



Expression of genes encoding interleukin 15 and its receptor subunits in the duodenal and colonic mucosae of dogs with chronic enteropathy

Rintaro Furukawa^{a,b}, Yuna Hara^a, Keiko Furuya^a, Kaho Takahashi^a, Rinka Nishimura^a, Tomoaki Shingaki^a, Hironari Osada^a, Hirotaka Kondo^c, Keitaro Ohmori^{a,*}

^a Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo, 183-8509, Japan

^b Advanced Animal Medical Center, 1075 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-0934, Japan

^c Laboratory of Veterinary Pathology, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa, 252-0880, Japan

ARTICLE INFO

Keywords:

Chronic enteropathy

Dog

Inflammatory bowel disease

Interleukin-15

ABSTRACT

A pro-inflammatory role of interleukin (IL)-15 and IL-15 receptor (R) in chronic intestinal inflammation, such as inflammatory bowel disease, has been reported in humans. However, the contribution of IL-15 signaling in the pathogenesis of canine chronic enteropathy (CE) remains unclear. Therefore, as a first step in elucidating the importance of IL-15 signaling in canine CE, we measured the mRNA expression of *IL-15* and *IL-15R* subunits, including *IL-15R α* , *IL-15R β* , and *IL-15R γ* , in the duodenal and colonic mucosae of healthy dogs and those with CE, including food-responsive enteropathy (FRE), antibiotic-responsive enteropathy (ARE), and immunosuppressant-responsive enteropathy (IRE). Real-time PCR analysis revealed significantly lower *IL-15R α* mRNA expression levels in the duodenal mucosa of dogs with IRE compared to healthy dogs. In contrast, the mRNA expression levels of *IL-15*, *IL-15R β* , and *IL-15R γ* in the duodenal mucosa and *IL-15*, *IL-15R α* , *IL-15R β* , and *IL-15R γ* in the colonic mucosa did not differ among healthy dogs and those with FRE, ARE, or IRE. These findings suggest that decreased mRNA expression of *IL-15R α* might be involved in the pathogenesis of duodenitis in dogs with IRE. Moreover, even in canine CE, IL-15 signaling appears to play different roles in duodenitis and colitis in dogs with FRE, ARE, and IRE. However, there were no correlations between the gene expression levels of *IL-15R α* and clinical severity or histopathological scores in the duodenum of dogs with IRE. Further studies are necessary to investigate the IL-15R α protein localization and to determine how impaired *IL-15R α* expression contributes to the development of duodenitis in dogs with IRE.

Abbreviations

ARE	antibiotic-responsive enteropathy
CCECAI	canine chronic enteropathy clinical activity index
CE	chronic enteropathy
FRE	food-responsive enteropathy
Foxp3	forkhead box P3
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GI	gastrointestinal
IL	interleukin
IRE	immunosuppressant-responsive enteropathy
IBD	inflammatory bowel disease
IEC	intestinal epithelial cell
IEL	intestinal intraepithelial lymphocyte

NK	natural killer
PO	per os
R	receptor
TBP	TATA-binding protein
UC	ulcerative colitis
Tregs	regulatory T cells
WSAVA	World Small Animal Veterinary Association.

1. Introduction

Canine inflammatory bowel disease (IBD), together with food-responsive enteropathy (FRE) and antibiotic-responsive enteropathy (ARE), constitute a chronic gastrointestinal (GI) disease called chronic enteropathy (CE) in dogs (Allenspach, Wieland, Gröne & Gaschen, 2007;

* Corresponding author.

E-mail address: k-ohmori@cc.tuat.ac.jp (K. Ohmori).

<https://doi.org/10.1016/j.vas.2022.100256>

Available online 16 June 2022

2451-943X/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Makielski, Cullen, O'Connor & Jergens, 2019; Simpson & Jergens, 2011). The pathogenesis of canine CE involves multiple factors, including dysregulated intestinal immune activation, dysbiosis, inappropriate reactions to dietary components, intestinal barrier dysfunction, and genetic factors; these are associated with chronic mucosal inflammation in the small and large intestines of dogs with CE (Eissa, Kittana, Gomes-Neto & Hussein, 2019; German, Hall & Day, 2003; Washabau et al., 2010). The treatment of canine IBD typically requires immunosuppressive drugs, including glucocorticoids. Therefore, canine IBD is called steroid-responsive disease (Allenspach & Mochele, 2022) or immunosuppressant-responsive enteropathy (IRE) (Dandrieux, 2016). In contrast, GI signs in dogs with FRE and ARE are improved with dietary or antibiotic treatments, respectively (Allenspach, Culverwell & Chan, 2016). The different treatment responses in dogs with CE suggest redundant and non-redundant pathogeneses in FRE, ARE, and IRE.

