1 **Lymphocyte subpopulations and mast** 2 **cells intestinal changes as indicators of** 3 **inflammatory bowel disease in dogs**

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11

12 **Abstract**

13 Inflammatory bowel disease (IBD) is a disease with recurring
14 gastrointestinal symptoms. Lymphocytes and mast cells are proposed as
15 important components in the immunopathology of IBD in dogs. Mast cells
16 depend on degranulation, a process that compromises mucosal
17 permeability and normal intestinal barrier function, which alters the normal
18 inflammatory process by allowing recruitment of lymphocytes in dogs with
19 IBD. In this study, T and B lymphocyte populations and mast cells were
20 examined in situ in 39 intestinal samples of dogs affected by IBD, by

immunohistochemistry. Both T lymphocytes and mast cells numbers were significantly higher in the lamina propria of the intestinal wall of dogs with IBD compared with control dogs. Out of the total number of mast cells detected by CD117 expression significantly less cells appear to be granulated according to granule staining with Toluidine Blue, suggesting that an important degranulation process takes place in IBD. Single and double immune staining for tryptase and chymase showed that mast cells can express only one or both enzymes. Tryptase positive cells were significantly higher in number than chymase positive and tryptase/chymase positive cells. T lymphocytes were concentrated mostly at the upper portion of the intestinal villi lamina propria while mast cells were distributed mainly among crypts. These results suggest that populations of T lymphocytes and mast cells play a role in the immunopathology and development of IBD in dogs, also these changes could be helpful as complementary indicators of canine IBD.

Keywords: dog; inflammatory bowel disease; immunohistochemistry; T lymphocytes; mast cells

Introduction

Canine inflammatory bowel disease (IBD) is a group of gastrointestinal disorders characterized by persistent or recurring gastrointestinal symptoms with histologic evidence of inflammatory cell infiltration (1).

44 There are different forms of IBD in dogs, depending on the main cell type
 45 involved and the affected portion of the intestine where the infiltration
 46 takes place, being lymphocytic-plasmacytic enteritis the most common
 47 form (2). The etiology of IBD remains unknown. Several studies suggest
 48 that these diseases result from inappropriate immune responses to the
 49 intestinal microbiome in genetically susceptible individuals (3–6). There
 50 are several factors involved including microbiome, environmental factors,
 51 genetic predisposition, and changes in the immune response of the
 52 individual, which may lead to loss of tolerance to the endogenous flora
 53 and the development of chronic inflammation of the gastrointestinal tract
 54 (7,8).

55 Different studies (9–12) suggest that a primary defect in the
 56 recognition of commensal bacteria or bacterial pathogens in the intestinal
 57 lumen, may lead to an increase in the production of IL-23, that induce
 58 naïve T cells to differentiate into T-helper (Th) lymphocytes, which release
 59 large amounts of proinflammatory cytokines (13,14). These cytokines
 60 damage the intestinal epithelium, allowing other pathogens to invade the
 61 lamina propria, driving more naïve T cell to differentiate into Th cells (4).
 62 Kleinschmidt and colleagues reported the increase of Th lymphocytes in
 63 IBD dogs, finding that lymphocytic-plasmacytic enteritis is mediated by
 64 Th1 lymphocytes, while eosinophilic gastroenteritis is mediated by
 65 hypersensitivity reactions modulated by Th2 lymphocytes, suggesting
 66 that there are different pathologic mechanisms (15). However, Heilmann
 67 and Suchodolski mentioned that in contrast to the results in human IBD,

68 a distinct T-helper lymphocyte profile has not been clearly demonstrated
69 to exist in canine or feline inflammatory bowel disease at this time (16).
70 Overall, these studies evidence the importance of T lymphocytes in the
71 adaptive immune system response during the development of IBD in
72 dogs.

73 Besides T lymphocytes, B cells had been related to immunologic
74 abnormalities in IBD in dogs too, but their number is usually lower
75 compared with T lymphocytes (17). B cells perform several immunological
76 functions but have been considered mainly as positive regulators of
77 immune responses and central contributors to the pathogenesis of
78 immune-related diseases because of their ability to produce antibodies,
79 especially during the period between innate and adaptive immunity
80 (18,19). The initial event that drives naïve cells to differentiate in B cells
81 in IBD remains unknown, however, abrogation of the oral tolerance to
82 commensal bacteria, lymphoid neogenesis, and hyperplasia in lesions
83 may be involved (20).

84 Different molecular markers are used to determinate the populations
85 of lymphocytes existing in the intestinal mucosa. The cluster of
86 differentiation 3 (CD3) is a protein complex located on T lymphocyte cell
87 surface and makes an ideal target for T lymphocyte in tissue sections. For
88 detecting B cells, CD79a which is expressed by B lymphocytes during
89 differentiation from early pre-B cell stage through to plasma cells, makes
90 it a useful marker for mature B lymphocyte (21), or CD20, which is
91 expressed in pre-, naïve and mature B-lymphocytes (22).

92 In addition to lymphocytes, mast cells and its mediators have also
 93 been associated with the pathogenesis of IBD. Mast cells are
 94 haematopoietic cells that arise from bone marrow and develop into
 95 mature mast cells under the influence of local growth factors, in particular,
 96 stem cell factor (SCF) (23). SCF, the ligand for the CD117/c-Kit receptor,
 97 is essential for mast cell proliferation, development, migration, survival
 98 and mediator release (24). Normally, mast cells can be found close to
 99 blood vessels or nerves beneath epithelial surfaces, where these cells are
 100 stimulated by environmental antigens (25). Within seconds of stimulation,
 101 mast cells can undergo degranulation, rapidly releasing pre-formed
 102 mediators present within cytoplasmic granules, including histamine,
 103 proteases, and tumor necrosis factor-alpha (TNF- α) (26). This affects the
 104 intestinal barrier function by increasing mucosal permeability. GI mast cell
 105 can contribute to an ongoing inflammatory process in the GI tract, allowing
 106 recruitment of granulocytes and lymphocytes to the site of the infection
 107 (27). Also, it had been speculated that interaction between commensal
 108 bacteria and mast cells affects the gastrointestinal barrier, promoting
 109 mucosal penetration of pathogens in the intestinal lamina propria
 110 perpetuating tissue damage and adaptive immune cells infiltration (28).

111 Commonly, toluidine blue staining of granules is the method used for
 112 the identification and quantification of mast cells in most tissues (29,30),
 113 but it is not always possible to detect fully degranulated mast cells with
 114 this technique (31). Alternately, CD117, which is a mast/stem cell grown
 115 factor receptor (SCFR) found in the membrane of mast cells, had been

116 targeted to determinate activated mast cell in several tissues, including
117 degranulated mast cells in the gastrointestinal mucosa (32,33).

