

Presence of the house dust mite allergen in the gastrointestinal tract of dogs with chronic enteropathy: A potential inducer of interleukin-1 β

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ABSTRACT

House dust mite (HDM) is an environmental allergen ubiquitously present indoors, causing allergic inflammation in dogs. However, it is unclear whether HDM allergens can be detected in the gastrointestinal (GI) tract of dogs. In addition, although expression of interleukin (IL)-1 β is increased in the intestinal mucosa of dogs with chronic enteropathy (CE), the role of HDM allergens in the production of IL-1 β has not been evaluated. The objectives of this study were to determine the presence of HDM allergens in the GI tract of dogs and to elucidate the effect of HDM on IL-1 β expression in canine macrophages. HDM allergen, *Dermatophagoides pteronyssinus* (Der p) 1, was quantified in the gastric and duodenal fluids and the duodenal and colonic mucosae of dogs with CE and healthy laboratory dogs, and faeces of dogs with CE, healthy laboratory dogs and healthy client-owned dogs. Gene expression and protein levels of IL-1 β were measured in HDM-stimulated canine peripheral macrophages from healthy laboratory dogs. Der p 1 was detected in the gastric and duodenal fluids of dogs with CE and healthy laboratory dogs, and faeces of all dogs examined. Der p 1 levels in the duodenal and colonic mucosae were significantly higher in dogs with CE than in healthy laboratory dogs. HDM increased both gene expression and protein levels of IL-1 β in canine macrophages. These findings demonstrate the presence of HDM allergens in the GI tract of dogs and the possible involvement of HDM allergens in the pathogenesis of CE by promoting IL-1 β expression in macrophages.

1. Introduction

Chronic enteropathy (CE) in dogs is a group of disorders characterized by persistent or recurrent gastrointestinal (GI) signs and mucosal inflammation in the GI tract in the absence of other well defined GI and extra-GI diseases (Allenspach et al., 2007; Dandrieux, 2016; Heilmann and Steiner, 2018; Washabau et al., 2010). Various factors, including mucosal barrier dysfunction, altered gut microbiota and inappropriate reactions to dietary components, may lead to chronic inflammation in the intestines of dogs with CE (Eissa et al., 2019; German et al., 2003). Environmental factors may also be involved in the development of CE; however, they have not been evaluated in dogs.

Previous studies reported a relative increase in interleukin (IL)-1 β protein, a proinflammatory cytokine, in the intestinal mucosa of dogs with inflammatory bowel disease (IBD), which is a subtype of CE (Hawes et al., 2018; Maeda et al., 2012; Ogawa et al., 2018). IL-1 β was shown to suppress mRNA expression of occludin, a tight junction component of the intestinal epithelial cells, in the colonic mucosa of dogs (Ogawa et al., 2018). These findings indicate that increased expression of IL-1 β plays a pivotal role in duodenitis and colitis in dogs with CE. Although lamina propria macrophages are considered as the main producer of IL-1 β in the intestine (Cappello et al., 1992; Mahida et al., 1989; Youngman et al., 1993), the kind of stimuli activating intestinal macrophages to induce IL-1 β expression in dogs with CE is not fully

Abbreviations: AEBSEF, 4-2-aminoethylbenzenesulfonyl fluoride hydrochloride; CE, chronic enteropathy; Der p, *Dermatophagoides pteronyssinus*; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GI, gastrointestinal; HDM, house dust mite; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; PAR-2, protease-activated receptor-2.

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understood.

House dust mite (HDM) is an environmental allergen ubiquitously and perennially present indoors, causing allergic inflammation in humans and dogs (Arlian and Platts-Mills, 2001; Nuttall et al., 2006). *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* are the most common HDMs (Arlian and Platts-Mills, 2001). Cysteine and serine proteases identified in HDM allergens have the potential to trigger allergic skin and airway inflammation by disrupting epithelial tight junctions and activating both innate and acquired immune systems (Reithofer and Jahn-Schmid, 2017). A previous study demonstrated that the group 1 allergen of Der p (Der p 1) was present in the human gut and induced intestinal barrier dysfunction and cytokine production in colon biopsies (Tulic et al., 2016). Human macrophages were found to produce intracellular pro-IL-1 β in response to HDM (Sundaram et al., 2015). Dogs are prone to lick and bite carpets and other objects in their living environments where HDMs are abundantly present. These findings bring us to hypothesize that HDM can be found in the GI tract of dogs and could contribute to the pathogenesis of CE by inducing IL-1 β expression.