Interleukin (IL)-15 is a member of the IL-2 family cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-21 (Allard-Chamard et al., 2020). IL-15 is produced by a wide range of immune and non-immune cells and is required for the development, function, and homeostasis of CD8⁺ T cells, natural killer (NK) cells, NKT cells, $\gamma\delta$ T cells, and intestinal intraepithelial lymphocytes (IELs) (Allard-Chamard et al., 2020). The IL-15 receptor (R) comprises three subunits: a ligand-specific α chain, β chain shared by IL-2R, and a common γ chain used by the IL-2 family cytokines (Allard-Chamard et al., 2020). IL-15 binds to IL-15R α with high affinity during intracellular biosynthesis and is shuttled to the cell surface. The IL-15/IL-15R α complex on the cell surface is trans-presented to target cells expressing a heterodimeric receptor composed of IL-15R β and γ , inducing IL-15 signaling (Allard-Chamard et al., 2020; Stonier & Schluns, 2010). To a lesser extent, the IL-15/IL-15R α complex can bind to IL-15R $\beta\gamma$ in the same cells as cis-presentation (Allard-Chamard et al., 2020; Stonier & Schluns, 2010). In the intestine, IL-15 produced by intestinal epithelial cells (IECs) maintains the biological functions of IELs, thereby playing a pivotal role in intestinal homeostasis (Yu et al., 2006; Zhu et al., 2020).

Dysregulated IL-15 signaling contributes to the pathogenesis of chronic intestinal inflammation (Pagliari et al., 2013). Previous studies have revealed the increased expression of IL-15 and IL-15R α at the mRNA and protein levels in the intestinal mucosa of human patients with IBD, including Crohn's disease and ulcerative colitis (UC) (León et al., 2009; Liu et al., 2000; Nishiwaki et al., 2005; Perrier et al., 2013; Vitale et al., 2017). In the intestine of human patients with IBD, the damaged IECs and lamina propria macrophages and dendritic cells produce IL-15, leading to the further release of inflammatory cytokines from myeloid and lymphoid cells (Allard-Chamard et al., 2020). Moreover, IL-15-deficient mice were protected from dextran sodium sulfate-induced colitis in a mouse model of UC (Yoshihara, Yajima, Kubo & Yoshikai, 2006). These findings indicate a pro-inflammatory role of IL-15 in intestinal inflammation in human IBD.

Based on the potential role of IL-15 in chronic intestinal inflammation, IL-15 is hypothesized to be involved in the pathogenesis of canine CE. However, no analyses have assessed IL-15 and IL-15R expression in dogs with CE. Therefore, as a first step toward elucidating the importance of IL-15 signaling in canine CE, this study measured the mRNA expression of *IL-15* and *IL-15R* subunits in the duodenal and colonic mucosae of healthy dogs and those with FRE, ARE, and IRE.

2. Material and methods

2.1. Dogs

This study included 30 dogs diagnosed with CE at Tokyo University of Agriculture and Technology Animal Medical Center. CE was diagnosed as we previously described (Hirokawa et al., 2021; Osada et al., 2017). The inclusion criteria for CE were chronic GI signs, such as vomiting, small bowel diarrhea (melena, normal fecal frequency, and increased fecal volume), and/or large bowel diarrhea (mucus,

hematochezia, tenesmus, increased frequency of defecation, and decreased fecal volume) for a duration of >3 weeks, as well as histopathological evidence of inflammation, such as lymphoplasmacytic enteritis, in the duodenal and colonic mucosae in endoscopic biopsy. Other possible causes of chronic GI signs, such as metabolic diseases; infectious diseases, including bacterial, viral, and parasitic diseases; exocrine pancreatic insufficiency; pancreatitis; hepatic diseases; renal diseases; and neoplasms, including alimentary lymphoma, were ruled out based on vaccination history and physical and clinical examinations, including blood tests, thoracic and abdominal radiography and ultrasonography, and fecal analysis. After confirmation of CE, FRE was diagnosed by the complete resolution of GI signs in a dietary trial using hydrolyzed or novel protein diets. ARE was diagnosed by the complete resolution of GI signs in response to antibiotic treatment with metronidazole (Flagyl, Shionogi & Co., Ltd., Osaka, Japan; 10–15 mg/kg, per os [PO], q12 h) or tylosin (Tylan, Eli Lilly Japan K.K., Kobe, Japan; 20 mg/kg, PO, q12 h). After exclusion of FRE and ARE, IRE was diagnosed by the partial or complete resolution of GI signs with prednisolone (Pfizer, Tokyo, Japan; 0.5–2.0 mg/kg, PO, q24 h). The clinical severity of CE in the 30 dogs was scored according to the canine chronic enteropathy clinical activity index (CCECAI) (Allenspach et al., 2007).