118 Mast cells contain preformed granules that include heparin,
119 histamine, cytokines, chemotactic factors, proteases and lipid derivatives
120 that fulfill several biological functions, among which are phagocytosis,
121 release of vasoactive substances and chemotaxis. (34). Mast cells are
122 particularly rich in serine proteases stored and released from the
123 secretory granules of mast cells. They present two subtypes, the mast
124 cells, which contain only chymase and the mast cells that produce only
125 tryptase and are located in the mucous membranes, close to T helper
126 cells (Th2 type that release IL-4, 5, 6 and 13) (35), particularly in the
127 intestinal lamina propria. These proteases promote vascular permeability
128 through several mechanisms. They contribute to tissue remodeling
129 through selective proteolysis of the matrix and the activation of
130 metalloproteinases (36), in addition they promote the chemotaxis of
131 inflammatory cells that surround the innate immune response (37).
132 Changes in the number of mast cells in several anatomical sites and/or
133 evidence of degranulation have been observed in a wide range of
134 responses including hypersensitivity reactions (36,38), fibrosis,
135 autoimmune pathology, inflammatory diseases, and neoplasia (39–41).

136 Lymphocytes and mast cells seem to play a role in the development
137 and maintenance of IBD in dogs, considering they are an important cell
138 component in lesions associated with chronic and autoimmune diseases.
139 Changes in lymphocyte and mast cells populations could help in the

140 diagnosis of IBD and contributes to improving understanding of this
141 disease. Thereby, this study aimed to evaluate if there is a specific
142 predominance of T or B lymphocytes and degranulated mast cells in the
143 intestinal wall from dogs with IBD when compared with healthy controls.

144

145 **Materials and methods**

146 **Dogs and Tissue Samples**

147 Archived formalin-fixed paraffin-embedded (FFPE) intestinal biopsy
148 pathology samples from dogs with a clinical and histopathological
149 diagnosis of IBD were used for the study. In addition, samples from ten
150 healthy dogs, with no history of antibiotic or immunosuppressive
151 treatments two weeks before biopsy were included as controls. Only
152 biopsy samples including both mucosa and submucosa, or the entire
153 intestinal wall were considered in this study. Clinical inclusion criteria for
154 these cases were vomiting, diarrhea, anorexia, weight loss, or some
155 combination of these signs for at least 3 weeks, with no recent history of
156 administration of immunosuppressive drugs or antibiotics. The
157 histopathological inclusion criteria considered lymphocytic plasmacytic
158 enteritis or colitis cases scored as marked, according to the
159 histopathologic guidelines for the evaluation of gastrointestinal
160 inflammation in companion animals recommended previously (42). Health
161 status was assessed considering normal physical examination, and blood
162 test results. Informed consent was obtained from all owners, and the

163 study protocol was approved by the Ethics Committee of the Faculty of
164 Life Sciences, Andres Bello University, Santiago, Chile. Endoscopic
165 biopsy samples were collected for diagnostic and research purposes as
166 described previously (43). All tissue samples were immediately placed in
167 10% neutral buffered formalin, processed conventionally and embedded
168 in paraffin wax.

169

170 **Histology and Immunohistochemistry**

171 All biopsies were cut (3µm) thick and stained with hematoxylin and eosin
172 (H&E), toluidine blue, to detect granulated mast cells, and Masson's
173 trichrome staining, to assess fibrosis in lesions. Serial sections from
174 samples were subjected to immunohistochemistry specific for T cells
175 (CD3), B cells (CD79a) and mast cells (CD117). Briefly, after dewaxing,
176 tissue sections were immersed in Tris-buffered saline (TBS 1X, pH 7.4).
177 For antigen retrieval, slides were incubated for 20 minutes in citrate buffer
178 (pH6.0) for CD3 and CD79a, or 15 minutes in proteinase K for CD117.
179 Endogenous peroxidase was quenched with 3% hydrogen peroxide for
180 15 minutes. Non-specific binding was blocked by incubating slides for 20
181 minutes with a 2.5% normal horse blocking serum. Slides were incubated
182 with the following primary antibodies: CD3 diluted 1:50 for 30 minutes,
183 CD79a diluted 1:300 for 40 minutes, or CD117 diluted 1:300 for 20
184 minutes. Then, all slides were incubated with ImmPRESS™ HRP
185 Reagent Kit Universal anti-mouse/rabbit IgG (Vector Laboratories,

186 Burlingame, CA, USA) for 30 minutes. Primary antibody reactivity was
187 detected by ImmPACT™ DAB Peroxidase Substrate Kit (Vector
188 Laboratories, Burlingame, CA, USA) according to the manufacturer's
189 instructions, and all slides were counterstained with Mayer's hematoxylin.
190 Table 1 shows a summary of the primary antibodies used in the study.

191

192 **Table 1. Primary antibodies used in the study.**

193

194 For mast cell chymase and tryptase detection samples were
195 incubated with MastCell Tryptase Abcam Rabbit monoclonal Clone
196 ERP8476, and MastCell ChymaseThermo Mouse monoclonal Clone
197 CC1. For double immune staining Polink TS-MMR-Hu A Kit, Polymer-
198 HRP &AP triple staining kit was used.

199

200 **Examination of sections**

201 Histological grading was assessed in H&E stained sections of intestine of
202 dogs with IBD (n=39), according to WSAVA Gastrointestinal
203 Standardization Group guidelines (42,44), by an experienced pathologist
204 (CG). Vascular, degenerative and inflammatory histologic parameters
205 were scored based on a scale from 0 to 3 as follows: normal (0), mild (1),
206 moderate (2) and marked (3). Then, each parameter was assigned an
207 importance factor (45,46) of 1 to 3 according to its histopathological
208 relevance. We gave the maximal score to lacteal dilation, intraepithelial

209 lymphocyte (IEL) and lamina propria lymphocyte infiltration compared to
 210 other features like fibrosis, villous stunting, epithelial injury and crypt
 211 distension. Also the height and width in both villi and lacteals was
 212 evaluated to assess villous stunting and crypt distention, as mentioned
 213 before (47). The assigned importance factor was multiplied by the score
 214 obtained in the first evaluation. Finally, we calculated the total corrected
 215 score by arithmetic means and classified cases into different severity
 216 groups as: normal ($\leq 25\%$ of the total score), mild (25–50% TS), moderate
 217 (50–75% TS) and marked ($> 75\%$ TS).

218 Samples were evaluated with an Olympus microscope FSX-100
 219 (Olympus, Center Valley, PA, USA) and then computer-assisted
 220 morphometric analyzed with the ImageProPlus TM 4.5 software (Media
 221 Cybernetics, Silver Springs, MD, USA). For scoring of intestinal CD3+ T
 222 lymphocytes, CD79a+ B lymphocytes, mast cells stained with blue
 223 toluidine and CD117+ mast cells, the number of cells in the lamina propria
 224 of ten appropriate fields (magnification at 40X) were quantified and
 225 arithmetic means were calculated for each. Results were expressed as
 226 the average of positive cells per high power field (HPF). Regarding for
 227 blue toluidine staining, we only considered granulated mast cells, those
 228 with visible metachromatic granules. Also morphologic features were
 229 taken into account that indicated that these cells were indeed mature
 230 granulated mast cells as described before (30).

