

## Intestinal IL-17 Expression in Canine Inflammatory Bowel Disease

Research Article

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### Abstract

IL-17 plays important roles in human inflammatory bowel disease (IBD). However, little is known about the possible relation of IL-17 in canine IBD. For comparative medicine and canine disease, it is useful to demonstrate the role of IL-17 in canine IBD. In this study, 7 cases of the dog with IBD typed of lymphocytic-plasmacytic enteritis without Intestinal lymphoma were examined immunohistochemically for possible relation with IL-17. Here, we found that IL-17 is expressed in intestinal mucosa of the dog with IBD. Among 7 cases, there are strong expressions of IL-17 in cases 2, moderate expression of it in cases 2, weak expression of it in cases 2, and very low expression of it in case 1. The score of IL-17 expression is not correlated with sex, age, and the breed. Using biopsy specimen, our results revealed the immunopathological relevance of IL-17 in the dog with IBD.

**Abbreviations:** CD: Crohn's Disease; IBD: Inflammatory Bowel Disease; IHC: Immunohistochemistry; UC: Ulcerative Colitis.

### Introduction

The gastrointestinal tract is crucial for the immunological system in host defense in addition to food intake, food digestion, and nutrient absorption within humans and animals. Epithelium barrier in the intestine plays to block pathogens from the lumen. Inflammatory bowel diseases (IBDs) in dogs, as well as humans, are characterized by dysregulated intestinal inflammation and mucosal tissue damage in parts of the gastrointestinal tract [9, 11-13, 15, 18, 24, 28]. Regarding of histologic criteria, ulcerative colitis (UC) and Crohn's disease (CD) are the two types of human IBD. Despite having a common basis in over responsiveness to mucosal antigens, UC and CD have considerably different pathophysiology. In contrast, IBD in dogs divided into the several types by histological criteria of inflammatory infiltrate such as neutrophilic, eosinophilic, lymphocytic, plasmacytic and granulomatous. However, little is known whether these types reveal the common basis or different basis. Some reports show the pathology is different between humans and dogs, whereas other reports show common mucosal immune system between

human and dogs [1, 6, 8, 19].

The maintenance of intestinal homeostasis and the development of IBD involve interactions among the intestinal microflora, epithelium, and host mucosal immune system [25, 28]. Especially, the host mucosal immune system was controlled by the balance among inflammatory mediators, anti-inflammatory mediators and regulatory cytokines then contributed to the intestinal homeostasis. Cytokines have important roles in the regulation of the mucosal immune system of dogs as well as humans. In human, CD is associated with a Th1 response mainly driven by IL-12 and IFN $\gamma$  [21], while UC is driven by Th2 cytokines, such as IL-4, IL-5, and IL-13 [27]. In contrast, dogs with IBD express with a mixture of both Th1 and Th2 cytokines, including IL2, IL4, IL5, IL10, IL12, IFN $\gamma$ , TNF $\alpha$ , and TGF $\beta$ , in intestinal mucosa [17]. However, little is known about the expression of IL-17 in the dog with IBD. Data implicating mucosal IL-17 in the pathogenesis of dog with IBD are limited. Th17 is responsible for the IL-17 production, a key inflammatory cytokine and increased in human IBD [29]. The aim of the present study was to report

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the expression and localization of IL-17 in 7 cases of dogs with IBD typed of lymphocytic-plasmacytic gastroenteritis.

## Materials and Methods

### The Case Animals

The details of 7 cases of dogs with IBD typed of lymphocytic-plasmacytic gastroenteritis are given in Table 1. In all cases, a biopsy was submitted for microscopical evaluation. A sample of biopsy was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin.

### Immunohistochemistry (IHC)

IHC of 3- $\mu$ m-thick paraffin-embedded sections was performed as previously described [20], with minor modifications. We used primary antibodies against IL-17 (ab79056; Abcam, Cambridge, MA, USA). To absorption the antibody, we used IL17 peptide (ab190773; Abcam). Immunoreactivity was detected using peroxidase-conjugated anti-rabbit polymers (Histofine, Nichirei, Tokyo, Japan) and a DAB substrate kit (Vector Laboratories Inc., Burlingame, CA, USA).