The objectives of this study were to determine whether HDM allergens were present in the GI tract of dogs and to elucidate the effect of HDM on IL-1 β expression in canine macrophages.

2. Materials and methods

2.1. Dogs

Thirty-eight dogs diagnosed with CE at the Tokyo University of Agriculture and Technology Animal Medical Center were included in this study. CE was diagnosed according to previous studies (Dandrieux, 2016; Osada et al., 2017; Washabau et al., 2010): (1) chronic GI signs, such as vomiting and diarrhoea, for over 3 weeks; (2) histopathological evidence of inflammation in the duodenal and colonic mucosae in specimens obtained by endoscopic biopsy in dogs that received endoscopic examination; (3) exclusion of other causes of chronic GI signs, including metabolic disease, infection, parasitic disease, pancreatic insufficiency, hepatic disease, renal disease and alimentary lymphoma. All dogs lived indoors with their owners. The clinical characteristics of the 38 dogs with CE are summarised in Table 1.

Eighteen healthy client-owned dogs and six healthy laboratory dogs were also enrolled in this study (Table 1). Healthy client-owned dogs lived indoors with their owners. Healthy laboratory dogs were housed in individual cages in our facility and fed a commercial diet (Science Diet Adult, Hill's-Colgate). Water was provided *ad libitum*. Physical and clinical examinations, including blood tests and faecal examination, did not identify any abnormalities in healthy client-owned dogs and healthy laboratory dogs.

All procedures were approved by the Institutional Animal Care and Use Committee of Tokyo University of Agriculture and Technology (approval number 28–34 and 30–132; date of approval 20 May 2016 and 18 January 2019).

2.2. Collection and preparation of the gastrointestinal specimens

Gastric and duodenal fluids were collected from six and four of the 38 dogs with CE, respectively, and from all healthy laboratory dogs by endoscopic aspiration. Faeces were collected from the rectum of all dogs by hand to avoid HDM contamination from the environment. Faeces were first weighed and then mixed with PBS at a 1:1 ratio to obtain a faecal solution. The gastric and duodenal fluids and the faecal solution were centrifuged at 20,000 g for 5 min at 4 °C, and pellets were discarded. This procedure was repeated, and the supernatant was then filtered through 0.8 μ m syringe filters (Pall Corporation) and stored at –30 °C until analysis.

Duodenal and colonic mucosal samples were obtained from 19 and 16 of the 38 dogs with CE, respectively, and from all healthy laboratory dogs using endoscopic biopsy forceps. Samples were stored at –80 °C

Table 1

Clinical characteristics of dogs examined in this study.

	Dogs with CE <i>n</i> = 38	Healthy client- owned dogs <i>n</i> = 18	Healthy laboratory dogs <i>n</i> = 6
Age (years)	8.3 (2.2–14.1)	6.7 (2.0–12.8)	9.0 (5.0–11.0)
Body weight (kg)	9.8 (1.8–33.6)	7.6 (2.9–13.8)	12.6 (11.3–13.7)
Sex (<i>n</i>)			
Castrated males	16	8	
Intact males	5	1	6
Spayed females	11	8	
Intact females	6	1	
Breed (<i>n</i>)			
Toy poodle	6	2	
Maltese	4		
Shiba Inu	4		
Mixed breed	3	4	
Papillon	3		
Pembroke Welsh Corgi	3		
Cavalier King Charles Spaniel	2	3	
French Bulldog	2		
American Pit Bull Terrier	1		
Beagle	1	3	6
Border Collie	1		
Chin	1		
Flat-Coated Retriever	1		
Golden Retriever	1		
Jack Russell Terrier	1		
Kaninchen Dachshund	1		
Labrador Retriever	1		
Miniature Schnauzer	1	1	
Shetland Sheepdog	1		
Pomeranian		2	
Chihuahua		1	
Italian		1	
Greyhound Miniature Dachshund		1	

until protein extraction. The mucosal specimens were homogenized in 600 μ L lysis buffer (EzRIPA Lysis kit, ATTO). After centrifugation, the supernatant was stored at –80 °C until analysis.

Sample collection by GI endoscopy was conducted under general anaesthesia. Food was withheld for at least 24 h before endoscopy. Colon cleansing with saline was also conducted under general anaesthesia before the endoscopic sample collection.