231

232 **Statistics**

233 The results were subjected to SPSS Statistics 22 software (SPSS Inc.,
234 Chicago, IL, USA) for analysis. Cell counts were assessed for normal
235 distribution and non-parametric tests were performed. Differences
236 between cell population numbers in dogs with IBD and controls were
237 analyzed using the Wilcoxon test, and $P \leq 0.05$ was considered significant.

239 **Results**

240 44,497 archived reports corresponding to all the tissues samples of dogs
241 received in a 10 years period (2008-2017), by a Diagnostic Veterinary
242 Laboratory (Citovet-Chile), were reviewed. Only 215 samples (0.48%)
243 that showed histopathological findings compatible with IBD. Seventy-
244 seven (88.5%) samples corresponded to a lymphocytic-plasmacytic
245 enteritis, 92 (71.9%) samples to lymphocytic-plasmacytic colitis, 4 (4.6%)
246 samples to eosinophilic enteritis, 5 (3.9%) samples to eosinophilic colitis
247 and the remaining 37 samples corresponded to cases classified as non-
248 specific enteritis or colitis. Distribution of the IBD cases from this study, is
249 shown in Table 2.

251 **Table 2. Distribution of anatomic regions affected and histological types of IBD**
252 **cases.**

253

254 A summary of the distribution of frequency of sex, age (Fig 1) and
255 breed of the dogs diagnosed with IBD during the 10 years period, is
256 shown in Table 3. Regarding healthy control dogs, 7 of them were
257 females, and 3 males, age range between 1 to 6 years and more than half
258 were crossbreed dogs.

259

260 **Figure 1. Age distribution of 215 dogs suffering from IBD.**

261

262 **Table 3. Distribution of frequency of sex, age and breed of dogs with IBD.**

263

264 Out of the 77 cases of dogs with a clinical and histopathological
265 diagnosis of IBD lymphocytic-plasmacytic enteritis or colitis, that met the
266 criteria established previously, thirty-nine FFPE intestinal samples,
267 suitable for immunohistochemical study were selected for this study.
268 Twenty-seven of thirty-nine intestinal samples (69.2%) corresponded to
269 lymphocytic-plasmacytic enteritis (LPE). Nine of them, were classified as
270 moderate LPE, and eighteen as marked LPE. Most of the marked cases
271 showed significantly villous stunting, epithelial injury, lacteal distention,
272 loss and dilation of crypts and a severe lymphocyte and plasma cells
273 infiltration (Fig 3A).

274 The remaining twelve samples (30.8%) corresponded to lymphocytic-
275 plasmacytic colitis (LPC). Ten of them were classified as moderate LPC,
276 and the other two as marked LPC. Marked cases commonly showed
277 villous stunting, dilatation, and loss of crypts, fibrosis, and a severe

278 lymphocyte infiltration. Intestinal samples from all control dogs showed a
279 normal histology.

280

281 **T and B lymphocyte populations**

282 CD3+ T lymphocytes and CD79a+ B lymphocytes were detected in all
283 cases. The median number of the different type of cells populations in IBD
284 and control dogs are shown in Table 4.

285

286 **Table 4. Comparison of mean lymphocyte and mast cells counts in dogs with IBD**
287 **and control dogs.**

288

289 The total number of CD3+ T lymphocytes in the lamina propria of the
290 intestine from dogs with IBD were significantly higher compared with
291 control dogs ($p < 0.001$). The number of CD3+ T lymphocytes of dogs with
292 IBD was higher in the villi (Fig 2 and Table 5) and its number reduced
293 toward the crypts in cases of LPE (Fig 3B), seeing a similar distribution in
294 the colonic mucosa in cases of LPC. The total number of CD79a+ B
295 lymphocytes in the lamina propria of dogs with IBD were significantly
296 higher compared with control dogs ($p < 0.001$). The distribution of CD79a+
297 B lymphocytes was similar in LPE and LPC cases, predominating near
298 the crypt areas (Fig 2 and Table 5). The total number of CD3+ T
299 lymphocytes was significantly higher ($p < 0.001$) than the total number of
300 CD79a+ B lymphocytes in the intestinal lamina propria of dogs with IBD.
301 Regarding controls dogs, the total number of CD3+ T lymphocytes was

302 significantly higher ($p < 0.001$) compared with the total number of CD79a+
303 B lymphocytes.

304

305 **Figure 2. The histological organization of the intestinal wall and lymphocyte**
306 **infiltration zones in canine IBD. Adapted from Martini et. al (2015) (48).**

307

308 **Table 5. Distribution of the infiltrative lymphocyte and mast cells subpopulations**
309 **in the intestinal wall mucosa in canine IBD and controls.**

310

311 **CD117+ and blue toluidine mast cells**

312 Mast cells were detected in all samples by both blue toluidine staining and
313 CD117 immunophenotyping (Fig 3C and D). The total number of CD117+
314 mast cells in the lamina propria of the intestine from dogs with IBD was
315 significantly higher compared with control dogs ($p < 0.013$). However, the
316 number of granulated mast cells detected with blue toluidine in samples
317 from dogs with IBD was lower compared with control dogs ($p < 0.014$). The
318 total number of CD117+ mast cells detected in the lamina propria of dogs
319 with IBD was significantly higher compared to the number of mast cells
320 detected with blue toluidine in IBD dogs. ($p < 0.001$). The distribution of
321 mast cells was similar in LPE and LPC cases, located between the villous
322 and close to the crypts (Fig 2 and Table 5).

323

324 **Figure 3. Lymphocytic-plasmacytic enteritis.**

325 **A. Biopsy from a dog with lymphoplasmacytic enteritis of marked severity. There**
 326 **is an increase of lymphocytes, plasma cells and some macrophages in the lamina**
 327 **propria, marked villus stunting and fusion. Hematoxylin and eosin stain. Bar 300**
 328 **µm. B. Large amount of CD3+ lymphocytes (brown color) are detected throughout**
 329 **the lamina propria of the villi using immunohistochemistry. Bar: 300 µm. C.**
 330 **CD117+ mast cells (arrowheads) scattered throughout the lamina propria of the**
 331 **villi using immunohistochemistry. Bar: 50 µm. D. Granulated mast cells**
 332 **(arrowheads) and degranulated mast cells (arrows) scattered throughout the**
 333 **lamina propria of the villi. Blue toluidine stain. Bar: 60 µm.**

334

335 **Chymase and tryptase detection in mast cells**

336 Mast cells showed chymase and tryptase cytoplasmic expression in
 337 intestinal lamina propria. Some mast cells were only tryptase positive both
 338 in single stained samples (Fig 4A) or double stained samples (Fig 4C and
 339 E). Also, some mast cells were only chymase positive both in single
 340 stained samples (Fig 4B) or double stained samples (Fig 4C and F). On
 341 the other hand, there were mast cell were tryptase and chymase double
 342 positive (Fig 4D, E and F). The number of mast cells positive only to
 343 tryptase was significantly higher ($p<0.001$) than both mast cells positive
 344 only to chymase and mast cells double positive for tryptase/chymase. The
 345 double positive cells were significantly lower in number ($p<0.001$) to
 346 single positive cells to either tryptase o chymase.