The control group comprised seven healthy intact male beagles (median age, 4.8 years; range, 1.2–6.4 years; median body weight, 11.3 kg; range, 10.1–11.6 kg). The dogs were housed in individual cages and fed a commercial diet. Water was provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Tokyo University of Agriculture and Technology (approval numbers: 28–34 and 30–132). Informed consent was obtained from the owners of the dogs included in this study.

2.2. Endoscopic examination and histopathological analysis

Endoscopic examination of the duodenum and colon was performed according to the procedure in our previous report (Hirokawa et al., 2021). Duodenal and colonic samples were collected from all dogs. More than 6 specimens were obtained from each region. The duodenal and colonic biopsy specimens were subjected to histopathological analysis and graded according to the guideline of the World Small Animal Veterinary Association (WSAVA) international GI standardization group (Washabau et al., 2010) by a board-certified veterinary anatomic pathologist (HK). A portion of each biopsy sample was stored immediately in RNAlater[®] solution (Thermo Fisher Scientific, Waltham, MA, USA) until RNA extraction.

2.3. Real-time PCR

Total RNA extraction, cDNA synthesis, and real-time PCR were performed as previously described (Hirokawa et al., 2021). The fluorescence intensities of the PCR products were measured in real-time using a Thermal Cycler Dice[®] Real Time System *Lite* (Takara Bio, Shiga, Japan). The primers for real-time PCR (Supplementary Table 1) were designed by a Perfect Real Time Support System (Takara Bio) and according to a previous report (Meyer, Gruber & Klopffleisch, 2013). The reference genes for the duodenal mucosa were glyceraldehyde 3-phosphate dehydrogenase (GAPDH), TATA-binding protein (TBP), and succinate dehydrogenase complex, subunit A, while those for the colonic mucosa were GAPDH, TBP, and hydroxymethylbilane synthase, according to our previous report (Osada et al., 2017). The relative mRNA expression of the target genes was determined using the 2^{- Δ Ct} method, wherein each value was presented as an *n*-fold difference relative to the geometric mean of the three reference genes.

2.4. Statistical analysis

The normality of all data was analyzed using the Shapiro–Wilk test. Data between 2 groups were compared using the unpaired *t*-test or the

Mann–Whitney *U* test, depending on the normality. Data among 4 groups were compared using the one-way analysis of variance or the Kruskal–Wallis test, followed by the Steel test, depending on the normality. Correlations between 2 parameters were evaluated using the Spearman's rank correlation coefficient (r_s). Statistical analysis was performed using the Jamovi version 2.2 computer software (The Jamovi project, 2021). $P < 0.05$ was considered statistically significant.

3. Results and discussion

The clinical and histopathological characteristics of the 30 dogs with CE are summarized in Supplementary Table 2. The median age of the dogs with CE was 8.2 years (range, 1.8–14.1 years), and the median body weight was 6.1 kg (range, 1.8–29.7 kg). The median CCECAI score at the first visit was 7 (range, 0–16). The median WSAVA scores in the duodenum and colon were 10 (range, 3–15) and 5 (range, 1–9), respectively. Among the 30 dogs with CE, 9, 4, and 17 were diagnosed with FRE, ARE, and IRE, respectively.

IL-15, *IL-15R α* , *IL-15R β* , and *IL-15R γ* mRNA expression levels in the duodenal and colonic mucosae were compared between healthy dogs and those with CE. Those results were then compared among healthy dogs and those with FRE, ARE, or IRE. In the duodenal mucosa, *IL-15R α* mRNA expression levels were significantly lower in dogs with CE than in healthy dogs (Fig. 1C; $P = 0.031$). Among healthy dogs and those with FRE, ARE, or IRE, *IL-15R α* mRNA expression levels were significantly lower in dogs with IRE than in healthy dogs (Fig. 1D; $P = 0.003$). In contrast, *IL-15*, *IL-15R β* , and *IL-15R γ* mRNA expression levels in the duodenal mucosa did not differ significantly between healthy dogs and those with CE (Fig. 1A, E, and G; $P > 0.05$, respectively) and among healthy dogs and those with FRE, ARE, or IRE (Fig. 1B, F, and H; $P > 0.05$, respectively). In the colonic mucosa, *IL-15R α* mRNA expression levels were significantly lower in dogs with CE than in healthy dogs (Fig. 2C; $P = 0.040$). However, *IL-15R α* mRNA expression levels did not differ significantly among healthy dogs and those with FRE, ARE, or IRE (Fig. 2D; $P > 0.05$). In addition, *IL-15*, *IL-15R β* , and *IL-15R γ* mRNA expression levels in the colonic mucosa did not differ significantly between healthy dogs and those with CE (Fig. 2A, E, and G; $P > 0.05$, respectively) and among healthy dogs and those with FRE, ARE, or IRE (Fig. 2B, F, and H; $P > 0.05$, respectively).