2.3. Stimulation of macrophages

Peripheral blood-derived macrophages were obtained from five of the six healthy laboratory dogs according to a previous study (Goto-Koshino et al., 2011). In brief, canine peripheral blood mononuclear cells (4×10^6 cells/well) were stimulated with 10 ng/mL phorbol 12-myristate 13-acetate (Sigma-Aldrich) in culture medium at 37 °C for 5 days under 5% CO₂. Similar to the previous report (Goto-Koshino et al., 2011), approximately 2×10^5 macrophages were yielded in each well. For gene expression analysis, macrophages were stimulated for 4 h at 37 °C with medium alone as the experimental control, 5 μ g/mL HDM (Der p extract, ITEA) or HDM pre-treated with the followings: 10 μ M E64 (Sigma-Aldrich) as a cysteine protease inhibitor; 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSE; Santa Cruz Biotechnology) as a serine protease inhibitor; or 30 μ g/mL polymyxin B

sulphate (Thermo Fisher Scientific) as a lipopolysaccharide (LPS) inhibitor. Macrophages were also stimulated for 4 h at 37 °C with medium alone (control), 10 ng/mL LPS (Sigma-Aldrich) or LPS pre-treated with 30 µg/mL polymyxin B. HDM was pre-incubated with E64 or AEBSF for 15 min at 37 °C (Tulic et al., 2016). HDM and LPS were pre-incubated with polymyxin B for 1 h at 37 °C (Cardoso et al., 2007). After stimulation, macrophages were immediately preserved in RNeasy lysis solution (Qiagen) until RNA extraction. For protein expression, macrophages were stimulated with medium alone (control), 5 µg/mL HDM or 10 ng/mL LPS for 24 h at 37 °C. After stimulation, macrophages were dissolved in 1 mL lysis buffer (EzRIPA Lysis kit, ATTO). After centrifugation, the supernatant was stored at -80 °C until analysis.

2.4. ELISA

The amount of Der p 1 protein was measured using a Der p 1 ELISA kit 5H8/4C1 (Indoor Biotechnologies) as previously described (Macchiaverni et al., 2014; Tulic et al., 2016). This ELISA kit specifically quantified Der p 1 using two different monoclonal antibodies (5H8 and 4C1) that were validated previously (Luczynska et al., 1989). Der p 1 levels in faeces (pg/g) were calculated by the following formula: the concentration of Der p 1 in the faecal solution (pg/mL) × volume of the solution (mL) ÷ weight of faecal samples used for faecal solution extraction (g). The concentrations of Der p 1 in the duodenal and colonic mucosae were normalized to the total protein concentration in each sample, which was determined using a commercial kit (TaKaRa BCA Protein Assay Kit, Takara Bio). The amount of IL-1β protein was measured using a commercial ELISA kit specific for canine IL-1β (Canine IL-1beta ELISA kit, Thermo Fisher Scientific).

2.5. Real-time PCR

Total RNA extraction, cDNA synthesis and real-time PCR analysis were performed as described previously (Ohmori et al., 2013). Primers for real-time PCR (Table 2) were designed by a Perfect Real Time support system (Takara Bio) and according to a previous report (Osada et al., 2017). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control. The relative mRNA expression of target genes was determined by the $2^{-\Delta\Delta CT}$ method, where $\Delta CT = CT^{\text{sample}} - CT^{\text{gapdh}}$. Each value was presented as *n*-fold change of mRNA expression relative to the control group.

2.6. Statistical analysis

The normality of data was analysed by the Shapiro-Wilk test. Differences between two groups were compared by the Mann-Whitney *U* test, the unpaired *t* test or the Wilcoxon signed-rank test, depending on the normality of data. For comparisons of more than two groups, the Kruskal-Wallis test was used, followed by the Steel-Dwass test or the Steel test. Statistical analyses were performed using BellCurve for Excel (Social Survey Research Information). *P* < 0.05 was considered statistically significant.

3. Results

3.1. Der p 1 in the gastric and duodenal fluids and faeces

Der p 1 was detected in all gastric and duodenal fluids and faecal samples of the tested dogs. There were no significant differences in Der p 1 levels in the gastric fluid (*P* = 0.70; Fig. 1A) or in the duodenal fluid (*P* = 0.87; Fig. 1B) between healthy laboratory dogs and dogs with CE. Der p 1 levels in faecal samples from healthy client-owned dogs were significantly higher than in samples from laboratory dogs (*P* = 0.01; Fig. 1C) and from dogs with CE (*P* = 0.02; Fig. 1C).