347

348 **Figure 4. Positive reaction to chymase or tryptase expression or both in mast cells**
 349 **in canine small bowel lamina propria. Single immune staining for tryptase can be**
 350 **seen in Fig. A (green color) and for chymase in Fig. B (red color). Figure C to F**
 351 **show double immune staining for tryptase/chymase. Figure C shows mast cells**
 352 **positive to either tryptase (green color) o chymase (red color). Figure D shows a**
 353 **mast cell positive to both tryptase/chymase (green/red color). Figure E shows a**
 354 **group of mast cells stained for tryptase (green color) and one double stained**
 355 **(arrow). Figure F shows a group of mast cells stained for chymase (red color) and**
 356 **one double stained (arrow). Figs A to D 1000x. Figs E and F 400x.**

357

358 **Discussion**

359 In this study, different types of leukocytes were examined extensively in
 360 the intestinal layer of dogs with inflammatory bowel disease. After a
 361 retrospective study analysis of 44,497 FFPE samples of dogs, from 2010
 362 to 2017, 215 samples presented histopathological findings compatible
 363 with IBD, giving a prevalence of 0,48% for this disease. Low IBD
 364 prevalence (1,99%) has also been reported Kathrani and colleagues,
 365 who found 546 patients diagnosed with IBD, after looking at the clinical
 366 records of 27463 patients treated at the Queen Mother Hospital for
 367 Animals, from the Royal Veterinary College during a six-year period (6).
 368 Although prevalence is similar in both studies, results are not necessarily
 369 comparable given the fact that our study considered only
 370 histopathological samples. German Shepherd dogs and crossbreeds
 371 were the most commonly affected breeds in our study. Similar results had

372 also been reported in the United Kingdom by several studies (6,49–51),
 373 suggesting that German shepherd dogs are at increased risk of
 374 developing IBD. Authors identified several candidate genes by genome-
 375 wide association studies, showing a genetic predisposition of this breed.
 376 However, our study did not include any genetic analysis, and results may
 377 support this hypothesis, but could also be reflecting a sample bias (See
 378 Table 3). No significant difference regarding gender or age was found in
 379 the IBD group, as similarly reported by other studies (6,52).

380 Unexpected difficulties were experienced when processing the
 381 endoscopic biopsy samples, particularly related to a low amount of
 382 obtained material, localization of the lesions and improper orientation of
 383 the tissue. Willard and colleagues and Hall reported that the quality and
 384 amount of endoscopically obtained tissue samples has a profound effect
 385 on their sensitivity for identifying certain lesions (43,53). In addition,
 386 almost all endoscopic biopsies were sampled from duodenum or/and
 387 colon, and few of them had ileum. This may be relevant as Casamian-
 388 Sorrosal and colleagues found poor agreement between
 389 histopathological findings from duodenal versus ileum biopsies with
 390 abnormalities sometimes being more readily detected in the ileum (54),
 391 thus the collection of concurrent duodenal and ileum endoscopic biopsies
 392 is recommended (55). As we were not able to control how the samples
 393 were collected and processed, only those showing lesions consistent with
 394 IBD were included in this study.

395 Most of IBD cases corresponded to lymphocytic-plasmacytic
 396 enteritis/colitis. Previous studies demonstrated that lymphocytic-
 397 plasmacytic enteritis/colitis is the most common form of IBD in dogs
 398 (1,56,57). Many researchers follow the endoscopic, biopsy, and
 399 histopathologic guidelines for the evaluation of gastrointestinal
 400 inflammation in companion animals (42,44) to assess the pathological
 401 lesions found in chronic gastroenteropathies in dogs with IBD. Villus
 402 atrophy, lacteal dilatation, and the presence of inflammatory infiltrate are
 403 evaluated and graded with this system, however, these guidelines do not
 404 rank the lesion according to how important it is to the organ function.
 405 Different studies proposed the use of a standardized method that allows
 406 the quantification of organ damage, extent and pathological importance
 407 of changes in a fish species (45,46,58). This standardized tool for the
 408 assessment of histological lesions can be applied to different organs.
 409 Hereby, a similar approach was put forward to classify IBD scoring in
 410 dogs. Using the proposed method, it was possible to categorize marked
 411 and moderate cases regarding the traditional guideline that classifies
 412 them initially as moderate and mild respectively.

413 Most LPE cases were classified as marked, while most LPC cases
 414 were scored as moderate. These findings might be related with the fact
 415 that most patients are referred for endoscopy and histopathological
 416 examination only when clinical signs have persisted for a long period of
 417 time or when patients do not respond to conventional treatment. In
 418 humans, diagnosis of IBD is often delayed, particularly in Crohn's disease

(CD) due to the variety of its clinical signs, being opposed to ulcerative colitis (UC), where symptoms appear earlier (59). Further, a long diagnostic delay was associated with a poor outcome when IBD-related surgery was performed in CD and UC patients (60). No recent studies regarding how a diagnosis delay affects the severity of IBD in dogs were found, however, it is possible that a similar scenario is seen, being moderate cases detected faster in lymphocytic-plasmacytic colitis than in lymphocytic-plasmacytic enteritis due to earlier signs manifestation. More studies are necessary to elucidate this issue.

A significantly higher number of CD3+ T lymphocytes, CD79a+ B lymphocytes, and CD117+ mast cells were detected in the lamina propria of dogs with IBD compared with healthy dogs. Changes in the number of CD3+ lymphocytes were similar to those found in other studies (61,62) in which CD3+ T cells were higher in the lamina propria of dogs with IBD. These changes in the lymphocytic composition could be related to a variety of components affecting the immune system of those individuals (5). Jergens found that a resistance to apoptosis by T cells could also contribute to the typical cell accumulation in IBD dogs (63). A higher number of T cells in the lamina propria of the gut compared with B cells was also found, which may be related to the cytokine profile present in this disease (16,64,65), facilitating the accumulation of T cells in the lamina propria. CD117+ mast cells were detected in a higher number in the lamina propria of the intestine from dogs with IBD similarly in other

442 study (66), and also observed in intestine samples from cats with IBD
443 (67).

444 Conversely, a decreasing number of metachromatically stained mast
445 cells was reported (15,68), suggesting reduced lamina propria mast cell
446 numbers in dogs with IBD. This apparently occurs because the staining
447 of mast cells by immunohistochemistry, but not by toluidine blue, is
448 independent of whether mast cells are degranulated or not. Our results
449 suggest that the number of mast cells in the lamina propria of intestine
450 from dogs with IBD does not necessarily increase significantly, but the
451 portion of degranulated mast cells is higher. These findings point out that
452 whether mast cells are increased in number or not they become activated
453 and degranulated during IBD in dogs. Mast cells degranulation will also
454 trigger the recruitment of more inflammatory cells, like T lymphocytes, in
455 the intestinal layer (26,36), intensifying the typical lymphocyte infiltrative
456 pattern seen in this disease.

