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Review

Cytokines in canine inflammatory bowel disease

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Abstract

Canine inflammatory bowel disease is a group of chronic enteropathies characterized by persistent or recurring gastric symptoms with an unknown etiology which are related to histopathological changes in the mucosa of the small and large bowel in the form of cellular infiltration in the mucosal lamina propria. Recent years have witnessed a growing number of investigations into the role of the immune system and, in particular, cytokines in the development of IBD. In this article, the expression of pro-inflammatory (IL-1, IL-2, IL-5, IL-6, IL-12, IL-18, IFN-γ, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) was compared in canine patients with IBD based on clinical presentation, breed, lamina propria cell infiltrate and histopathological grade. Only selected studies confirmed higher mRNA expression levels of cytokines IL-2, IL-4, IL-5, IL-12p40, IFN-γ, TNF-α and TGF-β in dogs with IBD in comparison with healthy subjects. GSD were strongly represented in most study populations. Dogs with LPE were characterized by elevated levels of IL-1α, IL-1β, IL-2, IL-5, IL-6, IL-12, TNF-α, TGF-β. The present studies of canine patients with LPC revealed the mRNA expression of cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p35, IL-12p40, IFN-γ, TNF-α, TGF-β. In the reviewed studies, the progression of IBD was not accompanied by changes in the mRNA expression of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, TNF-α, IFN-γ or TGF-β.

Key words: inflammatory bowel disease (IBD), cytokines, dog

List of Abbreviations:
ARE – antibiotic response enteropathy
CIBDAI – canine inflammatory bowel disease activity index
EGE – eosinophilic gastroenteritis
FRE – food response enteropathy
GSD – German shepherd dogs
HUC – histiocytic ulcerative colitis
IBD – inflammatory bowel disease
IFN-γ – interferon gamma
IL – interelukine/cytokine
LPC – lymphocytic-plasmacytic colitis
LPE – lymphocytic-plasmacytic enteritis
TGF-β – transforming growth factor beta
TNF-α – tumor necrotic factor alpha

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Canine inflammatory bowel disease is a group of chronic enteropathies characterized by persistent or recurring gastric symptoms with an unknown etiology. The disease is accompanied by cellular infiltration in the mucosal lamina propria of the small and large bowel in the form of lymphocytes, plasmocytes, eosinophils and neutrophils (Allenspach and Gaschen 2003, Jergens et al. 2003, Craven 2004, Day et al. 2008, Simpson and Jergens 2011, Jergens and Simpson 2012). The most common forms of IBD are lymphocytic-plasmacytic enteritis (LPE), lymphocytic-plasmacytic colitis (LPC) and eosinophilic gastroenteritis (EGE) (Simpson and Jergens 2011, Jergens and Simpson 2012). The inflammation may affect the intestinal region between the duodenum and the large intestine, but in dogs 75% of inflammatory processes are localized in the anterior section of the small intestine (German et al. 2003, Hall 2007).

The etiology of IBD has not been fully elucidated. It is believed that canine inflammatory bowel disease is conditioned by complex interactions between bacterial and environmental factors, genetic predispositions, the side effects of certain drugs and reduced intestinal immunity (Bhatia and Tandon 2005, Hall 2007, Simpson and Jergens 2011, Jergens and Simpson 2012). The key role in the pathogenesis of IBD is ascribed to the loss of tolerance for endogenous microflora, food antigens or endogenous antigens which lead to chronic inflammations of the gastrointestinal tract (Allenspach et al. 2007, Xenoulis et al. 2008). The loss of permeability in the gastrointestinal mucosa and immunological aberrations in the gastrointestinal system produce an incorrect immune response (German et al. 2001, Hall 2007, Mc Cann et al. 2007, Sauter et al. 2007).