3.2. Der p 1 in the duodenal and colonic mucosae

Der p 1 levels in the duodenal mucosa were significantly higher in dogs with CE than in healthy laboratory dogs (*P* < 0.01; Fig. 2A). In addition, Der p 1 levels in the colonic mucosa were significantly higher in dogs with CE than in healthy laboratory dogs (*P* < 0.01; Fig. 2B).

3.3. Effect of HDM on the expression of *IL1B* mRNA in canine macrophages

Expression of *IL1B* mRNA was significantly higher in macrophages cultured with HDM than in those cultured without HDM (*P* < 0.01; Fig. 3A). Expression of *IL1B* mRNA was also significantly higher in macrophages cultured with HDM pre-incubated with E64 or polymyxin B than in those cultured without HDM (*P* < 0.01 for both; Fig. 3A). In contrast, no significant difference was detected in *IL1B* mRNA expression between macrophages cultured without HDM and those cultured with HDM pre-incubated with AEBSF (*P* = 0.14; Fig. 3A). When LPS was used as the stimulus instead of HDM, expression of *IL1B* mRNA was significantly higher in macrophages cultured with LPS than in those cultured without LPS (*P* < 0.01; Fig. 3B). No significant difference was detected in *IL1B* mRNA expression between macrophages without LPS and those cultured with LPS pre-incubated with polymyxin B (*P* = 0.43; Fig. 3B).

3.4. Effect of HDM on IL-1β protein levels in canine macrophages

IL-1β protein levels were significantly higher in macrophages cultured with HDM than in those cultured without HDM (*P* = 0.04; Fig. 4A). Similarly, IL-1β protein levels were significantly higher in macrophages cultured with LPS than in those cultured without LPS (*P* = 0.04; Fig. 4B).

4. Discussion

The present study clearly demonstrated that the HDM allergen, Der p 1, was detected in the GI tract of all dogs tested. Der p 1 levels in the duodenal and colonic mucosae were significantly higher in dogs with CE than in healthy laboratory dogs. In addition, HDM was able to induce the expression of *IL1B* mRNA and increase IL-1β protein levels in canine macrophages. These findings suggest that HDM allergens in the GI tract may be involved in the pathogenesis of CE by promoting IL-1β expression in macrophages.

Der p 1 levels in faeces was significantly higher in healthy client-owned dogs than in healthy laboratory dogs. The healthy laboratory

Table 2
Sequences of oligonucleotide primers.

Gene	Primer	Sequence (5' - 3')	GenBank accession number	Size (base pairs)
<i>IL1B</i>	Forward	AGCTGATGGCCCTGGAATG	NM_001037971.1	124
	Reverse	CACGAAATGCCTCAGACTCTTGTTA		
<i>GAPDH</i>	Forward	CATTGCCCTCAATGACCACT	NM_001003142.2	105
	Reverse	TCCTTGGAGGCCATGTAGAC		