To investigate whether decreased *IL-15R α* mRNA expression levels in the duodenal mucosa of dogs with IRE were associated with clinical and histopathological severity, we assessed the correlations between the gene expression levels and CCECAI or WSAVA scores in the duodenum. The results showed no significant correlations between *IL-15R α* mRNA expression levels and CCECAI ($r_s = -0.219$, $P = 0.399$) or WSAVA score ($r_s = 0.031$, $P = 0.906$) in the duodenal mucosa of dogs with IRE.

The results of the present study demonstrated significantly lower *IL-15R α* mRNA expression levels in the duodenal mucosa of dogs with IRE compared to healthy dogs. In contrast, the mRNA expression levels of *IL-15*, *IL-15R β* , and *IL-15R γ* in the duodenal mucosa and *IL-15*, *IL-15R α* , *IL-15R β* , and *IL-15R γ* in the colonic mucosa did not differ significantly among healthy dogs and those with FRE, ARE, or IRE. These findings suggest that decreased *IL-15R α* expression might be involved in the pathogenesis of duodenitis in dogs with IRE.

Our findings are contrary to those of previous studies that reported increased *IL-15R α* mRNA expression in the intestine of human patients with IBD (Nishiwaki et al., 2005; Perrier et al., 2013). A pro-inflammatory function of IL-15 has been proposed in human IBD, whereas several studies have revealed an anti-inflammatory role of IL-15 signaling, such as IL-15-mediated suppression of pro-inflammatory cytokine production in the inflamed intestine of UC (Silva, Menezes, Deslandres & Seidman, 2005) and IL-15-induced protection against IEC apoptosis during chronic intestinal inflammation (Obermeier et al., 2006). In addition, IL-15 and IL-15R α have been shown to maintain the balance between forkhead box P3 (Foxp3)⁺ regulatory T cells (Tregs) and Th17 cells in the intestine (Tosiek, Fiette, El Daker, Eberl & Freitas,

2016). Disruption of this balance induced intestinal inflammation with prominent mucosal damage in mice (Tosiek et al., 2016). In the present study, we detected reduced *IL-15R α* mRNA expression in the duodenal mucosa of dogs with IRE. As IL-15 signals mainly by trans-presentation of the IL-15/IL-15R α complex to the target cells, decreased *IL-15R α* expression suggests impaired IL-15 signaling due to reduced complex formation. Dogs with IRE show a diminished number of FoxP3⁺ Tregs compared to healthy dogs (Maeda, Ohno, Fujiwara-Igarashi, Uchida & Tsujimoto, 2016). Considering the beneficial effect of IL-15 signaling on Treg numbers and functions (Tosiek et al., 2016), impaired IL-15 signaling due to reduced *IL-15R α* expression might be associated with decreased Treg numbers in the inflamed duodenal mucosa of dogs with IRE. Further studies are needed to confirm this hypothesis.

In this study, we did not detect significant differences in the mRNA expression of *IL-15* and *IL-15R* subunits in the duodenal mucosa of healthy dogs and those with FRE or ARE, or in the colonic mucosa of healthy dogs and those with FRE, ARE, or IRE. These results suggest that IL-15 signaling may not be pivotal in the development of duodenitis in dogs with FRE and ARE, and colitis in dogs with FRE, ARE, and IRE. Thus, other factors may shape the pathogenesis of duodenitis and colitis in these dogs.

Although we analyzed the gene expression of *IL-15* and *IL-15R* subunits in dogs with CE, this study had four major limitations. First, IL-15 and IL-15R subunits were measured at the mRNA level. To identify the distribution and cellular sources of IL-15 and IL-15R subunits, immunohistochemical analysis using appropriate antibodies is required. Second, the present study evaluated a relatively small population of dogs with CE, especially those with ARE. Further analysis should be performed in a larger population. Third, owing to a lack of available samples, we could not assess mRNA expression in dogs with non-responsive enteropathy, which is classified as a type of CE and does not respond to immunosuppressive drugs (Dandrieux, 2016). Fourth, the control group comprised healthy intact male beagles. Age-, sex-, and breed-matched healthy dogs would be more appropriate for the control group. To further clarify the significance of IL-15 signaling in the pathogenesis of canine CE, these potential limitations should be addressed in future studies.

4. Conclusion

The results of the present study revealed decreased mRNA expression of *IL-15R α* in the duodenal mucosa of dogs with IRE. Our findings clarified that the gene expression profiles of *IL-15* and *IL-15R* subunits in canine CE differed from those in human IBD. Moreover, even in canine CE, IL-15 signaling appears to play different roles in the pathogenesis of duodenitis and colitis in dogs with FRE, ARE, and IRE. Further studies are necessary to determine how impaired *IL-15R α* expression contributes to the development of duodenitis in dogs with IRE.