Recent years have witnessed a growing number of investigations into the role of the immune system and, in particular, cytokines in the development of IBD (Table 1). It is believed that a defective immune response to antigens present in the gastrointestinal system (microorganisms, nutrients) leads to aberrations in the inflammatory response, non-specific damage to intestinal tissue and inflammatory cell infiltration (Simpson and Jergens 2011, Jergens and Simpson 2012). When antigens come into contact with the intestinal wall, they can damage the epithelial barrier, thus enabling bacterial and/or food antigens to penetrate deeper intestinal layers. The above causes a local inflammatory reaction and non-specific damage to intestinal tissue. The arachidonic acid cascade is activated to release inflammatory mediators: prostaglandins, leukotrienes, thromboxanes and free radicals (Fuss et al. 1996). Activated B cells synthesize immunoglobulins, and stimulated T-helper (Th) lymphocytes enhance the production of cytokines which regulate the immune response (Powrie et al. 1993, Powrie et al. 1994, Berg et al. 1996, Shanahan 2002). The resulting imbalance between pro-inflammatory and anti-inflammatory cytokines disrupts the mechanisms of intestinal immunity (Jump 2004, Owczarek et al. 2009, Polińska et al. 2009).

Table 1. Biologically active factors responsible for the development of human and canine inflammatory bowel disease (IBD).

Growth factors	– Transforming growth factor beta (TGF-β) – Platelet- activating factor – Keratinocyte growth factor
Pro-inflammatory cytokines	– Interleukin 1 (IL-1) – Interleukin 2 (IL-2) – Interleukin 6 (IL-6) – Interleukin 9 (IL-9) – Interleukin 12 (IL-12) – Interleukin 18 (IL-18) – Interleukin 23 (IL-23) – Tumor necrosis factor-alpha (TNF-α) – Interferon gamma (IFN-γ)
Anti-inflammatory cytokines	– Interleukin 4 (IL-4) – Interleukin 10 (IL-10) – Interleukin 13 (IL-13)
Chemokines	– Interleukin 8 (IL-8) – Monocyte chemotactic protein 1 (MCP-1)

The results of recent research suggest that cytokines play a key role in inflammatory bowel disease. Cytokines are glycoproteins with molecular mass of several to more than 10 kDa, and they are released by activated cells of various tissues, including gastrointestinal epithelial cells. Cytokine networks are part of extensive control systems in various tissues, they affect the nervous system and connective tissue, and they control numerous processes, including cell proliferation, differentiation and mobility. Cytokines have hormone-like properties, they participate in the formation of blood cells, they influence the function of all bodily cells and condition their mutual interactions. Cytokines are mediators of inflammatory processes, immune responses, tissue repair and healing processes (Naito et al. 2004, Yen et al. 2006, Xavier and Podolsky 2007, Holtta et al. 2008, Owczarek et al. 2009, Polińska et al. 2009). Subject to their properties, cytokines are classified as either pro-inflammatory or anti-inflammatory (Sartor 1995, Polińska et al. 2009). Pro-inflammatory cytokines participate in IBD by initiating, enhancing and maintaining the inflammatory process. This group of cytokines includes IL-1, IL-2, IL-5, IL-6, IL-8, IL-9, IL-12, IL-17, IL-18, IL-23,

IFN-γ and TNF-α (Rogler and Andus 1998, Sartor 2006, Sanchez-Munoz et al. 2008). Anti-inflammatory cytokines IL-4, IL-10, IL-11 and IL-13 inhibit the inflammatory process in intestinal mucosa. They are produced mainly by stimulated Th2 lymphocytes (Rogler and Andus 1998, Fuss et al. 2004, Sartor 2006, Sanchez-Munoz et al. 2008).

