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Research paper

Immunohistochemical characterization of gastrointestinal macrophages/phagocytes in dogs with inflammatory bowel disease (IBD) and non-IBD dogs

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ABSTRACT

Intestinal Mϕ play a pivotal role in the maintenance of gut homeostasis, but can also contribute to inflammation such as inflammatory bowel disease (IBD). In contrast to human tissues, little is known about phenotypes of Mϕ in the canine gastrointestinal tract. Therefore, an immunohistochemical study was performed using Abs against Mϕ-associated molecules (Cluster of differentiation (CD)64, CD163, CD204, ionized calcium-binding adaptor molecule 1, L1 Ag, and MHC II) on stomach, duodenum, jejunum, ileum and colon from non-IBD dogs. In addition, marker-expression in the stomach, duodenum and colon of the non-IBD dogs was compared to that in dogs with IBD. Results revealed predominance of resident Mϕ displaying an anti-inflammatory phenotype represented by expression of CD163 as well as CD204 in the gut of non-IBD dogs with high Mϕ numbers especially present in the small intestinal villus area. Compared to non-IBD tissue counterparts, stomach, duodenum, and colon from dogs with IBD showed reduced Mϕ numbers with the exception of slightly increased numbers of CD64 + Mϕ. Correlation analyses between marker-expression of Mϕ and the Canine Inflammatory Bowel Disease Activity Index as well as histological scores failed to reveal relevant relationships. The present study provides evidence of the canine steady state gastrointestinal tract being dominated by Mϕ with anti-inflammatory properties maintaining gut homeostasis. A significant reduction in these resident Mϕ may reflect disturbances in homeostatic capacity that could contribute to the development of canine IBD. In contrast to human IBD and murine disease models, infiltration of pro-inflammatory Mϕ does not significantly contribute to the inflammatory process of canine IBD, which may illustrate possible species-specific differences in IBD pathogenesis.

1. Introduction

Harboring a high load of microbiota and nutrient contents the gut represents the organ with the most challenging exposure to exogenous Ags (Sanders et al., 2017). Intestinal Mϕ act together with other cell types of the innate (e.g. dendritic cells, DCs) and adaptive immune system (e.g. regulatory T-cells) to balance between tolerance towards beneficial microbiota and food Ags and protection against harmful pathogens. In the steady state, resident Mϕ in the intestinal lamina propria (LP) contribute to homeostasis by secreting cytokines, particularly IL-10, as well as factors which are essential for the integrity of the epithelial layer, e.g. prostaglandin E2. In case of infection, bone marrow-derived monocytes are recruited to the intestinal LP where they mature into pro-inflammatory Mϕ that fulfill protective functions by phagocytosis and destruction of pathogens such as bacteria, but also secrete chemotactic cytokines resulting in recruitment of leukocytes that contribute to tissue damage (Grainger et al., 2017). In contrast to resident Mϕ in other tissues, intestinal monocyte-derived Mϕ do not respond to stimuli received from toll-like receptor activation with initiation of an inflammatory reaction (Smythies et al., 2005). This anergic status is gained by down-regulation of downstream signaling pathways and protects the host from ongoing inflammation induced by harmless commensals (Bain et al., 2013; Smith et al., 2005). Disbalances of this vulnerable condition can lead to permanent Mϕ activation and chronic inflammatory diseases such as inflammatory bowel disease (IBD) (Magnusson et al., 2016).

Abbreviations: CIBDAI, Canine Inflammatory Bowel Disease Activity Index; CD, cluster of differentiation; DC(s), dendritic cell(s); EGE, eosinophilic gastroenteritis; HE, hematoxylin and eosin; HUC, histiocytic ulcerative colitis; Iba-1, ionized calcium-binding molecule 1; IHC, immunohistochemistry; IBD, inflammatory bowel disease; LP, lamina propria; LPE/C, lymphoplasmacytic enteritis/colitis; MC, Morbus Crohn; PAS, periodic acid-Schiff; SR, scavenger receptor; UC, ulcerative colitis; WSAVA, World Small Animal Veterinary Association

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Table 1
Detailed information about IBD cases and non-IBD dogs.

Dog no.	Group	Sex	Age (years)	Breed	Diagnosis
1	IBD	F	3	Golden Retriever	LPGE
2	IBD	M	2	Australian Shepherd	LPGE
3	IBD	NM	8	Crossbreed	LPGE
4	IBD	NM	3	Bernese Mountain Dog	LPEC
5	IBD	M	5	Golden Retriever	LPGEC
6	IBD	F	11	Airedale Terrier	LPGEC
7	IBD	NF	3.5	Spitz	LPGEC
8	IBD	M	6	Jack Russel Terrier	LPGEC
9	IBD	M	5.5	Jack Russel Terrier	LPGEC
10	IBD	F	8	Jack Russel Terrier	LPGEC
11	IBD	NF	7	Jack Russel Terrier	LPGEC
12	IBD	M	2	Yorkshire Terrier	EGE
13	IBD	F	7	Crossbreed	EEC
14	IBD	M	3	Berger Blanc Suisse	EEC
15	IBD	NF	2.25	Crossbreed	EEC
16	IBD	NM	4	Fox Terrier	EEC
17	IBD	NM	3	Maltese	EGEC
18	IBD	NF	6	Australian Shepherd	EGEC
19	IBD	F	8	Irish Terrier	EGEC
20	IBD	NF	5	Miniature Schnauzer	EGEC
21	IBD	NF	12	Dachshund	EGEC
22	Control	F	12.75	German Shepherd Dog	Hemangiosarcoma
23	Control	M	4	Siberian Husky	Idiopathic epilepsy
24	Control	NF	14.75	West Highland White Terrier	HCM
25	Control	M	10	Crossbreed	GME
26	Control	M	4.25	Kuvasz	Spindle cell sarcoma
27	Control	M	4.5	Australian Shepherd	GME
28	Control	M	6.25	Yorkshire Terrier	NLE
29	Control	F	15.25	Crossbreed	Pancreatic adenocarcinoma
30	Control	F	9	Beagle	Tonsillar SSC
31	Control	NM	5.5	Labrador Retriever	Osteosarcoma

