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Research paper

Association between lymphocyte antigen receptor gene rearrangements and histopathological evaluation in canine chronic enteropathy

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ABSTRACT

Although definitive diagnosis of chronic enteropathy (CE) and gastrointestinal (GI) lymphoma requires histopathological evaluation of the GI tract, these conditions are often still difficult to differentiate from each other. Polymerase chain reaction (PCR) for antigen receptor gene rearrangements (PARR) has been applied recently as an adjunctive for diagnosis of lymphoid tumors; however, its clinical value in canine CE and GI lymphoma remains unclear. The purpose of this study was to investigate the relationship between PARR and histopathological diagnosis, degree of enteritis or lymphoma, and long-term prognosis in dogs, in order to evaluate the clinical significance of PARR. Endoscopic biopsy specimens obtained from 96 dogs with chronic enteritis (mild, $n = 14$; moderate, $n = 20$; marked, $n = 62$) and 21 dogs with GI lymphoma were used. Clonality was observed in 51% of the animals with chronic enteritis; interestingly, it was found in 29% of those with only mild enteritis. In dogs with marked enteritis, the rate of PARR was higher in those with lymphocyte epitheliotropism than in those without epitheliotropism. The sensitivity of PARR in animals with GI lymphoma was 76%. There was no significant prognostic difference between chronic enteritis with or without clonal rearrangements. In contrast, dogs histopathologically diagnosed with marked enteritis had a significantly shorter survival time than did those with mild or moderate enteritis. While the significance of PARR in the diagnosis of GI lymphoma remains uncertain, the pathological roles of clonally expanding lymphocytes in canine CE should be investigated further.

1. Introduction

Chronic enteropathy (CE) is one of the common clinical diagnoses in dogs, characterized by persistent or recurrent gastrointestinal (GI) symptoms such as diarrhea, vomiting, and weight loss. The common causes of CE include food-responsive enteropathy,

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antibiotic-responsive enteropathy, and inflammatory bowel disease (IBD) (German et al., 2003). The diagnosis of canine CE requires various diagnostic evaluations in order to rule out other diseases causative of chronic GI symptoms, including metabolic diseases, infection, parasitism, or neoplastic diseases (Allenspach, 2013). Although the pathogenesis of canine CE is not yet fully elucidated, interaction between the intestinal microenvironment (bacterial or dietary antigens), mucosal immune system dysfunction, and genetic factors is considered to be involved in its etiology (German et al., 2003; Simpson and Jergens, 2011). Therapeutic trials of dietary modification, antibiotics, or immunomodulatory agents are needed to improve diagnosis and control of the disease (Allenspach, 2013). If animals with the condition are treated appropriately, the prognosis of canine CE is relatively favorable, as it has been reported that the 3-year survival rate was 97% in food-responsive dogs with diarrhea and 57% in steroid-treated dogs with IBD (Allenspach et al., 2007).

GI lymphoma should also be taken into consideration in dogs with chronic GI symptoms. Although GI lymphoma is less common in dogs than in cats, it accounts for approximately 7% of all canine GI neoplasms (Patnaik et al., 1977). Despite treatment with multidrug chemotherapy, the prognosis of dogs with GI lymphoma is usually poor, as median survival time was reported to be 77 days (Rassnick et al., 2009).

Because of differences in treatment strategies and outcomes, it is essential to distinguish between CE and GI lymphoma. As there is no specific laboratory test for differentiating between these diseases, definitive diagnosis requires histopathological evaluation of GI biopsies. Flexible endoscopy is the preferred method for obtaining specimens as it is less invasive than surgery (Washabau et al., 2010). However, as endoscopically obtained tissues are often limited to mucosa, this can lead to pathologists overlooking cases of lymphoma, since this condition can involve transmural expansion of neoplastic cells. In addition, GI lymphoma is frequently accompanied by infiltration of inflammatory lymphocytes and plasma cells that can cause misdiagnosis of lymphocytic–plasmacytic enteritis (Kleinschmidt et al., 2006).

Since its advent, polymerase chain reaction (PCR) for antigen receptor gene rearrangements (PARR) has been introduced into veterinary practice as a useful adjunctive for the diagnosis of lymphoma. This method is used to detect clonal lymphocytic expansion by amplifying the T-cell receptor gamma-chain (*TCRγ*) gene and the immunoglobulin heavy-chain (*IgH*) gene. It has a high accuracy for the detection of clonal lymphocytes, and its sensitivity in diagnosing lymphoid malignancies was reported to be more than 90% (Burnett et al., 2003; Gentilini et al., 2009). There are several studies on the application of PARR in GI biopsy specimens. One of these reports suggested that PARR was a useful diagnostic tool for detecting latent GI lymphoma, which cannot be histopathologically diagnosed using endoscopic biopsy specimens (Kaneko et al., 2009). In another study, the sensitivity of PARR in diagnosing GI lymphoma was reported to be 66.7%, which was lower than that for lymphoma affecting other anatomical sites; thus, it may be useful for detecting lymphoma when combined with histopathological examination (Fukushima et al., 2009). On the other hand, a more recent study reported that clonal lymphocytic infiltration was also detected in the GI tract of dogs with IBD, and reduced diversity of lymphocytic infiltrates significantly correlated with one-year survival rate (Olivero et al., 2011). In these studies, however, PARR was performed using different primers or analyzing systems. Therefore, the clinical value of PARR in canine CE remains unclear.

The purpose of this study was to investigate the relationship between PARR and histopathological diagnosis, degree of enteritis or lymphoma, and long-term prognosis in dogs, in order to evaluate the clinical significance of PARR.

2. Materials and methods

2.1. Cases

Medical records of dogs investigated for chronic GI diseases at the Veterinary Medical Center of the University of Tokyo (from May 2011 to December 2012) and the Japan Small Animal Medical Center (from April 2012 to March 2013) were reviewed retrospectively. The following inclusion criteria were used: (1) a history of chronic GI signs with a duration of >3 weeks and/or hypoalbuminemia (<2.7 g/dL); (2) exclusion of other causes of chronic GI signs (metabolic diseases, infection, parasitism, and pancreatic insufficiency) or hypoalbuminemia (hepatic disease, renal disease, and blood loss) by complete blood cell count, serum biochemistry, urinalysis, fecal examination for parasites and bacteria, and abdominal ultrasonography. Viral infection was ruled out based on patients' age, clinical symptoms, and PCR analysis using feces; (3) availability of histopathological evaluation of mucosal specimens obtained via upper GI tract endoscopic examination alone or in combination with lower GI tract endoscopic examination; and (4) availability of genomic DNA extracted from duodenal mucosa obtained via endoscopy. Food-responsive enteropathy and antibiotic-responsive enteropathy were not ruled out before endoscopic examination if rapid worsening of clinical signs was observed in animals. Cases were included regardless of treatments given.

According to the inclusion criteria, the medical records of 120 dogs were reviewed. Three of the animals were histopathologically diagnosed with gastric adenocarcinoma and so were excluded from the present study. Of the remaining 117 dogs, 96 were diagnosed with chronic enteritis and 21 were diagnosed with GI lymphoma by histopathological evaluation. Cases were excluded from survival analysis if concurrent neoplastic diseases other than GI lymphoma were confirmed. The referring veterinarians or the owners were contacted to obtain follow-up data.

2.2. Histopathology

Endoscopy of the upper GI tract was performed in all dogs, and mucosal biopsy specimens were collected from the stomach and the duodenum. In cases undergoing lower