Cytokines in Canine Inflammatory Bowel Disease

There is a general scarcity of research studies evaluating the role of pro-inflammatory and anti-inflammatory cytokines in the pathogenesis of canine inflammatory bowel disease. In a recent study, German described balanced mRNA expression of pro-inflammatory and anti-inflammatory cytokines in German shepherds dogs with small intestinal enteropathy, but this condition was observed in only four dogs with lymphocytic-plasmacytic enteritis LPE (German et al. 2000). Ridyard analyzed the mRNA expression of intestinal mucosal cytokines in dogs with lymphocytic-plasmacytic colitis LPC and observed enhanced expression of IL-2 and TNF-α (Ridyard et al. 2002). Elevated levels of IL-4 and IL-12 in dogs with IBD in comparison with healthy subjects were confirmed only by Jergens (Jergens et al. 2009). Peters did not report differences in cytokine expression levels in the duodenal mucosa of healthy dogs and subjects affected by chronic diarrhea, but the subjects were not divided into groups with IBD, antibiotic response enteropathy (ARE) or food response enteropathy (FRE) in analyses of their response to treatment (Peters et al. 2005). Due to variations in the reported results, the cytokines that control or stimulate the local immune response in canine IBD have not been fully identified. In this article, the expression of pro-inflammatory and anti-inflammatory cytokines was compared in dogs with inflammatory bowel disease based on clinical presentation, breed, lamina propria cell infiltrate and histopathological grade.

Classification based on clinical presentation

Jergens investigated dogs with symptoms of inflammatory disease of the small and large intestine (Jergens et al. 2009). The control group comprised of patients which had not shown any symptoms of gastrointestinal disease over a period of 42 days before an endoscopic examination. Jergens demonstrated that in dogs affected by an inflammation of the small intestine, the mRNA expression of cytokines IL-1α, IL-1β, IL-2, IL-10, TNFα and IFN-γ was reduced, whereas the mRNA expression of cytokine IL-12 was enhanced in comparison with the values noted in the control group. Dogs diagnosed with an inflammation of the large bowel showed reduced mRNA expression of IL-2 and TGF-β and elevated mRNA expression of IL-4 in comparison with control. The small intestinal mucosa of healthy subjects was characterized by higher concentrations of cytokines IL-2, IL-4 and IL-10 than in the large intestinal mucosa, whereas the concentrations of TGF-β were higher in the large intestinal mucosa of healthy dogs than in sections of small intestinal mucosa. De Majo compared cytokine expressions in healthy dogs and in subjects with IBD (De Majo et al. 2008). In a clinical test, the experimental dogs showed symptoms of inflammation of the small intestine (tarry stool), the large intestine (mucus in stool) or mixed symptoms (mucus and blood in stool, higher frequency of bowel movements, vomiting, weight loss). The control group comprised dogs without chronic diarrhea. No significant differences in the mRNA expression of cytokines TNF-α and IFN-γ were observed in biopsy specimens from small and large intestinal mucosa. In a study by Peters, dogs were divided into two groups (Peters et al. 2005). The control group consisted of dogs without symptoms of chronic diarrhea which were subjected to endoscopic examinations of the gastrointestinal tract for other reasons (gastritis, esophageal hiatus hernia, megaesophagus, uncontrolled aggression, spinal cord tumors). The experimental group consisted of dogs with chronic diarrhea. No significant differences were determined in the expression of mRNA encoding cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFN-γ, TNF-α and TGF-β between dogs with IBD and healthy patients. In Ridyard's study, the experimental group consisted of dogs with prolonged gastrointestinal symptoms (diarrhea, higher frequency of bowel movements, mucus and blood in stool, vomiting, abdominal pain) lasting longer than four weeks (Ridyard et al. 2002). Control group dogs did not show chronic gastrointestinal symptoms and were euthanized on account of behavioral problems, old age, diabetes insipidus and urinary incontinence. In this study significantly elevated levels of IL-2 and TNF-α were reported in the group of dogs with LPC in comparison with control. In the work of German, the experimental group comprised of German shepherd dogs with gastrointestinal symptoms (diarrhea, vomiting, weight loss, varied appetite and coprophagia) persisting for more than three months (German et al. 2000). Control group dogs did not show any gastrointestinal symptoms. The mRNA expression of cytokines IL-2, IL-5, IL-12p40, IFN-γ, TNF-α and TGF-β was higher in experimental dogs than in control. Elevated levels of pro-inflammatory cytokines (IL-2, IL-5, IL-12p40,