Funding

This study was supported by JSPS KAKENHI (grant number 22H02516).

Ethical approval

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Tokyo University of Agriculture and Technology (approval numbers: 28–34 and 30–132). Informed consent was obtained from the owners of the dogs included in this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. None of the authors has any financial or

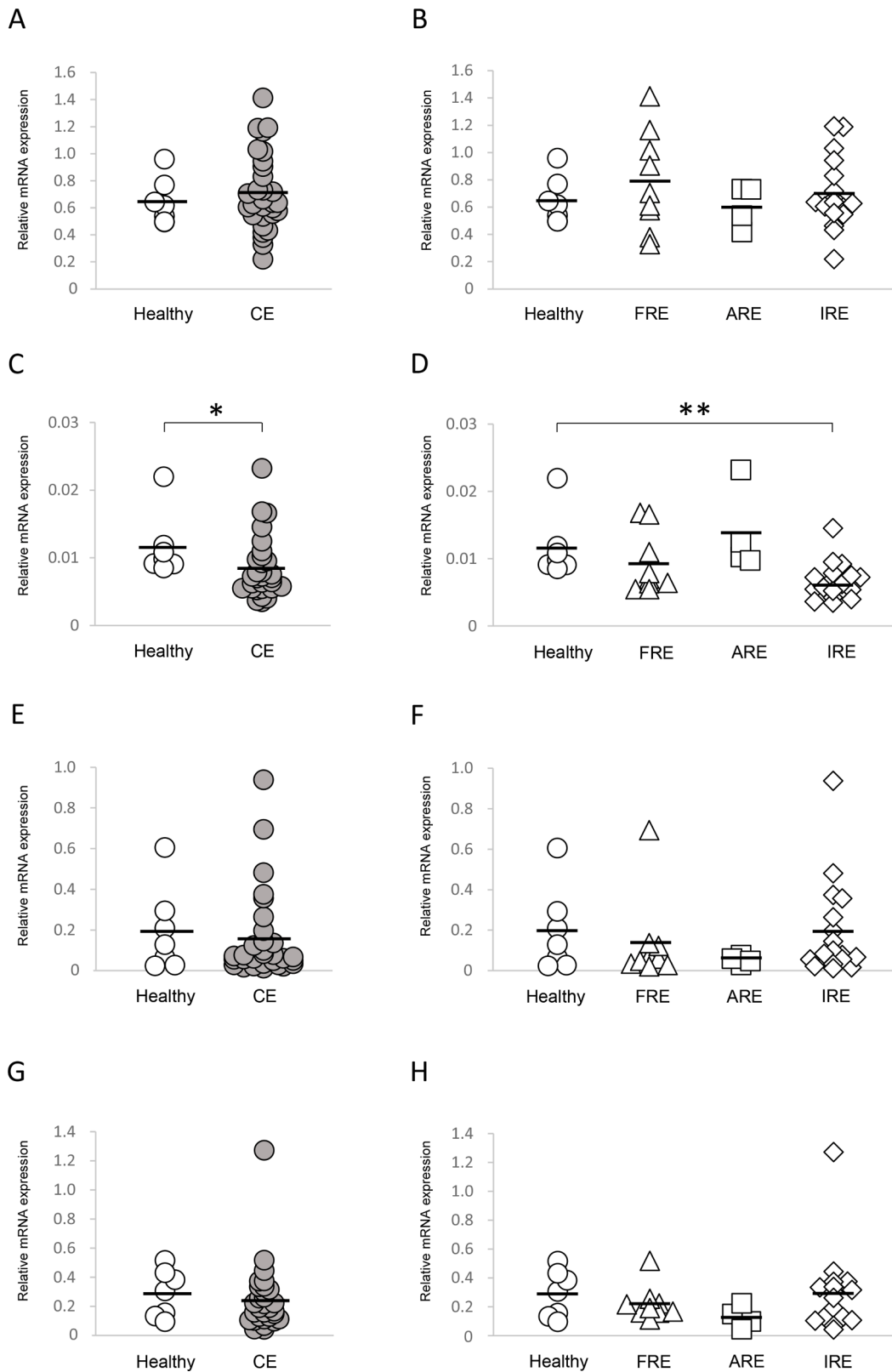


Fig. 1. Relative mRNA expression of interleukin (IL)-15 and its receptor (R) subunits in the duodenal mucosa of dogs with chronic enteropathy (CE). The expression levels of *IL-15* (A and B), *IL-15R α* (C and D), *IL-15R β* (E and F), and *IL-15R γ* (G and H) mRNA were analyzed by real-time PCR in healthy dogs ($n = 7$) and those with CE ($n = 30$), which included dogs with food-responsive enteropathy (FRE) ($n = 9$), antibiotic-responsive enteropathy (ARE) ($n = 4$), and immunosuppressant-responsive enteropathy (IRE) ($n = 17$). The horizontal lines in each group represent the mean values. The relative mRNA expression levels were compared between healthy dogs and those with CE using the unpaired *t*-test (A) or the Mann-Whitney *U* test (C, E, and G). The relative mRNA expression levels were compared among healthy dogs and those with FRE, ARE, or IRE using the one-way analysis of variance (B) or the Kruskal-Wallis test (D, F, and H), followed by the Steel-test (D). * $P < 0.05$, ** $P < 0.01$.

