Veterinary Immunology and Immunopathology 159 (2014) 1–10

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Expression profiling of pattern recognition receptors and selected cytokines in miniature dachshunds with inflammatory colorectal polyps

Hirotaka Igarashi[a], Koichi Ohno[a,*], Shingo Maeda[a], Hideyuki Kanemoto[a], Kenjiro Fukushima[a], Kazuyuki Uchida[b], Hajime Tsujimoto[a]

[a] *Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*
[b] *Department of Veterinary Pathology, Graduate School of Agricultural and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 21 February 2014
Accepted 3 March 2014

Keywords:
Inflammatory colorectal polyp
Miniature dachshund
Pattern recognition receptor
Proinflammatory cytokine
Innate immunity

ABSTRACT

Inflammatory colorectal polyps (ICRPs) are commonly seen in miniature dachshund (MD) dogs; typically, multiple polyps form with severe neutrophil infiltration. ICRP is thought to be a novel form of inflammatory bowel disease (IBD), but its etiology has not been investigated. The innate immune system is implicated in the pathogenesis of both human and canine IBD. Therefore, the aim of the current study was to evaluate the messenger RNA (mRNA) expression profiles of pattern recognition receptors (PRRs) and cytokines in ICRPs. Polyp tissues were collected by colonoscopic biopsies from 24 MDs with ICRPs. Non-polypoid colonic mucosa was collected from all MDs with ICRPs and 21 clinically healthy beagles (as the controls). The expression levels of the mRNAs encoding toll-like receptors (TLRs) 1–10; nucleotide-binding oligomerization domain (NOD)-like receptors NOD1 and NOD2; and cytokines IL-1β, IL-6, IL-8/CXCL8, and TNF-α were evaluated by quantitative real-time RT-PCR. Three of the 10 well-known candidate reference genes were selected as housekeeper genes based on analyses from the GeNorm, NormFinder, and BestKeeper programs. Levels of TLR1, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TLR10, NOD2, and all cytokines were significantly upregulated in the polyps relative to those in the controls. There was significant decrease in the expression levels of TLR3 and NOD1 in the polyp tissues compared to the non-polypoid colonic mucosa obtained from MDs with ICRPs. All upregulated PRR mRNAs were positively correlated with all proinflammatory cytokine mRNAs. This study demonstrated the dysregulation of PRRs and proinflammatory cytokines in ICRPs of MDs, which may play an important role in the pathogenesis of this disease.

Abbreviations: MD, miniature dachshund; ICRP, inflammatory colorectal polyp; IBD, inflammatory bowel disease; PRR, pattern recognition receptor; TLR, toll-like receptor; NOD, nucleotide-binding oligomerization domain; NLR, NOD-like receptor; mRNA, messenger RNA; NF-κB, nuclear factor-kappa B; qPCR, quantitative real-time PCR; cDNA, complementary DNA; B2M, β-2 microglobulin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMBS, hydroxymethylbilane synthase; HPRT1, hypoxanthine phosphoribosyltransferase 1; RPL13A, ribosomal protein L13a; RPL32, ribosomal protein L32; RPS18, ribosomal protein S18; SDHA, succinate dehydrogenase complex subunit A; TBP, TATA box binding protein.

* Corresponding author. Tel.: +81 3 5841 5402; fax: +81 3 5841 5640.
E-mail address: aohno@mail.ecc.u-tokyo.ac.jp (K. Ohno).

http://dx.doi.org/10.1016/j.vetimm.2014.03.003
0165-2427/

1. Introduction

Colorectal polyps are relatively common in dogs, and most of these polyps are neoplastic polyps. Recently, we reported that the miniature dachshund (MD) is the breed most commonly affected by inflammatory colorectal polyps (ICRPs) (Ohmi et al., 2012). ICRPs in MDs typically develop multiple polyps that are restricted to the colorectal region, have severe inflammatory infiltration (predominantly with the neutrophils), and respond relatively well to immunosuppressive therapy (Ohmi et al., 2012). Therefore, ICRPs are thought to represent a novel form of canine inflammatory bowel disease (IBD) (Ohta et al., 2013).

One of the etiologies of human and canine IBD is hypothesized to result from the inappropriate activation of mucosal immunity by various environmental factors such as intestinal microbiota (Xavier and Podolsky, 2007). Innate immune mechanisms recognize microorganisms and are thought to be implicated in many inflammatory conditions (Drexler and Foxwell, 2010). Pattern recognition receptors (PRRs) are the key regulators of the innate immune system in gastrointestinal mucosa that induce proinflammatory and immunomodulatory responses in various cell types, including immune and epithelial cells (Akira and Takeda, 2004). Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are the most characterized classes of PRRs, and many studies have revealed that the dysregulation of TLRs and/or NLRs can lead to inflammation (Becker and O'Neill, 2007; Franchi et al., 2008; Shibolet and Podolsky, 2007).