IFN-γ, TNF-α,), anti-inflammatory cytokines (IL-4) and transforming growth factor β (TGF-β) in the group of dogs with IBD were reported only in selected studies (German et al. 2000, Ridyard et al. 2002, Jergens et al. 2009). As the knowledge of the immune background of canine inflammatory bowel diseases is being expanded, assessment of immune markers raises hopes for noninvasive diagnostic methods. The basic problem in the presented studies is the lack of correlation between elevated levels of pro-inflammatory and anti-inflammatory cytokines and the type and intensity of clinical symptoms (canine inflammatory bowel disease activity index CIBDAI). Despite insufficient correlation of the aforementioned immunological assays and the clinical assessment of the intensity of the disease, these assays may be important auxiliary elements in the diagnostics of canine IBD.

Classification based on breed

German shepherd dogs were strongly represented in the majority of the studied populations. In the work of Ridyard, 5 out of 14 dogs with LPC were GSD. In the studies carried out by Peters, the control group comprised 4 GSD and 14 dogs of other breeds without clinical gastrointestinal symptoms, whereas the experimental group included 12 GSD and 27 representatives of other breeds with prolonged diarrhea (Peters et al. 2005). No significant variations in the mRNA expression of cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFN-γ, TNF-α and TGF-β were observed between groups. In the work of German, the control group was made up of 2 GSD and 10 dogs of other breeds, and the experimental group consisted of 16 GSD with chronic enteropathy (German et al. 2000). In German shepherd dogs with LPE, the mRNA expression of cytokines IL-2, IL-5, IL-12p40, IFN-γ, TNF-α and TGF-β was higher than in control. German shepherd dogs were overrepresented in three out of the seven reviewed experiments, and elevated levels of IL-2, IL-5, IL-12p40, IFN-γ, TNF-α and TGF-β in the above breed were noted only by German (German et al. 2000). The conducted studies showed no unambiguous correlation between overexpression of pro-inflammatory and anti-inflammatory cytokines and the development of IBD in German shepherd dogs. Recent studies showed that etiopathogenesis of IBD in GSD may be related to the impact of toll-like receptor TLR2, TLR4 and TLR5 gene polymorphisms on the capability to produce various cytokines in lymphocytes involved in the immune reaction within the gastrointestinal tract (Allenspach et al. 2010). Such polymorphisms may negatively influence the immune processes and disturb composition of intestinal microflora, thus contributing to the development of inflammatory bowel disease in GSD.

