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SPONTANEOUSLY ARISING DISEASE

CD11c$^+$ Cells are Significantly Decreased in the Duodenum, Ileum and Colon of Dogs with Inflammatory Bowel Disease

A. Kathrani[*], S. Schmitz[*], S. L. Priestnall[†], K. C. Smith[†], D. Werling[†], O. A. Garden[*] and K. Allenspach[*]

** Department of Veterinary Clinical Sciences and † Department of Pathology and Infectious Diseases, Royal Veterinary College, University of London, London, UK*

Summary

CD11c serves as a marker for human and murine dendritic cells (DCs) and cells expressing this marker have been shown to have similar morphological and functional characteristics in the canine immune system. The aim of this study was to quantify CD11c$^+$ cells in the duodenum, ileum and colon of healthy dogs and dogs with inflammatory bowel disease (IBD). Endoscopic biopsies from the duodenum ($n = 12$ cases), ileum ($n = 8$ cases) and colon ($n = 12$ cases) were obtained from dogs diagnosed with IBD. Intestinal tissue from 10 healthy beagle dogs was used as control. Immunofluorescence microscopy was carried out using an anti-canine CD11c monoclonal antibody. Labelled cells were recorded as cells per 120,000 μm^2. The canine chronic enteropathy clinical activity index (CCECAI) was calculated for all dogs with IBD. In addition, sections from all dogs with IBD were evaluated according to the guidelines of the World Small Animal Veterinary Association Gastrointestinal Standardization Group. The number of CD11c$^+$ cells in the duodenum, ileum and colon of dogs with IBD was significantly reduced compared with controls ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively). There was a significant negative correlation between the number of CD11c$^+$ cells in the colon of dogs with IBD and the CCECAI ($P = 0.044$, $r^2 = -0.558$). Chronic inflammation in canine IBD appears to involve an imbalance in the intestinal DC population. Future studies will determine whether reduced expression of CD11c could be a useful marker for the diagnosis and monitoring of canine IBD.

Keywords: CD11c; dendritic cell; dog; inflammatory bowel disease

Introduction

The healthy gut is able to mount an inflammatory response to pathogenic bacteria while maintaining the ability to tolerate commensal and dietary antigens (Rakoff-Nahoum *et al.*, 2004; Abreu *et al.*, 2005; Magalhaes *et al.*, 2007). This important distinction is thought to occur via the action of dendritic cells (DCs).

Inflammatory bowel disease (IBD) occurs in man and dogs and results from an inappropriate immune response to commensal and dietary antigens (Abreu *et al.*, 2005). The two main types of human IBD are Crohn's disease (CD) and ulcerative colitis (UC), but in dogs IBD includes a spectrum of chronic gastrointestinal (GI) diseases (Jergens *et al.*, 1992; Xavier and Podolsky, 2007). Accumulating evidence suggests that DCs may be responsible for the breakdown of tolerance that occurs in human IBD and murine models of IBD. For example, many studies have demonstrated an increase in the number of DCs in inflamed gut mucosa in both people and mice, and studies have also demonstrated that DCs isolated from IBD tissue are phenotypically more mature and produce more pro-inflammatory cytokines than cells isolated from control tissue (Bernstein *et al.*, 1998; Krajina *et al.*, 2003; Sands *et al.*, 2005; Baumgart *et al.*, 2009; Strauch *et al.*, 2010).

Correspondence to: K. Allenspach (e-mail: kallenspach@rvc.ac.uk).

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Canine IBD is similar to human IBD in that both are chronic relapsing conditions with underlying excessive immune responsiveness and inappropriate inflammation (Cerquetella *et al.*, 2010). DCs may also play a role in the breakdown of tolerance seen in dogs with this disease. Investigating the number and phenotype of DCs in dogs with IBD may help with future diagnostic and therapeutic interventions.

