, except for IgM$^+$ plasma cells, no differences in lamina propria immune cell populations between healthy and diseased cats were found (Waly *et al.*, 2004).

The aim of the present study was to characterize the distribution and numbers of CD3$^+$ T lymphocytes, immunoglobulin (Ig)$^+$ plasma cells and macrophages in full-thickness biopsy samples from different segments of the small intestine and colon of cats with clinical signs of IBD and a histopathologically confirmed diagnosis of LPE or lymphoplasmacytic enterocolitis (LPEC) and clinically normal cats.

Materials and Methods

Animals and Tissue Samples

Full-thickness biopsy samples of the intestine were obtained from nine cats admitted to the Small Animal Clinic of the University of Veterinary Medicine Hannover between 1998 and 2002 with the following clinical signs: vomiting (cats 1, 2, 3, 4 and 7), diarrhoea (cats 5, 6, 8 and 9) or anorexia (cats 1 and 6). The animals were part of a study described elsewhere (Kleinschmidt *et al.*, 2010). The age of the animals ranged from 2 to 12 years (mean 9.5 years). Eight animals were domestic shorthair cats and one (cat 4) was a Norwegian forest cat. All cats had clinical signs present for at least 3 weeks. All animals underwent a detailed clinical work up including case history, clinical investigation, haematological examination, serum biochemistry, faecal examination for parasites as well as radiographic and ultrasonographic examinations.

Samples from 12 cats of various breeds and sex served as controls. Their age range was 3–12 years (mean 7.3 years). These cats had diseases unrelated to the gastrointestinal tract and were humanely destroyed due to poor prognosis. Further details of these animals are given in Marsilio *et al.* (2011).

Transmural biopsy samples from diseased cats were collected during laparotomy for diagnostic purposes. The control cats were humanely destroyed because of diseases unrelated to the gastrointestinal tract. Samples from control animals were collected immediately after death. Samples were taken from the duodenum, the mid jejunum, the ileum and the descending colon with a 4 mm biopsy punch (GE Healthcare, Garbsen, Germany). In diseased cats, samples from the ileum were available from only four cats and in eight cats biopsy samples were also taken from the ascending colon (Table 1). All tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax.

Table 1
Histopathological diagnosis of cats with inflammatory bowel disease

Cat number	Duodenum	Jejunum	Ileum	Colon
1	+ +, LPE	+ +, LPE	NS	NS
2	+, LPE	+ +, LPE	NS	+, LPEC
3	AL	+ +, LPE	NS	AL
4	+, LPE	+ +, LPE	AL	+, LPEC
5	+ +, LPE	+, LPE	AL	AL
6	+ +, LPE	+ +, LPE	NS	AL
7	+ +, LPE	+ +, LPE	+ +, LPE	AL
8	+, LPE	+, LPE	AL	+ +, LPEC
9	Normal	+ +, LPE	NS	AL

+, mild; + +, moderate; LPE, lymphoplasmacytic enteritis; LPEC, lymphoplasmacytic enterocolitis; NS, no biopsy sample available; AL, architectural lesions without increased infiltration; Normal, without histological changes.

Histology and Immunohistochemistry

Sections (2–4 µm) were stained with haematoxylin and eosin (HE). Serial sections were subjected to immunohistochemistry (IHC) with primary antibodies specific for the T lymphocyte marker CD3 (rabbit anti-human CD3; Dako, Hamburg, Germany; dilution 1 in 800) and antibodies specific for feline IgG, IgA and IgM all conjugated directly to fluorescein isothiocyanate (FITC; Bethyl Laboratories, Frankfurt, Germany; dilution 1 in 9,000 for anti-IgG, 1 in 12,000 for anti-IgA and 1 in 9,000 for anti-IgM). The immunohistochemical procedures and controls are described in Marsilio *et al.* (2011). Briefly, after dewaxing, the tissue sections were immersed in H_2O_2 0.5% in methanol. Antigen retrieval was performed by microwave heating in citrate buffer pH 6.0 for CD3 or by using demasking solution G (BioLogo, Kronshagen, Germany) for immunoglobulins. After antigen retrieval, nonspecific binding was blocked with inactivated goat serum (non-commercial origin) diluted 1 in 5 in phosphate buffered saline (PBS, pH 7.1). The blocking serum was drained and replaced by the first antibody. After washing, the tissue sections were incubated with a biotin-conjugated goat anti-rabbit IgG for CD3 labelling or biotin-conjugated goat anti-FITC (both from Vector Laboratories, Burlingame, California, USA). The avidin–biotin–peroxidase reagent (Vector Laboratories) was used according to the manufacturer's instructions.

Labelling for macrophages/monocytes with mouse anti-human MAC387 (L1 protein) (Dako) was performed similarly. For antigen retrieval and as secondary antibody, however, demasking solution G (BioLogo) and biotin-conjugated anti-mouse IgG (Vector Laboratories) were applied, respectively.