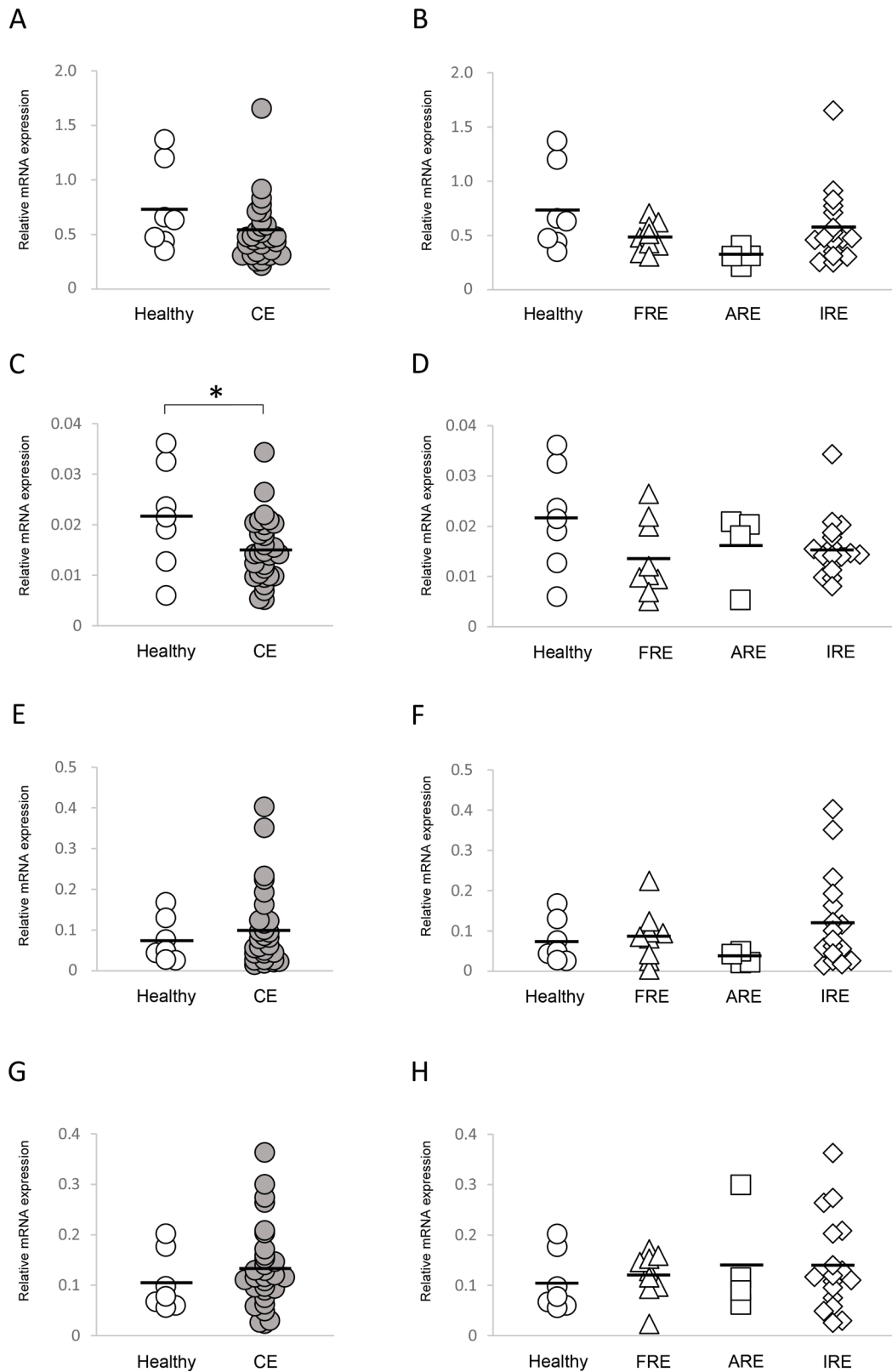


Fig. 2. Relative mRNA expression of interleukin (IL)-15 and its receptor (R) subunits in the colonic mucosa of dogs with chronic enteropathy (CE). The expression levels of *IL-15* (A and B), *IL-15R α* (C and D), *IL-15R β* (E and F), and *IL-15R γ* (G and H) mRNA were analyzed by real-time PCR in healthy dogs ($n = 7$) and those with CE ($n = 30$), which included dogs with food-responsive enteropathy (FRE) ($n = 9$), antibiotic-responsive enteropathy (ARE) ($n = 4$), and immunosuppressant-responsive enteropathy (IRE) ($n = 17$). The horizontal lines in each group represent the mean values. The relative mRNA expression levels were compared between healthy dogs and those with CE using the unpaired *t*-test (C) or the Mann-Whitney *U* test (A, E, and G). The relative mRNA expression levels were compared among healthy dogs and those with FRE, ARE, or IRE using the Kruskal-Wallis test (B, D, F, and H). * $P < 0.05$.

personal relationships that could inappropriately influence or bias the content of the paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.vas.2022.100256](https://doi.org/10.1016/j.vas.2022.100256).