### Histology

The histological score of IL-17 expression was graded by ranging from 0 to 3 as follows: 0, no expression; 1, low expression; 2, moderate expression; 3, high expression. Scores were obtained by a pathologist.

### Statistical Analysis

Histological score was statistically analyzed using the Mann-Whitney U test. Differences with P values of less than 0.05 were considered significant.

## Results

All of 7 cases investigated in this study (Table 1) were diagnosed IBD typed of lymphocytic-plasmacytic enteritis by histological criteria of inflammatory infiltrate. In addition, these 7 cases did not include intestinal lymphoma. IHC was performed to investigate the expression and localization of IL-17-positive cells in the intestinal mucosa of dogs with IBD. The treatment of recombinant IL-17 in addition to IL-17 antibody indicated as negative controls (each right panel). In Figure 1, the breed was all Dachshund. In case 1, the IL-17 was expressed in lamina propria (Figure 1). The expression of IL-17 in case 2 is stronger than it in case 1 (Figure 1). In contrast, the IL-17 was weakly expressed in lamina propria of case 3 (Figure 1). These results indicate that IL-17-positive cells are present in the lamina propria, and the expression level of IL-17 varies by case.

In case 4, there are dense IL-17-positive cells in lamina propria (Figure 2). In case 5, the IL-17 was expressed in lamina propria (Figure 2). In case 6, there is a little expression of IL-17 in lamina propria (Figure 2). In case 7, there is no marked expression of IL-17 in lamina propria (Figure 2). These results also indicate that the expression level of IL-17 varies by case.

A histological score of the IL-17 expression in all 7 cases is shown in Table 2. Regarding of the score of IL-17 expression in lamina propria, there are no significant differences in sex of dogs with IBD (Figure 3 upper). Furthermore, no significant differences in age were observed in the score of IL-17 expression in lamina propria (Figure 3 lower). These results suggest that the expression level of IL-17 may vary by the case rather than the breed.

## Discussion

In this study, we defined the mucosal IL-17 expression in intestinal biopsies from dogs with IBD. Namely, we observed marked expression of IL-17 and the different pattern of localization of

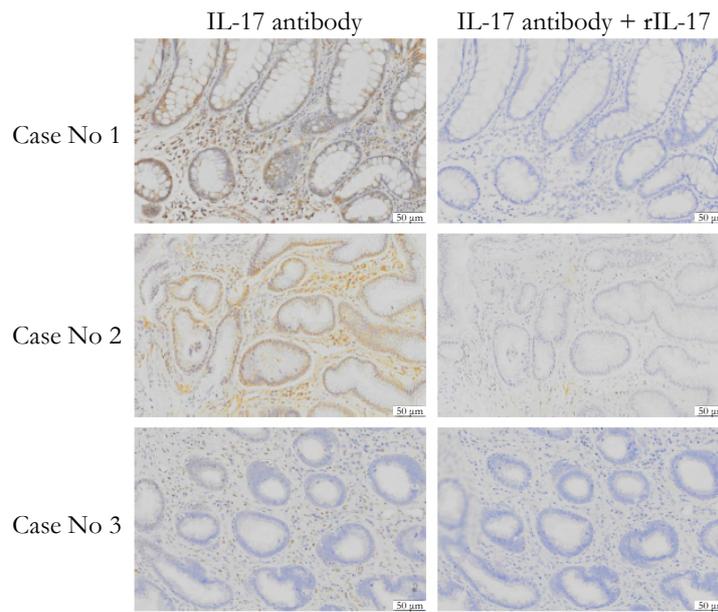
**Table 1. Details of Dogs with Lymphocytic - Plasmacytic Gastroenteritis.**

Case no.	Age (years)	Breed	Sex
1	11	Dachshund	Male
2	10	Dachshund	Female neutered
3	11	Dachshund	Male neutered
4	7	Papillon	Male neutered
5	2	Toy Poodle	Female
6	8	Shiba	Female neutered
7	10	Labrador retriever	Female neutered