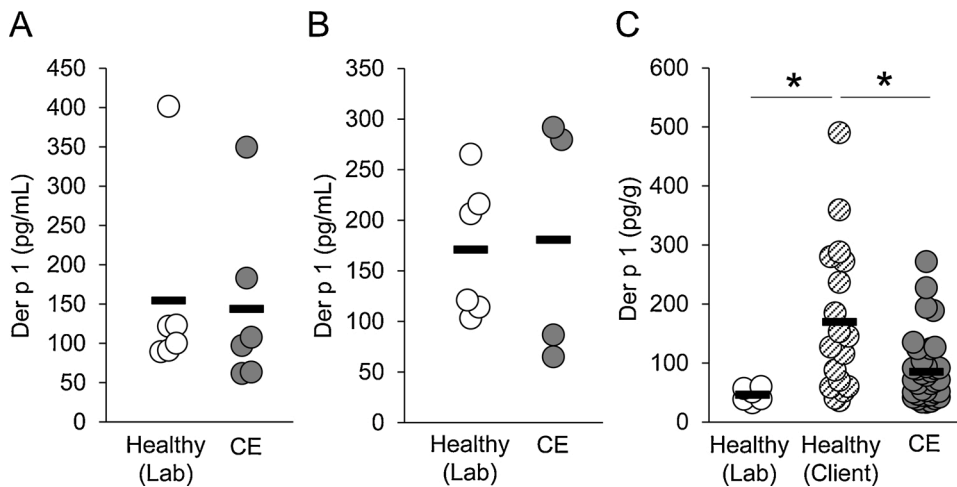


Fig. 1. Presence of *Dermatophagoides pteronyssinus* (Der p 1) in the gastrointestinal tract of dogs. Der p 1 levels in the gastric (A) and duodenal (B) fluids and faeces (C) were measured by ELISA in healthy laboratory dogs ($n = 6$), healthy client-owned dogs ($n = 18$) and dogs with chronic enteropathy (CE; gastric fluid, $n = 6$; duodenal fluid, $n = 4$; faeces, $n = 38$). The horizontal lines in each graph represent the mean value. Differences between two groups were compared by the Mann-Whitney U test (A) or the unpaired t test (B). Differences among three groups were compared by the Kruskal-Wallis test, followed by the Steel-Dwass test (C). * $P < 0.05$.

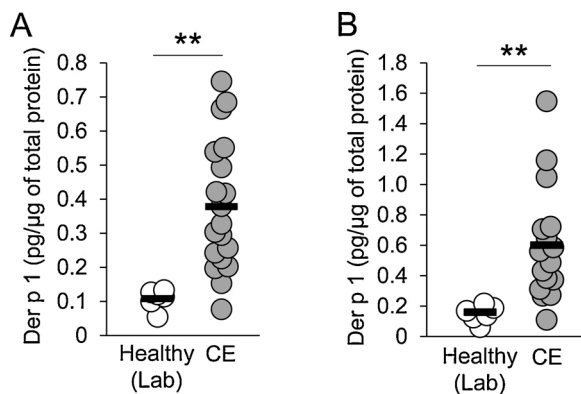


Fig. 2. Presence of *Dermatophagoides pteronyssinus* (Der p 1) in the intestinal mucosa of dogs. Der p 1 levels in the duodenal (A) and colonic (B) mucosae were measured by ELISA and normalized to the total protein concentration in each sample of healthy laboratory dogs ($n = 6$) and dogs with chronic enteropathy (CE; duodenal mucosa, $n = 19$; colonic mucosa, $n = 16$). The horizontal lines in each graph represent the mean value. Differences between two groups were compared by the unpaired t test. ** $P < 0.01$.

dogs in this study were housed in individual cages in our facility where there was little house dust and no carpets, which can be sources of HDM. In contrast, healthy client-owned dogs and dogs with CE lived indoors with their owners. In addition, the standard deviations in Der p 1 levels

in faeces were high in both healthy client-owned dogs and dogs with CE. These findings suggest that Der p 1 levels in the GI tract of dogs might be determined by their living environments. We also found that Der p 1 levels in faeces were significantly higher in healthy client-owned dogs than in dogs with CE, and the levels in the gastric and duodenal fluids did not differ between healthy laboratory dogs and dogs with CE. Since some dogs with CE showed lethargy and anorexia, ingestion of HDM may have been reduced in these dogs when compared with healthy laboratory dogs and healthy client-owned dogs. Furthermore, vomiting, diarrhoea and frequent defecation possibly lead to excretion of HDM from the GI tract of dogs with CE compared with healthy laboratory dogs and healthy client-owned dogs. To determine factors associated with the amounts of HDM allergens in the GI tract of dogs, further studies, including the measurement of HDM in the living environments, are warranted in a larger population of healthy dogs and dogs with CE.

In the current study, we found for the first time that Der p 1 levels in the duodenal and colonic mucosae were higher in dogs with CE than in healthy laboratory dogs. Intestinal permeability has been reported to be increased in dogs with IBD (Sørensen et al., 1997) and in dogs with lymphocytic-plasmacytic enteritis, a histopathological feature of CE (Kobayashi et al., 2007). In the barrier-disrupted intestines of mice with colitis, fluorescein isothiocyanate (FITC)-dextran (the molecular weight: 40 kDa) was shown to pass through the paracellular space between intestinal epithelial cells (Vijay-Kumar et al., 2007). The molecular weight of Der p 1 was 24 kDa (Gregory and Lloyd, 2011). Electrophoresis of LPS showed a ladder-like pattern ranging between 14 and 95 kDa, with most below the 45 kDa (Jürgens and Fehrenbach, 1997; Sindhu et al., 1990).