In human IBD, many studies have reported the upregulation of TLR2 and TLR4 messenger RNAs (mRNA) and proteins (Cario and Podolsky, 2000; Frolova et al., 2008; Szebeni et al., 2008). In a recent study, TLR5, TLR8, and TLR9 mRNA levels were also upregulated in ulcerative colitis patients and correlated with inflammatory activity (as determined by endoscopy, histology, and transcription levels of proinflammatory cytokines including IL-6 and TNF-α) (Sánchez-Muñoz et al., 2011). Another study also identified the hyperexpression of NOD2 in intestinal epithelial cells, macrophages, and mast cells in Crohn's disease patients (Berrebi et al., 2003; Okumura et al., 2009).

Several reports have shown similar results in veterinary medicine. TLR2, TLR4 and TLR9 mRNA levels were also upregulated in the duodenal mucosa, and TLR2 was weakly correlated with histological activity in dogs with IBD (Burgener et al., 2008; McMahon et al., 2010). In German shepherds with chronic enteropathy, TLR4 mRNA expression was also upregulated, while TLR5 (expressed mainly in CD11+ dendric cells inducing anti-inflammatory cytokines) expression was downregulated (Allenspach et al., 2010). More recently, NOD2 mRNA and nuclear factor-kappa B (NF-κB) activity were upregulated in dogs with lymphocytic–plasmacytic colitis (Okanishi et al., 2013).

Recently, a study reported an increase in proinflammatory cytokines in the colorectal mucosa of MDs with ICRPs (Tamura et al., 2013). However, to date, there are no reports on the status of PRRs in MDs with ICRPs. Therefore, we hypothesized that the dysregulation of PRRs exists in polypoid lesions in MDs with ICRPs, which could be correlated with the expression of proinflammatory cytokines. We conducted quantitative real-time PCR (qPCR) analyses to quantify the expression levels of PRRs and selected cytokines in polypoid lesions and non-polypoid colonic mucosa in MDs with ICRPs and in healthy dogs. It is essential to select the appropriate multiple reference genes for accurate quantification (Peters et al., 2007). Although Peters et al. (2007) have reported stable genes in the colon, duodenum, and duodenal endoscopic biopsies, previous studies have not investigated the appropriate combination of stable reference genes in colonic mucosa. Thus, we also assessed combinations of the most stably expressed reference genes.

2. Materials and methods

2.1. Animals

Tissue samples were obtained from MDs referred to the Veterinary Medical Center of the University of Tokyo for investigation of chronic hematochezia and/or tenesmus and detected colorectal polyps endoscopically between July 2011 and October 2013. Dogs diagnosed histopathologically with ICRPs were included in this study, while dogs with colorectal adenoma or adenocarcinoma were excluded.

As healthy controls, 21 beagles were used in this study. These dogs had no clinical signs of gastrointestinal disease and showed no abnormalities, as determined by blood test, fecal examination, and ultrasound. The use of dogs in this study was approved by the Animal Care Committee of the University of Tokyo (Approval No. P11-530, 2 June 2011).

2.2. Sample collection

Colonoscopy was performed in all dogs under sedation (butorphanol) or general anaesthesia (premedication of butorphanol and midazolam, propofol and isoflurane) using a VQ-8143B flexible videoendoscope (Olympus Medical Systems Co., Tokyo, Japan). Mucosal specimens of polypoid lesions were collected from MDs with ICRPs. As controls, colonic mucosa without macroscopic polypoid lesions was collected at the descending colon of MDs with ICRPs and from healthy beagles. Multiple mucosal biopsies were taken by using the FB-54Q-1 biopsy forceps (Olympus Medical Systems Co.) or an electrosurgical snare (ICC 200, ERBE Co., Tubingen, Germany). One to two mucosal specimens collected from polypoid lesion or non-polypoid colonic mucosa were used for RNA extraction, and at least 4 mucosal specimens or a large polypoid tissue resected by polypectomy were submitted for histopathology. Samples for total RNA extraction were immediately submerged in RNAlater (Qiagen Inc., Valencia, CA) and stored at −80 °C until use. Samples for histopathology were placed in 10% formalin, and hematoxylin and eosin-stained sections were prepared.

2.3. Evaluation of candidate reference genes

In total, 30 tissue samples were derived from 10 polypoid samples, 10 non-polypoid colonic mucosa samples