References

- Allard-Chamard, H., Mishra, H. K., Nandi, M., Mayhue, M., Menendez, A., Ilangumaran, S., et al. (2020). Interleukin-15 in autoimmunity. *Cytokine*, 136, Article 155258. <https://doi.org/10.1016/j.cyto.2020.155258>
- Allenspach, K., Culverwell, C., & Chan, D. (2016). Long-term outcome in dogs with chronic enteropathies: 203 cases. *Veterinary Record*, 178, 368. <https://doi.org/10.1136/vr.103557>
- Allenspach, K., & Mochel, J. P. (2022). Current diagnostics for chronic enteropathies in dogs. *Veterinary Clinical Pathology*, 50, 18–28. <https://doi.org/10.1111/vcp.13068>
- Allenspach, K., Wieland, B., Gröne, A., & Gaschen, F. (2007). Chronic enteropathies in dogs: Evaluation of risk factors for negative outcome. *Journal of Veterinary Internal Medicine*, 21, 700–708. <https://doi.org/10.1111/j.1939-1676.2007.tb03011.x>
- Dandrieux, J. R. S. (2016). Inflammatory bowel disease versus chronic enteropathy in dogs: Are they one and the same? *Journal of Small Animal Practice*, 57, 589–599. <https://doi.org/10.1111/jsap.12588>
- Eissa, N., Kittana, H., Gomes-Neto, J. C., & Hussein, H. (2019). Mucosal immunity and gut microbiota in dogs with chronic enteropathy. *Research in Veterinary Science*, 122, 156–164. <https://doi.org/10.1016/j.rvsc.2018.11.019>
- German, A. J., Hall, E. J., & Day, M. J. (2003). Chronic intestinal inflammation and intestinal disease in dogs. *Journal of Veterinary Internal Medicine*, 17, 8–20. <https://doi.org/10.1111/j.1939-1676.2003.tb01318.x>
- Hirokawa, M., Takahashi, K., Miyajima, M., Furukawa, R., Sugita, K., Kondo, H., et al. (2021). Expression of genes encoding inflammasome sensor subunits in the duodenal and colonic mucosae of dogs with chronic enteropathy. *Journal of Veterinary Medical Science*, 83, 1161–1166. <https://doi.org/10.1292/jvms.20-0519>
- León, A. J., Gómez, E., Garrote, J. A., Bernardo, D., Barrera, A., & Marcos, J. L. (2009). High levels of proinflammatory cytokines, but not markers of tissue injury, in unaffected intestinal areas from patients with IBD. *Mediators of Inflammation*, 2009, Article 580450. <https://doi.org/10.1155/2009/580450>
- Liu, Z., Geboes, K., Colpaert, S., D'Haens, G. R., Rutgeerts, P., & Ceuppens, J. L. (2000). IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production. *Journal of Immunology*, 164, 3608–3615. <https://doi.org/10.4049/jimmunol.164.7.3608>
- Maeda, S., Ohno, K., Fujiwara-Igarashi, A., Uchida, K., & Tsujimoto, H. (2016). Changes in Foxp3-positive regulatory T cell number in the intestine of dogs with idiopathic inflammatory bowel disease and intestinal lymphoma. *Veterinary Pathology*, 53, 102–112. <https://doi.org/10.1177/0300985815591081>
- Makielski, K., Cullen, J., O'Connor, A., & Jergens, A. E. (2019). Narrative review of therapies for chronic enteropathies in dogs and cats. *Journal of Veterinary Internal Medicine*, 33, 11–22. <https://doi.org/10.1111/jvim.15345>
- Meyer, A., Gruber, A. D., & Klopfeisch, R. (2013). All subunits of the interleukin-2 receptor are expressed by canine cutaneous mast cell tumours. *Journal of Comparative Pathology*, 149, 19–29. <https://doi.org/10.1016/j.jcpa.2012.11.232>
- Nishiwaki, T., Ina, K., Goto, H., Watanabe, O., Tsuzuki, T., Furuta, R., et al. (2005). Possible involvement of the interleukin-15 and interleukin-15 receptor system in a heightened state of lamina propria B cell activation and differentiation in patients with inflammatory bowel disease. *Journal of Gastroenterology*, 40, 128–136. <https://doi.org/10.1007/s00535-004-1510-y>