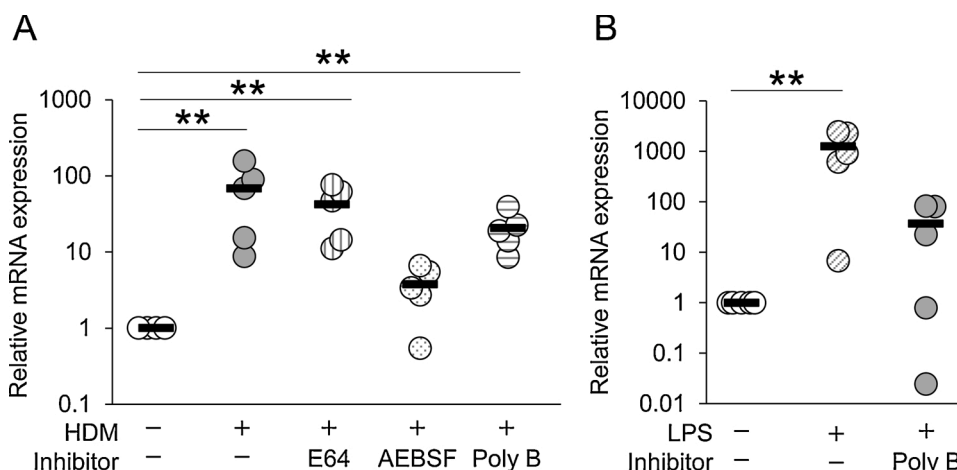


Fig. 3. Effect of house dust mite (HDM) on the expression of *IL1B* mRNA in canine macrophages. (A) Peripheral blood-derived macrophages of healthy laboratory dogs ($n = 5$) were incubated for 4 h with medium alone (control), HDM (*Dermatophagoides pteronyssinus* extract), HDM pre-treated with E64, HDM pre-treated with 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) or HDM pre-treated with polymyxin B (Poly B). (B) Peripheral blood-derived macrophages of healthy laboratory dogs ($n = 5$) were incubated for 4 h with medium alone (control), lipopolysaccharide (LPS) or LPS pre-treated with Poly B. Relative mRNA levels of *IL1B* in canine macrophages were determined by real-time PCR. The horizontal lines in each graph represent the mean value. Differences among groups were compared by the Kruskal-Wallis test, followed by the Steel test. ** $P < 0.01$.

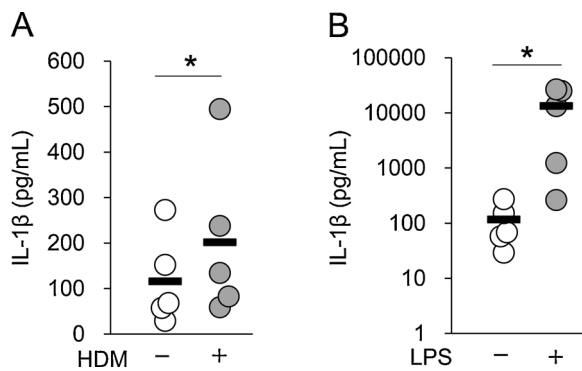


Fig. 4. Effect of house dust mite (HDM) on IL-1 β protein levels in canine macrophages. (A) Peripheral blood-derived macrophages of healthy laboratory dogs ($n = 5$) were incubated for 24 h with medium alone (control) or HDM (*Dermatophagoides pteronyssinus* extract) (B) Peripheral blood-derived macrophages of healthy laboratory dogs ($n = 5$) were incubated for 24 h with medium alone (control) or lipopolysaccharide (LPS). IL-1 β protein concentrations in cell lysates were measured by ELISA. The horizontal lines in each graph represent the mean value. Differences between two groups were compared by the Wilcoxon signed-rank test. * $P < 0.05$.