Classification based on lamina propria cell infiltrate

Jergens investigated dogs with lymphocytic-plasmacytic colitis and enteritis (Jergens et al. 2009). The mRNA expression of cytokines was more frequently observed in sections of the duodenal mucosa. Transcriptional responses to cytokines IL-1α, IL-1β, IL-2, IL-5, IL-12, TNF-α and TGF-β were reported in 27 out of 37 dogs with lymphocytic-plasmacytic enteritis. In this study in the experimental group, the mRNA expression of cytokines IL-1β, IL-4, IL-5, TNF-α and TGF-β was detected in 5 out of 11 patients with lymphocytic-plasmacytic colitis. De Majo studied subjects with lymphocytic-plasmacytic duodenitis, eosinophilic duodenitis, lymphocytic-plasmacytic colitis and histiocytic ulcerative colitis (De Majo et al. 2008). Very high TNF-α levels were noted in a patient with HUC. The mRNA expression of cytokines TNF-α and IFN-γ was reported in all duodenal mucosa sections from experimental dogs. In a study by Peters, dogs were divided into groups based on the predominant type of cellular infiltration in the lamina propria of the duodenal mucosa (Peters et al. 2005). In experimental group dogs, a histopathological analysis revealed predominant infiltration of lymphocytes, plasmocytes, eosinophils and various inflammatory cells in the lamina propria of duodenal mucosa. No significant differences were observed in the mRNA expression of cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFN-γ, TNF-α and TGF-β between groups. In Ridyard's experiment, a histopathological examination of biopsy sections from the studied dogs confirmed lymphocytic-plasmacytic colitis (Ridyard et al. 2002). In the experimental group, the mRNA expression of cytokines IFN-γ, TNF-α, IL-2 and IL-6 was reported in colonic mucosa specimens obtained from all dogs, TGF-β and IL-12p40 transcripts were found in 13 out of 14 dogs, and IL-10 and IL-12p35 in 12 out of 14 subjects. In another study pro-inflammatory (IL-6) and anti-inflammatory cytokines (IL-10) were found to participate in the pathogenesis of canine IBD (Chrząstowska 2009). The patients were diagnosed with LPE and LPC. High levels of pro-inflammatory cytokine IL-6 and low levels of anti-inflammatory cytokine IL-10 were observed. Other experiments revealed significant differences in the serum levels of pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 in dogs with IBD (Malewska 2010). A histopathological analysis of intestinal mucosa sections supported diagnoses of lymphocytic-

-plasmacytic duodenitis and jejunitis. In the above experiment, high levels of IL-6 and low levels of IL-10 were noted in dogs with IBD. High IL-6 levels were correlated with high CIBDAI scores (4-8 points). To sum up, the mRNA expression of pro-inflammatory and anti-inflammatory cytokines in dogs with IBD was more frequently observed in specimens of small intestinal mucosa. Dogs with LPE were characterized by elevated levels of IL-1α, IL-1β, IL-2, IL-5, IL-12, TNF-α, TGF-β (Jergens et al. 2009) and IL-6 (Chrząstowska 2009, Malewska 2010). No significant variations were found in the mRNA expression of pro-inflammatory and anti-inflammatory cytokines between dogs with lymphocytic-plasmacytic duodenitis and eosinophilic duodenitis (Peters et al. 2005). The reviewed studies of canine patients with lymphocytic-plasmacytic colitis revealed the mRNA expression of cytokines IL-1β, IL-4, IL-5, TNF-α, TGF-β (Jergens et al. 2009), cytokines IL-2, IL-6, IL-10, IL-12p35, IL-12p40, IFN-γ, TNF-α, TGF-β (Ridyard et al. 2002) and cytokine IL-6 (Chrząstowska 2009). Highly elevated levels of TNF-α were reported in a dog with HUC (De Majo et al. 2008). The above observation can be attributed to extensive damage of large bowel mucosa or the presence of cells which participate in immunological processes (macrophages).

Classification based on histopathological grade

In the work of Jergens, the intensity of LPC and LPE in the experimental group was not accompanied by the mRNA expression of cytokines IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-12, TNF-α and IFN-γ (Jergens et al. 2009). The results of a histopathological analysis performed in experimental dogs revealed mild and moderate forms of LPC and LPE. Peters did not observe differences in the mRNA expression of cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFN-γ, TNF-α and TGF-β between groups characterized by varying degrees of pathological changes in the duodenal mucosa. A histopathological examination revealed mild, moderate or severe duodenitis in the subjects (Peters et al. 2005). Malewska did not observe significant differences in the levels of cytokines IL-6 and IL-10 (Malewska 2010). In this study the analyzed dogs were diagnosed with mild or moderate lymphocytic-plasmacytic duodenitis and jejunitis. In the reviewed studies, the intensity of LPC and LPE in experimental dogs was not accompanied by the mRNA expression of pro-inflammatory and anti-inflammatory cytokine mRNA (Peters et al. 2005, Jergens et al. 2009, Malewska 2010).