457 In this study the number of mast cells positive only to tryptase
458 was significantly higher ($p < 0.001$) than both mast cells positive only to
459 chymase and mast cells double positive for tryptase/chymase. Tryptase
460 is a potent growth factor for epithelial cells, smooth muscle and
461 fibroblasts, it also regulates gastrointestinal smooth muscle activity and
462 intestinal transport (69). It also develops as an anticoagulant, fibrinolytic
463 agent, activator of PAR-2 (Protease-Activated Receptor) (activated by
464 trypsin and mast cell tryptase, associated with physiological processes
465 such as mediators and cytokine releasers, vasodilation, platelet

466 aggregation, cell proliferation, and in contraction or relaxation of the
467 smooth muscle) (70), improvement of vascular permeability,
468 angiogenesis, inflammation and hyperactivity of the smooth muscle of the
469 airways (71). Whereas chymase helps maintain the function of the
470 intestinal barrier (37), participates in defense against parasites (72),
471 promotes epithelial permeability, and maintains blood pressure during
472 anaphylaxis by the generation of angiotensin II, Chymase and Cathepsin
473 G destroy several cytokines associated with inflammation, which causes
474 the decrease or end of inflammation (37).

475 In our study CD3+ T leucocytes were predominantly located in the
476 villous region, however, CD79a+ B cells and CD117+ mast cells were
477 located close to crypt region, very similar to other studies (61,73). There
478 is no clear reason for this distribution, but it is possible that T lymphocytes
479 are higher in villi because of a higher presentation of antigen associated
480 to the molecule histocompatibility complex by mononuclear cells (74), and
481 this could be related to the close contact of stimulant bacteria in the villi
482 and the crypts. In mice, mast cells reside in the muscle/submucosa region
483 (75) and after they are activated by substance P produced by
484 lymphocytes and macrophages (76), they move into the lamina propria
485 and the epithelium where inflammatory mediators such as serotonin and
486 proteases are released (25). Mast cells located around the crypts could
487 be associated with close contact of environmental antigens, because they
488 recognize molecular motifs (pattern recognition molecules) which trigger
489 a subsequent immune response attracting lymphocytes to the site of the

490 lesion (77,78). Thus, a clear and strong relation between mast cells and
491 T lymphocytes could possibly explain the changes observed in the
492 intestinal layer of dogs affected with IBD.

493 In summary, our results show that CD3+ lymphocytes and CD117+
494 mast cells were the predominant leukocyte population in the intestine of
495 dogs with IBD compared to control dogs and found that CD3+
496 lymphocytes mainly occupy villous tips while CD79a+ lymphocyte and
497 CD117+ are distributed close to the crypts. Changes in the number of T
498 lymphocytes and mast cells may be considered as an additional criteria
499 tool for the diagnosis of IBD in dogs, as increased numbers of both cell
500 types were found as an indicator of disease severity. Also, the number of
501 activated mast cells detected by CD117 immunophenotyping was higher
502 than the number of granulated mast cells detected by blue toluidine
503 staining, showing that degranulation is probably occurring in this disease.
504 The information generated from this study suggest an important role of
505 both T lymphocytes and mast cells in the immunopathology of IBD in
506 dogs. The specific role of these leukocytes populations in IBD onset and
507 development should be further investigated to allow the development of
508 medical treatments focused on their regulation. Although diagnostic
509 guidelines for IBD report the parameters for mild IBD scoring, in our
510 experience, differences between structural and infiltrative changes
511 between normal cases and mild IBD are difficult to demonstrate, and
512 misclassification may occur. Considering this, only moderate and severe
513 cases were included in this study. However, according to the results

514 obtained in his study differences in both infiltration by lymphocyte
515 subpopulations and their tissue location as well as estimation of mast cell
516 number should be also investigated in mild cases.

517

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522

523 **Conflict of Interest Statement**

524 All the authors declare that they do not have any conflicts of interest
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526

527 **References**

- 528 1. Jergens AE. Inflammatory Bowel Disease. Vet Clin North Am
529 Small Anim Pract. 1999;29(2):501–21.
- 530 2. Cerquetella M, Spaterna A, Laus F, Tesei B, Rossi G, Antonelli E,
531 et al. Inflammatory bowel disease in the dog: Differences and
532 similarities with humans. World J Gastroenterol. 2010;16(9):1050–
533 6.
- 534 3. Van Limbergen J, Wilson DC, Satsangi J. The genetics of Crohn's

- 535 disease. Annu Rev Genomics Hum Genet. 2009;10:89–116.
- 536 4. Allenspach K. Clinical Immunology and Immunopathology of the
537 Canine and Feline Intestine. Vet Clin North Am Small Anim Pract
538 [Internet]. 2011;41(2):345–60. Available from:
539 <http://dx.doi.org/10.1016/j.cvsm.2011.01.004>
- 540 5. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology
541 of inflammatory bowel disease. World J Gastroenterol.
542 2014;20(1):6–21.
- 543 6. Kathrani A, Werling D, Allenspach K. Canine breeds at high risk of
544 developing inflammatory bowel disease in the South-Eastern UK.
545 Vet Rec. 2011;169(24):1–4.
- 546 7. Allenspach K, Wieland B, Gröne A, Gaschen F. Chronic
547 enteropathies in dogs: Evaluation of risk factors for negative
548 outcome. J Vet Intern Med. 2007;21(4):700–8.
- 549 8. Malewska K, Rychlik A, Nieradka R, Kander M. Treatment of
550 inflammatory bowel disease (IBD) in dogs and cats. Pol J Vet Sci.
551 2011;14(1):165–70.
- 552 9. Burgener IA, König A, Allenspach K, Sauter SN, Boisclair J,
553 Doherr MG, et al. Upregulation of Toll-Like Receptors in Chronic
554 Enteropathies in Dogs. J Intern Med. 2008;22(3):553–60.
- 555 10. Matricon J, Barnich N, Ardid D. Immunopathogenesis of
556 inflammatory bowel disease. Self Nonself [Internet].