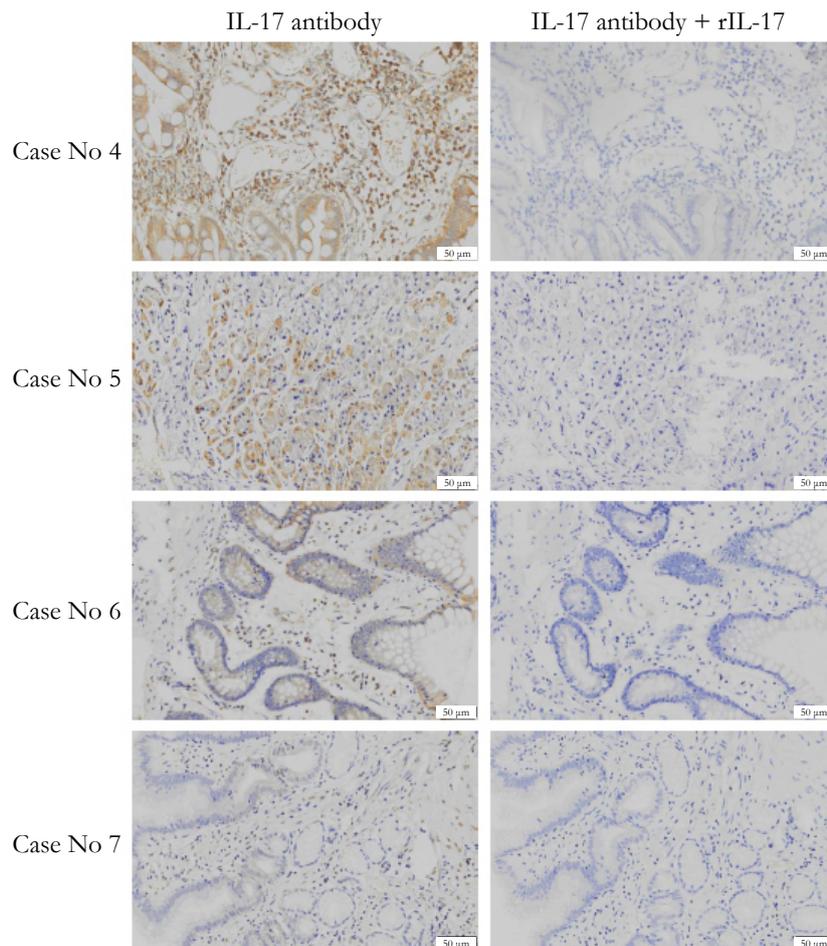
**Table 2. Histological Score of IL-17 Expression.**

Case no.	IL-17 expression score
1	++
2	+++
3	+
4	+++
5	++
6	+
7	±

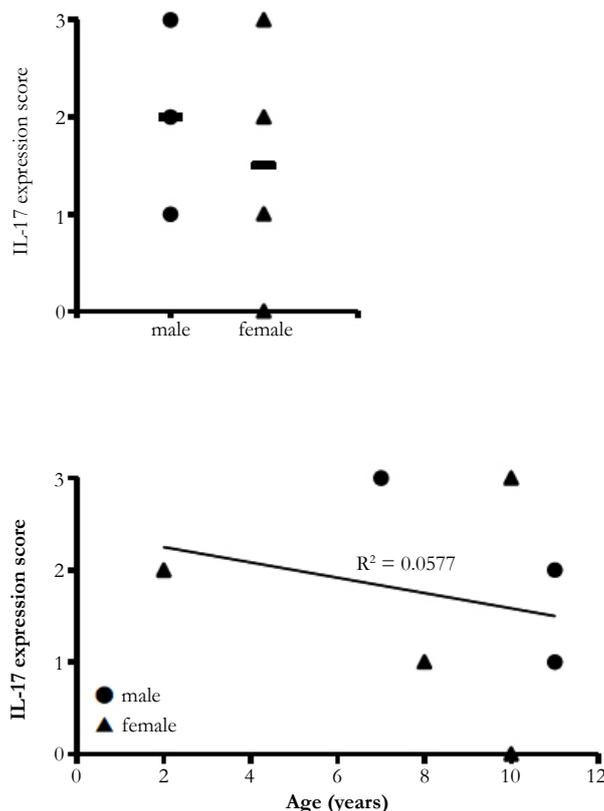
**Figure 1. Cases 1-3. Expression of IL-17 in intestinal mucosa by biopsy specimen. Expressions of IL-17 are present in the lamina propria (left panels), which are the loss by the absorption of IL-17 antibody by recombinant IL-17 (right panels). Scale bar, 50  $\mu$ m.**