Conclusions

The reviewed body of literature indicates that the immune system plays an important role in the pathogenesis of inflammatory bowel disease. The analyzed studies pointed to the activation of T lymphocytes, monocytes and macrophages which are a source of cytokines affecting the inflammatory or immune response. Drugs which directly target immune responses and the inflammatory process offer new hope in the treatment of inflammatory bowel disease. The above claim is based on the observation that blocking a single cytokine which is vital for the process can produce profound effects in IBD treatment (Sandborn and Targan 2002, Chojnacki and Wichan 2004). Canine IBD is characterized by altered mucosal cytokine profiles compared to healthy animals showing mixed Th1/Th2 cytokine activation (German et al. 2000, Peters et al. 2005, Jergens et al. 2009, Jergens and Simpson 2012). In the reviewed studies (Ridyard et al. 2002, Peters et al. 2005, Jergens et al. 2009, Malewska 2010), the expression of pro-inflammatory and anti-inflammatory cytokines in dogs with IBD was not significantly correlated with breed or histopathological grade. In selected studies, no variations in the mRNA expression of pro-inflammatory and anti-inflammatory cytokines were observed between healthy subjects and dogs with IBD (Peters et al. 2005, De Majo et al. 2008). Higher levels of cytokines IL-4 and IL-12 in IBD patients in comparison with healthy dogs were reported only by Jergens (Jergens et al. 2009). In the work of Ridyard, the levels of cytokines IL-2 and TNF-α were significantly higher in dogs with LPC than in the control group (Ridyard et al. 2002). German observed higher mRNA expression levels of cytokines IL-2, IL-5, IL-12p40, IFN-γ, TNF-α and TGF-β in GSD, but the above results were reported in only 4 dogs with LPE (German et al. 2000). A comparison of expression levels of pro-inflammatory and anti-inflammatory cytokines in healthy dogs and subjects with chronic enteropathy is presented in Table 2. Cytokine levels do not seem to be a vital marker of IBD in canine patients because the mRNA expression of pro-inflammatory and anti-inflammatory cytokines was also observed in sections of small and large intestinal mucosa obtained from healthy patients (De Majo et al. 2008, Jergens et al. 2009). The mechanism of cytokine activity should be further studied to expand our understanding of the pathogenesis of inflammatory bowel disease and to develop effective forms of treatment. Such efforts require more studies investigating larger groups of patients characterized by greater uniformity in breed, clinical symptoms, type of cellular infiltration in the lamina propria and disease progression.

Table 2. Overview of studies evaluating the mRNA expression of intestinal cytokines in dogs with IBD and healthy dogs.

Reference	Dogs	IL-1	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IL-18	IFNγ	TNFα	TGFβ
Jergens et al. (2009) Small intestinal data	– 9 disease-free – 48 with IBD	↓	↓	↔	↔	NA	↓	↑	NA	↓	↓	↔
Jergens et al. (2009) Colonic data		↔	↓	↑	↔	NA	↔	↔	NA	↔	↔	↓
De Majo et al. (2008)	– 4 disease-free – 11 with IBD	NA	NA	↔	NA	NA	NA	NA	NA	↔	↔	NA
Peters et al. (2005)	– 18 disease-free – 39 with chronic enteropathy	NA	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
Ridyard et al. (2002)	– 6 disease-free – 14 with IBD – 6 non-IBD diarrheic	NA	↑	↔	NA	↔	↔	↔	NA	↔	↑	↔
German et al. (2000)	– 12 disease-free – 4 with IBD – 10 ARE – 2 non-responders	NA	↑	↔	↑	NA	↔	↑	NA	↑	↑	↑

mRNA expression levels of cytokines in dogs with IBD and healthy dogs.
↑ – increased cytokine expression in diseased tissues
↓ – decreased cytokine expression in diseased tissues
↔ – no difference in transcript expression between diseased and control tissues
NA – not evaluated

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