- 2010;1(4):299–309. Available from:
<http://www.tandfonline.com/doi/abs/10.4161/self.1.4.13560>
11. Suchodolski JS. Companion animals symposium: Microbes and gastrointestinal health of dogs and cats. *J Anim Sci*. 2011;89(5):1520–30.
 12. Hold GL, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhyaya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: What have we learnt in the past 10 years? *World J Gastroenterol*. 2014;20(5):1192–210.
 13. Xenoulis PG, Palculict B, Allenspach K, Steiner JM, Van House AM, Suchodolski JS. Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiol Ecol*. 2008;66(3):579–89.
 14. Sartor RB, Mazmanian SK. Intestinal Microbes in Inflammatory Bowel Diseases. *Am J Gastroenterol Suppl* [Internet]. 2012;1(1):15–21. Available from: <http://www.nature.com/doi/abs/10.1038/ajgsup.2012.4>
 15. Kleinschmidt S, Meneses F, Nolte I, Hewicker-Trautwein M. Characterization of mast cell numbers and subtypes in biopsies from the gastrointestinal tract of dogs with lymphocytic-plasmacytic or eosinophilic gastroenterocolitis. *Vet Immunol*

- 579 Immunopathol. 2007;120(3–4):80–92.
- 580 16. Heilmann RM, Suchodolski JS. Is inflammatory bowel disease in
581 dogs and cats associated with a Th1 or Th2 polarization? Vet
582 Immunol Immunopathol [Internet]. 2015;168(3–4):131–4. Available
583 from: <http://dx.doi.org/10.1016/j.vetimm.2015.10.008>
- 584 17. Haas E, Rütgen BC, Gerner W, Richter B, Tichy A, Galler A, et al.
585 Phenotypic Characterization of Canine Intestinal Intraepithelial
586 Lymphocytes in Dogs with Inflammatory Bowel Disease. J Vet
587 Intern Med. 2014;28(6):1708–15.
- 588 18. Defendenti C, Sarzi-Puttini P, Grosso S, Croce A, Senesi O,
589 Saibeni S, et al. B Lymphocyte intestinal homing in inflammatory
590 bowel disease. BMC Immunol. 2011;12.
- 591 19. Mauri C, Bosma A. Immune Regulatory Function of B Cells. Annu
592 Rev Immunol [Internet]. 2012;30(1):221–41. Available from:
593 [http://www.annualreviews.org/doi/10.1146/annurev-immunol-](http://www.annualreviews.org/doi/10.1146/annurev-immunol-020711-074934)
594 [020711-074934](http://www.annualreviews.org/doi/10.1146/annurev-immunol-020711-074934)
- 595 20. Brandhorst G, Petrova DT, Weigand S, Eberle C, Von Ahsen N,
596 Schmitz J, et al. Lack of correlation between Treg quantification
597 assays in inflammatory bowel disease patients. World J
598 Gastroenterol. 2015;21(11):3325–9.
- 599 21. Fernandez NJ, West KH, Jackson ML, Kidney BA.
600 Immunohistochemical and histochemical stains for differentiating

- 601 canine cutaneous round cell tumors. Vet Pathol. 2005;42(4):437–
602 45.
- 603 22. Ernst JA, Li H, Kim HS, Nakamura GR, Yansura DG, Vandlen RL.
604 Isolation and characterization of the B-cell marker CD20.
605 Biochemistry. 2005;44(46):15150–8.
- 606 23. Wernersson S, Pejler G. Mast cell secretory granules: Armed for
607 battle. Nat Rev Immunol. 2014;14(7):478–94.
- 608 24. da Silva EZM, Jamur MC, Oliver C. Mast Cell Function: A New
609 Vision of an Old Cell. Vol. 62, Journal of Histochemistry and
610 Cytochemistry. 2014. 698–738 p.
- 611 25. Ramsay DB, Stephen S, Borum M, Voltaggio L, Doman DB. Mast
612 Cells in Gastrointestinal Disease. Gastroenterol Hepatol.
613 2010;6(12):772–7.
- 614 26. Urb M, Sheppard DC. The role of mast cells in the defence
615 against pathogens. PLoS Pathog. 2012;8(4):2–4.
- 616 27. Bischoff SC. Mast cells in gastrointestinal disorders. Eur J
617 Pharmacol [Internet]. 2016;778:139–45. Available from:
618 <http://dx.doi.org/10.1016/j.ejphar.2016.02.018>
- 619 28. Anbazhagan AN, Priyamvada S, Alrefai WA, Dudeja PK.
620 Pathophysiology of IBD associated diarrhea. Tissue Barriers
621 [Internet]. 2018;0(0):1–21. Available from:
622 <https://doi.org/10.1080/21688370.2018.1463897>

- 623 29. Sridharan G, Shankar A. Toluidine blue: A review of its chemistry
624 and clinical utility. J Oral Maxillofac Pathol [Internet].
625 2012;16(2):251. Available from:
626 <http://www.jomfp.in/text.asp?2012/16/2/251/99081>
- 627 30. Ribatti D. The Staining of Mast Cells: A Historical Overview. Int
628 Arch Allergy Immunol. 2018;176(1):55–60.
- 629 31. Puebla-Osorio N, Sarchio SNE, Ullrich SE, Byrne SN. Detection of
630 Infiltrating Mast Cells Using a Modified Toluidine Blue Staining. In:
631 Fibrosis [Internet]. 2017. p. 213–22. Available from:
632 <http://link.springer.com/10.1007/978-1-4939-7113-8>
- 633 32. Hauswirth AW, Florian S, Schernthaner G-H, Krauth M-T,
634 Sonneck K, Sperr WR, et al. Expression of cell surface antigens
635 on mast cells: mast cell phenotyping. Methods Mol Biol [Internet].
636 2005;315:77–90. Available from:
637 <http://ebooks.cambridge.org/ref/id/CBO9781107415324A009%5C>
638 <http://www.ncbi.nlm.nih.gov/pubmed/16110150>
- 639 33. Schmetzer O, Valentin P, Church MK, Maurer M, Siebenhaar F.
640 Murine and human mast cell progenitors. Eur J Pharmacol
641 [Internet]. 2016;778:1–9. Available from:
642 <http://dx.doi.org/10.1016/j.ejphar.2015.07.016>
- 643 34. Metcalfe DD, Baram D, Mekori YA. Mast Cells. Physiol Rev.
644 1997;77(4):1033–79.

- 645 35. Galli SJ, Tsai M, Weshil BK, Tam S, Costa JJ. Regulation of
646 mouse and human mast cell development, survival and function
647 by stem cell factor, the ligand for the c-kit receptor. *Int Arch*
648 *Allergy Immunol.* 1995;107(1–3):51–3.
- 649 36. Miller HRP, Pemberton AD. Tissue-specific expression of mast
650 cell granule serine proteinases and their role in inflammation in the
651 lung and gut. *Immunology.* 2002;105(4):375–90.
- 652 37. Caughey GH. Mast cell proteases as protective and inflammatory
653 mediators. *Adv Exp Med Biol.* 2011;716:212–34.
- 654 38. Weidner N, Austen KF. Evidence for morphologic diversity of
655 human mast cells. An ultrastructural study of mast cells from
656 multiple body sites. *Lab Invest.* 1990 Jul;63(1):63–72.
- 657 39. Nonomura N, Takayama H, Nishimura K, Oka D, Nakai Y, Shiba
658 M, et al. Decreased number of mast cells infiltrating into needle
659 biopsy specimens leads to a better prognosis of prostate cancer.
660 *Br J Cancer.* 2007;97(7):952–6.
- 661 40. Theoharides TC, Conti P. Mast cells: the JEKYLL and HYDE of
662 tumor growth. *TRENDS Immunol.* 2004;25(5):235–41.
- 663 41. Dyduch G, Kaczmarczyk K, Okoń K. Mast cells and cancer:
664 Enemies or allies? *Polish J Pathol.* 2012;63(1):1–7.
- 665 42. Washabau RJ, Day MJ, Willard MD, Hall EJ, Jergens AE, Mansell
666 J, et al. Endoscopic, Biopsy, and Histopathologic Guidelines for