IBD, inflammatory bowel disease; F, female; M, male; NM, neutered male; NF, neutered female; LPGE, lymphoplasmacytic gastroenteritis; LPEC, lymphoplasmacytic enterocolitis; LPGEC, lymphoplasmacytic gastroenterocolitis; EGE, eosinophilic gastroenteritis; EEC, eosinophilic enterocolitis; EGEC, eosinophilic gastroenterocolitis; HCM, hypertrophic cardiomyopathy; GME, granulomatous meningoencephalitis; NLE, necrotizing leucoencephalitis; SSC, squamous cell carcinoma.

In dogs the term IBD is used for a group of different chronic enteropathies with unknown etiology (Jergens and Simpson, 2012). Clinically, affected dogs show permanent or recurrent gastrointestinal symptoms like diarrhea, vomiting, anorexia and weight loss for at least a three-week period. To assess clinical disease activity a scoring index (Canine Inflammatory Bowel Disease Activity Index, CIBDAI) was established which includes six typical signs of gastrointestinal disorder (attitude/activity, appetite, vomiting, stool consistency, stool frequency, and weight loss) (Jergens et al., 2003). Although the exact etiology is still not known, a disturbed balance between commensal bacteria and host immunological response in a genetically predisposed individual is assumed to play a crucial role in disease development (Jergens and Simpson, 2012). Usually, in addition to the ruling out of other causes, the diagnosis of IBD requires histopathological examination of intestinal biopsies. The hallmark of the disease is a slightly to markedly increased infiltration of the LP, mainly consisting of lymphocytes and plasma cells (lymphoplasmacytic enteritis/colitis, LPE/C) which might be accompanied by salient amounts of eosinophilic granulocytes (eosinophilic gastroenteritis, EGE) (Jergens and Simpson, 2012). A standardized scoring system is widely accepted for the evaluation of intestinal biopsies of the stomach, duodenum, and colon of diseased animals including architectural changes like villus atrophy, crypt distension, and fibrosis as well as LP infiltration of lymphocytes, plasma cells, eosinophils and neutrophils (Day et al., 2008). Studies dealing with the involvement of Mϕ in canine IBD are missing until now.

IBD in humans mainly occurs in two different forms: ulcerative colitis (UC), and Morbus Crohn (MC). Infiltrating Mϕ in MC are supposed to exhibit a pro-inflammatory phenotype (Kamada et al., 2008; Thiesen et al., 2014), while the phenotype of Mϕ in UC is less obvious and cells exhibiting both pro- and anti-inflammatory properties are present (Isidro and Appleyard, 2016).

Since little is known about phenotypes and distribution of canine intestinal Mϕ we performed an immunohistochemical study on tissue sections of the stomach, small intestine and colon of non-IBD dogs. Thereby, the study aimed to evaluate if, like in murine tissues (Bain et al., 2013), there is a predominance of anti-inflammatory (resident) Mϕ in the gut of non-IBD dogs and if newly recruited, pro-inflammatory Mϕ accumulate in the intestinal LP of dogs with IBD. Different Mϕ markers were used including Cluster of Differentiation (CD)163 and CD204 (anti-inflammatory Mϕ), CD64 (pro-inflammatory Mϕ), and L1 Antigen (newly recruited Mϕ). Moreover, Abs against Iba-1 and MHC II were used, which both are expressed by Mϕ but also other antigen presenting cells such as DCs.

2. Material and methods

2.1. Case material

For this study, material from IBD dogs no. 1–21 and non-IBD dogs no. 24–31 was reused from previous studies and detailed information concerning clinical data and work-up is available elsewhere (Junginger et al., 2014). Briefly, endoscopic biopsies from 21 dogs diagnosed with IBD were received from the Small Animal Clinic Duisburg-Asterlagen, Germany. Prior to diagnosis, these dogs showed chronic (> 3 weeks) or recurrent clinical signs of gastrointestinal disease. Detailed clinical work-up did not reveal any appreciable findings and diagnostic antimicrobial treatment as well as dietary trials did not lead to any clinical improvement. Therefore, etiologic differentials (e.g., bacterial or parasitic infection, extra-intestinal diseases, food-responsive enteropathy, antibiotic-responsive enteropathy) were ruled out. During enteroscopy under general anesthesia adequate (Jergens et al., 2016)