The first aim of the present study was to determine whether there is a significant difference in the number of DCs in the duodenum, ileum and colon of dogs with IBD compared with healthy controls. CD11c is a β-integrin that is used as a cell surface marker for the identification of DCs in mice (Henri *et al.*, 2001). This marker is also used to identify human DCs, but is commonly combined with other markers as it is known to be expressed also by human macrophages and other cell types (Springer, 1994; Petty and Todd, 1996; Ihanus *et al.*, 2007). Canine cells that express CD11c have a phenotype and function consistent with DCs (Isotani *et al.*, 2006; Mielcarek *et al.*, 2007; Wang *et al.*, 2007). Unlike in man, CD11c is more widely associated with canine DCs than tissue macrophages (Danilenko *et al.*, 1992). Therefore, for the purpose of this study, CD11c was used as a marker for canine DCs.

The World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group has recently published guidelines for the evaluation and scoring of canine intestinal inflammation (Day *et al.*, 2008; Washabau *et al.*, 2010); however, further studies have suggested that this system correlates poorly with the severity of clinical activity (Allenspach *et al.*, 2010; Willard *et al.*, 2010). The second aim of the present study was therefore to determine whether there is a correlation between CD11c expression, the canine chronic enteropathy clinical activity index (CCECAI) and the WSAVA intestinal histopathology score in dogs with IBD. If such a correlation existed, CD11c expression might serve as a potential marker of the severity of canine IBD.

Materials and Methods

Case Material

Endoscopically-derived tissue samples of duodenum ($n = 12$ cases), ileum ($n = 8$ cases) and colon ($n = 12$ cases) were collected from dogs during the diagnostic investigation of IBD. The samples from duodenum, ileum and colon generally did not come from the same dogs. Only one dog with IBD had all three sites sampled. One dog with IBD had both duodenal and ileal biopsy tissues collected and five dogs had both ileal and colonic biopsies collected. Therefore, a total of 26 dogs with IBD were investigated in this study.

All cases had a detailed medical history taken at the time of first examination and at subsequent re-examinations, haematological and serum biochemical analyses, ultrasonography and histopathological examination of biopsy samples. Known causes of GI inflammation were ruled out by these examinations and by measurement of the serum concentration of trypsin-like immunoreactivity. Faecal flotation, including zinc flotation to detect *Giardia* spp., and culture of faeces for *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp. and *Yersinia* spp. was carried out in all dogs. Definitive diagnosis of IBD was based on microscopical evidence of an inflammatory infiltrate within the lamina propria of endoscopically-obtained samples of intestinal mucosa. None of the dogs recruited into the study had prior glucocorticoid or other anti-inflammatory medication within 3 months of being diagnosed. The CCECAI was calculated for all dogs with IBD (Allenspach *et al.*, 2007).

Control tissues comprised full-thickness and endoscopic samples from the duodenum, ileum and colon of 10 healthy beagle dogs. These dogs were humanely destroyed for reasons unrelated to the present study. The health of these dogs had been monitored daily (including evaluation of faecal consistency). Necropsy examinations were carried out while harvesting the intestinal tissues and no gross lesions were visible in any of the dogs.

Histology and Immunofluorescence Microscopy

Formalin-fixed and paraffin wax-embedded tissues from each dog with IBD were used to prepare sections stained by haematoxylin and eosin (HE). These sections were reviewed and scored according to the WSAVA guidelines by a board-certified veterinary pathologist. As there are guidelines only for the stomach, duodenum and colon, ileal sections were not scored. Sections of intestinal tissue from the healthy beagle dogs were also assessed.

Tissues for immunofluorescence (IF) microscopy were embedded in optimal cutting temperature (OCT) compound (Miles Inc., Elkhart, Indiana, USA) and frozen in liquid nitrogen, followed by storage at -80°C until further use. Frozen tissue blocks were warmed to -20°C and serial sections (8–10 μm) were prepared and stored at -80°C in air-tight boxes until further use.

Slides were removed from storage and warmed to room temperature. Sections were fixed for 10 min in cold (-20°C) acetone and the tissues circled with a hydrophobic pen and rehydrated with 600 μl of Tris-buffered saline (TBS) per section for 10 min. Sections