**Figure 2. Cases 4-7. Expression of IL-17 in intestinal mucosa by biopsy specimen. Expressions of IL-17 are present in the lamina propria (left panels), which are the loss by the absorption of IL-17 antibody by recombinant IL-17 (right panels). Scale bar, 50  $\mu$ m.**



**Figure 3. The relation analysis. (Upper) The relation of IL-17 expression score with sex. The horizontal bar means the mean. (Lower) The relation of IL-17 expression score with age (years).**



IL-17 even the expressed in the intestinal mucosa in dogs with IBD. We press that the expression pattern of intestinal mucosa IL-17 did not depend on the breed type of dogs with IBD. These indicate that IL17-mediated immune responses are related to the development of dogs with IBD, similar to human UC, at large. The classified etiology of dogs with IBD is not well understood although dogs with IBD are compared with human IBD such as UC and CD [5, 15]. Similar to human IBD, histologic features of dogs with IBD show infiltration of mononuclear cells into the lamina propria, goblet cell depletion, crypt hyperplasia and epithelial erosion [15]. T-cells also are related to onset and development of dogs with IBD, because CD<sup>3+</sup>, CD<sup>4+</sup>, and CD<sup>8+</sup> T cells are elevated in the mucosa [10, 14, 16, 26].

Our strength of this study is that dog with IBD expressed IL-17 in duodenal mucosa. Up to the present, little has been reported on possible relation of IL-17 in canine diseases including IBD. These previous reports and our result show that IL-17 plays important role in canine as well as human. Our other strength is that a well-normalized and validated methodology of IHC is useful to evaluate the expression of interleukins in endoscopic biopsy [8, 23].

Cases 1, 2, 4 and 5 show high expression of IL-17, indicating increased infiltration of Th17 into the intestinal mucosa. In contrast, cases 3, 6 and 7 indicate low expression of IL-17, suggesting two possibilities. The first possibility is that Th17 population is low in spite of increased infiltration of the T-cells. The second possibility is that infiltration of the T-cells is not increased into the mucosa.

Unbalance among inflammatory mediators, anti-inflammatory mediators and regulatory cytokines result in IBD. Dysregulated

balance between Th1/Th17-mediated inflammatory cytokines and Th2/Treg-mediated immunoregulatory cytokines results in patients with IBD and mice models with colonic inflammation like human IBD [2-4, 7, 10, 20, 22, 23].

A limitation of our results is that dogs with IBD in each case were not almost same by age (years). Aging dogs make a spontaneous progress of terrible and prolonged enterocolitis with diarrhea and body weight loss. Further studies will be needed to assess the relation of IL-17 expression level and age of dogs. Like a human with IBD, genetic factors may contribute to the pathogenesis of dogs with IBD, because an enhanced risk for the development of IBD is related to especial breeds including Basenjis, French bulldogs, German shepherd dogs and soft-coated Wheaten terriers [10, 15]. Further studies are also needed for the expression levels of IL-17 in these breed types.

Our results indicate the increased expression of IL-17 as an inflammatory cytokine in dogs with IBD, and constitute one of the several potential immune mechanisms that can contribute to canine IBD pathology. In conclusion, our results provide the strong insight into the important roles of IL-17 in dogs with IBD. Our results are also able to contribute the relationship among IL-17 expression, canine IBD pathology, drug therapeutics, and clinical outcomes, due to the reduced number of cases. IL-17 and its receptor will be an important consideration in the design of therapeutic agents in dogs with IBD.

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