- 667 the Evaluation of Gastrointestinal Inflammation in Companion
668 Animals. J Vet Intern Med. 2010;24:10–26.
- 669 43. Hall E. Endoscopy of the gastrointestinal tract in dogs and cats. In
670 Pract. 2015;37(4):155–68.
- 671 44. Day MJ, Bilzer T, Mansell J, Wilcock B, Hall EJ, Jergens A, et al.
672 Histopathological Standards for the Diagnosis of Gastrointestinal
673 Inflammation in Endoscopic Biopsy Samples from the Dog and
674 Cat: A Report from the World Small Animal Veterinary Association
675 Gastrointestinal Standardization Group. J Comp Pathol.
676 2008;138(SUPPL. 1):1–43.
- 677 45. Zimmerli S, Bernet D, Burkhardt-Holm P, Schmidt-Posthaus H,
678 Vonlanthen P, Wahli T, et al. Assessment of fish health status in
679 four Swiss rivers showing a decline of brown trout catches. Aquat
680 Sci. 2007;69(1):11–25.
- 681 46. Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T.
682 Histopathology in fish: Proposal for a protocol to assess aquatic
683 pollution. J Fish Dis. 1999;22(1):25–34.
- 684 47. Rossi G, Cerquetella M, Antonelli E, Pengo G, Magi GE, Villanacci
685 V, et al. The importance of histologic parameters of lacteal
686 involvement in cases of canine lymphoplasmacytic enteritis.
687 Gastroenterol Hepatol from Bed to Bench. 2015;8(1):33–41.
- 688 48. Martini F, Timmons M, Tallitsch R. Human Anatomy. 8th editio.

- 689 2015. 901 p.
- 690 49. Schmitz S, Garden OA, Werling D, Allenspach K. Gene
691 expression of selected signature cytokines of T cell subsets in
692 duodenal tissues of dogs with and without inflammatory bowel
693 disease. Vet Immunol Immunopathol [Internet]. 2012;146(1):87–
694 91. Available from: <http://dx.doi.org/10.1016/j.vetimm.2012.01.013>
- 695 50. Kołodziejska-Sawerska A, Rychlik A, Depta A, Wdowiak M,
696 Nowicki M, Kander M. Cytokines in canine inflammatory bowel
697 disease. Pol J Vet Sci. 2013;16(1):165–71.
- 698 51. Peiravan A, Bertolini F, Rothschild MF, Simpson KW, Jergens AE,
699 Allenspach K, et al. Genome-wide association studies of
700 inflammatory bowel disease in German shepherd dogs. PLoS One
701 [Internet]. 2018;13(7):e0200685. Available from:
702 <https://doi.org/10.1371/journal.pone.0200685>
- 703 52. Jergens AE, Simpson KW. Inflammatory bowel disease in
704 veterinary medicine. Front Biosci [Internet]. 2012;4:1404–19.
705 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22201965>
- 706 53. Willard MD, Jergens AE, Duncan RB, Leib MS, McCracken MD,
707 Denovo RC, et al. Interobserver variation among histopathologic
708 from dogs and cats. J Am Vet Med Assoc [Internet].
709 2002;220(April 15):1177–82. Available from:
710 <http://www.ncbi.nlm.nih.gov/pubmed/20345772>

- 711 54. Casamian-Sorrosal D, Willard MD, Murray JK, Hall EJ, Taylor SS,
712 Day MJ. Comparison of histopathologic findings in biopsies from
713 the duodenum and ileum of dogs with enteropathy. J Vet Intern
714 Med. 2010;24(1):80–3.
- 715 55. Procoli F, Mötsküla PF, Keyte S V., Priestnall S, Allenspach K.
716 Comparison of histopathologic findings in duodenal and ileal
717 endoscopic biopsies in dogs with chronic small intestinal
718 enteropathies. J Vet Intern Med. 2013;27(2):268–74.
- 719 56. Craven M, Simpson JW, Ridyard AE, Chandler ML. Canine
720 inflammatory bowel disease: retrospective analysis of diagnosis
721 and outcome in 80 cases (1995-2002). J Small Anim Pract
722 [Internet]. 2004;45(7):336–42. Available from:
723 papers2://publication/uuid/D1EDEDAC-C22E-4687-AD17-
724 EDAA17DED80C
- 725 57. Crespo R, Cámara P, Buendía A, Ayala I. Enfermedad
726 inflamatoria crónica intestinal canina: Hallazgos endoscópicos,
727 bioquímicos y anatomopatológicos del tracto gastrointestinal
728 anterior. Arch Med Vet. 2015;47(3):355–64.
- 729 58. Cruces P, Lillo P, Salas C, Salomon T, Lillo F, González C, et al.
730 Renal Decapsulation Prevents Intrinsic Renal Compartment
731 Syndrome in Ischemia-Reperfusion–Induced Acute Kidney Injury.
732 Crit Care Med [Internet]. 2018;46(2):1. Available from:

- 733 <http://insights.ovid.com/crossref?an=00003246-9000000000-96438>
- 734 59. Oliveira SB, Monteiro IM. Diagnosis and management of
- 735 inflammatory bowel disease in children. BMJ [Internet].
- 736 2017;357:j2083. Available from:
- 737 <http://www.bmj.com/lookup/doi/10.1136/bmj.j2083>
- 738 60. Lee DW, Koo JS, Choe JW, Suh SJ, Kim SY, Hyun JJ, et al.
- 739 Diagnostic delay in inflammatory bowel disease increases the risk
- 740 of intestinal surgery. World J Gastroenterol. 2017;23(35):6474–81.
- 741 61. German AJ, Hall EJ, Day MJ. Analysis of leucocyte subsets in the
- 742 canine intestine. J Comp Pathol. 1999;120(2):129–45.
- 743 62. Stonehewer J, Simpson JW, Else RW, Macintyre N. Evaluation of
- 744 B and T lymphocytes and plasma cells in colonic mucosa from
- 745 healthy dogs and from dogs with inflammatory bowel disease. Res
- 746 Vet Sci. 1998;65(1):59–63.
- 747 63. Jergens AE. Clinical assessment of disease activity for canine
- 748 inflammatory bowel disease. J Am Anim Hosp Assoc.
- 749 2004;40(6):437–45.
- 750 64. Xu X-R, Liu C-Q, Feng B-S, Liu Z-J. Dysregulation of mucosal
- 751 immune response in pathogenesis of inflammatory bowel disease.
- 752 World J Gastroenterol [Internet]. 2014;20(12):3255–64. Available
- 753 from: <http://www.wjgnet.com/1007-9327/full/v20/i12/3255.htm>
- 754 65. Zundler S, Neurath MF. Immunopathogenesis of inflammatory