Thus, HDM allergens as well as enteric bacteria-derived components, such as LPS, may be able to penetrate the impaired epithelial barrier and invade into intestinal mucosa of dogs with CE, but not the mucosa of healthy dogs. Because general anaesthesia and endoscopic biopsies of intestinal mucosa could not be performed in healthy client-owned dogs in this study, we could not measure Der p 1 levels in the intestinal mucosa of these dogs. Therefore, we cannot rule out the possibility that Der p 1 levels in the intestinal mucosa are determined by luminal Der p 1 levels in the GI tract, and not by the impaired intestinal barrier function. However, the lack of significant differences in Der p 1 levels in the duodenal fluid and faeces between healthy laboratory dogs and dogs with CE suggests that higher levels of Der p 1 in the duodenal and colonic mucosae of dogs with CE were derived from the impaired intestinal barrier. Although Der p 1 was detected in the gastric and duodenal fluids and faeces of healthy dogs and dogs with CE, our findings raise the possibility that HDM allergens invading into the barrier-impaired intestinal mucosa may exacerbate enteritis in dogs with CE but not healthy dogs.

Our *in vitro* experiments showed that HDM and LPS induced the expression of *IL1B* mRNA and increased IL-1 β protein levels in canine macrophages. HDM contains LPS in its allergens (Gregory and Lloyd, 2011). LPS present in HDM, but not HDM itself, was reported to induce IL-1 β protein release from human monocytes (Sundaram et al., 2015). HDM was also shown to induce pro-IL-1 β expression in human macrophages, but it was not determined whether LPS present in HDM affected the expression in these cells (Sundaram et al., 2015). The present study revealed that the serine protease, but not the cysteine protease or LPS present in HDM, mediated the effect of HDM on the expression of *IL1B* mRNA. It can be speculated that due to small amounts of LPS in HDM, a LPS inhibitor, polymyxin B, did not affect HDM-induced upregulation of *IL1B* mRNA in canine macrophages under the experimental conditions of this study.

Der p 1 has cysteine protease activity whereas Der p 3, 6 and 9 (groups 3, 6 and 9 allergens of Der p, respectively) show serine protease activity (Reithofer and Jahn-Schmid, 2017). In this study, although we demonstrated that the serine protease of HDM induced the expression of *IL1B* mRNA in canine macrophages, we could not quantify Der p 3, 6 or 9 in the GI tract of dogs with CE. Alternatively, we detected Der p 1 in the intestinal lumen and mucosa of dogs with CE. The molecular weights of Der p 3, 6 and 9 were 31, 25 and 29 kDa, respectively (Gregory and Lloyd, 2011). Their molecular weights are lower than that of FITC-dextran (40 kDa) and close to that of Der p 1 (24 kDa). Therefore,

even though we did not measure these HDM allergens, they could co-exist with Der p 1 in the GI tract of dogs with CE. In a mouse model, proteases from HDM were shown to survive gut digestion and remain functional until the distal gut (Tulic et al., 2016). Collectively, these findings suggest that the serine protease of HDM may induce IL-1 β production in the intestinal mucosa of dogs with CE.

The receptor for the serine protease of HDM is protease-activated receptor-2 (PAR-2) (Gregory and Lloyd, 2011; Reithofer and Jahn-Schmid, 2017). Human macrophages, but not monocytes and dendritic cells, express PAR-2 on their cell surfaces (Colognato et al., 2003). A previous study demonstrated that trypsin, a kind of serine protease, and PAR-2 agonist peptide enhanced *IL1B* mRNA expression in *ex vivo*-cultured canine duodenum (Maeda et al., 2014). These reports, together with our findings, raise the possibility that serine protease-induced activation of PAR-2 may transduce IL-1 β production in HDM-stimulated canine macrophages. Duodenal PAR-2 expression was shown to be significantly higher in dogs with IBD than in healthy dogs (Maeda et al., 2014); therefore, the serine protease of HDM may upregulate IL-1 β through increased expression of PAR-2 in the intestinal mucosa of dogs with CE.

5. Conclusions

The present study revealed the presence of the HDM allergen in the intestinal lumen of healthy dogs and dogs with CE. The levels of the HDM allergen in the intestinal mucosa were higher in dogs with CE than in healthy dogs. We also demonstrated that the serine protease of HDM had an effect on IL-1 β production in canine macrophages. These findings suggest that the presence of HDM in the gut may exacerbate enteritis by the production of IL-1 β from intestinal macrophages in the barrier-impaired intestines of dogs with CE. Additional studies are required to further clarify the specific role of HDM as an environmental factor for the pathogenesis of CE in dogs.

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Declaration of Competing Interest

None of the authors have any financial or personal relationship that could inappropriately influence or bias the content of the paper.

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