- Obermeier, F., Hausmann, M., Kellermeier, S., Kiessling, S., Strauch, U. G., Duitman, E., et al. (2006). IL-15 protects intestinal epithelial cells. *European Journal of Immunology*, 36, 2691–2699. <https://doi.org/10.1002/eji.200535173>
- Osada, H., Ogawa, M., Hasegawa, A., Nagai, M., Shirai, J., Sasaki, K., et al. (2017). Expression of epithelial cell-derived cytokine genes in the duodenal and colonic mucosae of dogs with chronic enteropathy. *Journal of Veterinary Medical Science*, 79, 393–397. <https://doi.org/10.1292/jvms.16-0451>
- Pagliari, D., Cianci, R., Frosali, S., Landolfi, R., Cammarota, G., & Newton, E. E. (2013). The role of IL-15 in gastrointestinal diseases: A bridge between innate and adaptive immune response. *Cytokine & Growth Factor Reviews*, 24, 455–466. <https://doi.org/10.1016/j.cytogfr.2013.05.004>
- Perrier, C., Arijis, I., Staelens, D., Breynaert, C., Cleynen, I., Covens, K., et al. (2013). Interleukin-15 receptor α expression in inflammatory bowel disease patients before and after normalization of inflammation with infliximab. *Immunology*, 138, 47–56. <https://doi.org/10.1111/imm.12014>
- Silva, M. A., Menezes, J., Deslandres, C., & Seidman, E. G. (2005). Anti-inflammatory role of interleukin-15 in Crohn's disease. *Inflammatory Bowel Diseases*, 11, 219–230. <https://doi.org/10.1097/01.MIB.0000160804.52072.6a>
- Simpson, K. W., & Jergens, A. E. (2011). Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. *Veterinary Clinics of North America: Small Animal Practice*, 41, 381–398. <https://doi.org/10.1016/j.cvs.2011.02.003>
- Stonier, S. W., & Schluns, K. S. (2010). Trans-presentation: A novel mechanism regulating IL-15 delivery and responses. *Immunology Letters*, 127, 85–92. <https://doi.org/10.1016/j.imlet.2009.09.009>
- The jamovi project (2021). jamovi (Version 2.2) [Computer Software]. Retrieved from <https://www.jamovi.org>.
- Tosiek, M. J., Fiette, L., El Daker, S., Eberl, G., & Freitas, A. A. (2016). IL-15-dependent balance between Foxp3 and ROR γ t expression impacts inflammatory bowel disease. *Nature Communications*, 7, 10888. <https://doi.org/10.1038/ncomms10888>
- Vitale, S., Strisciuglio, C., Pisapia, L., Miele, E., Barba, P., Vitale, A., et al. (2017). Cytokine production profile in intestinal mucosa of paediatric inflammatory bowel disease. *Plos One*, 12, Article e0182313. <https://doi.org/10.1371/journal.pone.0182313>
- Washabau, R. J., Day, M. J., Willard, M. D., Hall, E. J., Jergens, A. E., Mansell, J., & WSAVA International Gastrointestinal Standardization Group. (2010). Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. *Journal of Veterinary Internal Medicine*, 24, 10–26. <https://doi.org/10.1111/j.1939-1676.2009.0443.x>
- Yoshihara, K., Yajima, T., Kubo, C., & Yoshikai, Y. (2006). Role of interleukin 15 in colitis induced by dextran sulphate sodium in mice. *Gut*, 55, 334–341. <https://doi.org/10.1136/gut.2005.076000>
- Yu, Q., Tang, C., Xun, S., Yajima, T., Takeda, K., & Yoshikai, Y. (2006). MyD88-dependent signaling for IL-15 production plays an important role in maintenance of CD8 alpha TCR alpha beta and TCR gamma delta intestinal intraepithelial lymphocytes. *Journal of Immunology*, 176, 6180–6185. <https://doi.org/10.4049/jimmunol.176.10.6180>
- Zhu, Y., Cui, G., Miyachi, E., Nakanishi, Y., Mukohira, H., Shimba, A., et al. (2020). Intestinal epithelial cell-derived IL-15 determines local maintenance and maturation of intra-epithelial lymphocytes in the intestine. *International Immunology*, 32, 307–319. <https://doi.org/10.1093/intimm/dxz082>