- 755 bowel diseases: functional role of T cells and T cell homing. Clin
756 Exp Rheumatol [Internet]. 2015;33(92):19–28. Available from:
757 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=26458165)
758 PubMed&dopt=Citation&list_uids=26458165
- 759 66. Locher C, Tipold A, Welle M, Busato A, Zurbriggen A, Griot-Wenk
760 ME. Quantitative assessment of mast cells and expression of IgE
761 protein and mRNA for IgE and interleukin 4 in the gastrointestinal
762 tract of healthy dogs and dogs with inflammatory bowel disease.
763 Am J Vet Res [Internet]. 2001;62(2):211–6. Available from:
764 <http://www.ncbi.nlm.nih.gov/pubmed/11212030>
- 765 67. Kleinschmidt S, Harder J, Nolte I, Marsilio S, Hewicker-Trautwein
766 Marion M. Phenotypical characterization, distribution and
767 quantification of different mast cell subtypes in transmural biopsies
768 from the gastrointestinal tract of cats with inflammatory bowel
769 disease. Vet Immunol Immunopathol. 2010;137(3–4):190–200.
- 770 68. German AJ, Hall EJ, Day MJ. Immune Cell Populations within the
771 Duodenal Mucosa of Dogs with Enteropathies. J Vet Intern Med
772 [Internet]. 2001;15:14–25. Available from:
773 papers3://publication/uuid/023B3AEB-2988-4D0D-AE7D-
774 AF5DC5F03B93
- 775 69. Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial
776 cells. Stimulation of IL-8 production and intercellular adhesion

- 777 molecule-1 expression. J Immunol. 1996 Jan;156(1):275–83.
- 778 70. Asokanathan N, Graham PT, Fink J, Knight DA, Bakker AJ,
779 McWilliam AS, et al. Activation of Protease-Activated Receptor
780 (PAR)-1, PAR-2, and PAR-4 Stimulates IL-6, IL-8, and
781 Prostaglandin E2 Release from Human Respiratory Epithelial
782 Cells. J Immunol [Internet]. 2002;168(7):3577–85. Available from:
783 <http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.168.7.3577>
- 784 71. Schwartz LB. Diagnostic Value of Tryptase in Anaphylaxis and
785 Mastocytosis. Vol. 26, Immunology and Allergy Clinics of North
786 America. 2006. p. 451–63.
- 787 72. Ruitenerg EJ, Elgersma A, Kruizinga W, Leenstra F. Trichinella
788 spiralis infection in congenitally athymic (nude) mice. Immunology.
789 1977;33(4):581–7.
- 790 73. Kleinschmidt S, Meneses F, Nolte I, Hewicker-Trautwein M.
791 Distribution of mast cell subtypes and immune cell populations in
792 canine intestines: Evidence for age-related decline in T cells and
793 macrophages and increase of IgA-positive plasma cells. Res Vet
794 Sci. 2008;84(1):41–8.
- 795 74. Junginger J, Lemensieck F, Moore PF, Schwittlick U, Nolte I,
796 Hewicker-Trautwein M. Canine gut dendritic cells in the steady
797 state and in inflammatory bowel disease. Innate Immun.
798 2014;20(2):145–60.

- 799 75. Friend DS, Ghildyal N, Austen LKF, Gurish MF, Matsumoto R,
800 Stevens RL. Mast cells that reside at different locations in the
801 jejunum of mice infected with *Trichinella spiralis* exhibit sequential
802 changes in their granule ultrastructure and chymase phenotype. *J*
803 *Cell Biol.* 1996;135(1):279–90.
- 804 76. Weinstock J V. Substance P and the regulation of inflammation in
805 infections and inflammatory bowel disease. *Acta Physiol.*
806 2015;213(2):453–61.
- 807 77. Bischoff SC. Physiological and pathophysiological functions of
808 intestinal mast cells. *Semin Immunopathol.* 2009;31(2):185–205.
- 809 78. Boeckxstaens G. Mast cells and inflammatory bowel disease. *Curr*
810 *Opin Pharmacol.* 2015;25:45–9.
- 811

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Cell Type	Antibody	Dilution	Source
T lymphocyte	Mouse anti-human CD3, clone F7.2.38	1:300	Dako, Glostrup, Denmark
B lymphocyte	Mouse anti-CD79a	1:50	Thermo Fisher Scientific, Waltham, MA, USA
Mast cell	Rabbit anti-CD117	1:300	Thermo Fisher Scientific, Waltham, MA, USA

Table 1

Histopathological diagnosis	Enteritis (n=87)	Colitis (n=128)
IBD (n =215)		
Lymphocytic plasmacytic	77 (88.5%)	92 (71.9%)
Eosinophilic	4 (4.6%)	5 (3.9%)
Non-specific	6 (6.9%)	31 (24,2%)

Table 2

Characteristics	IBD (n=215)	Enteritis (n=87)	Colitis (n=128)
Sex, No. (%)			
Male	128 (59.5)	52 (59.8)	76 (59.4)
Female	87 (40.5)	35 (40.2)	52 (40.6)
Age in years (range), No. (%)			
0-1	18 (8.4)	8 (9.2)	10 (7.8)
1-3	60 (27.9)	21 (24.1)	39 (30.5)
3-5	33 (15.3)	19 (21.8)	14 (10.9)
5-7	24 (11.2)	9 (10.3)	15 (11.7)
7-9	29 (13.5)	15 (17.2)	14 (10.9)
9-11	29 (13.5)	8 (9.2)	21 (16.4)
11-13	22 (10.2)	7 (8.0)	15 (11.7)
Breed, No. (%)			
Crossbreed	56 (26.0)	22 (25.3)	34 (26.6)
German Shepherd	38 (18.1)	13 (14.9)	26 (20.3)
Labrador	11 (5.1)	5 (5.7)	6 (4.7)
Poodle	10 (4.7)	2 (2.3)	8 (6.3)
Cocker Spaniel	10 (4.7)	3 (3.4)	7 (5.5)
French Bulldog	9 (4.2)	2 (2.3)	7 (5.5)
Other breeds	80 (37.2)	40 (46.0)	40 (31.3)

Table 3

Cell type	Category	IBD group (n=39)	Control group (n=10)	P value
T lymphocyte	CD3+	90.6 ± 77.20	40.0 ± 3.75	<0.001
B lymphocyte	CD79a+	28.5 ± 18.3	15.0 ± 5.0	<0.001
Mast cell	CD117+	12.0 ± 8.8	8.5 ± 3.28	0.013
Mast cell	Toluidine Blue	2.2 ± 7.5	7.95 ± 2.25	0.014

Table 4

	LYMPHOCYTES				MAST CELLS			
	CD3+		CD79a+		CD117+		Toluidine Blue+	
	IBD	CONTROL	IBD	CONTROL	IBD	CONTROL	IBD	CONTROL
ZONE 1	3	2	1	2	2	2	1	1
ZONE 2	1	2	3	2	2	2	3	2
ZONE 3	1	1	1	1	1	1	1	2

*0 (0% infiltrative cells) ,1 (<25% infiltrative cells) , 2 (25-50% infiltrative cells), 3 (>50% infiltrative cells)

Table 5