学 位 論 文 の 要 旨

氏名　古谷　聡史

学　位　論　文　名　Association Between Toll-like Receptor 9 Signaling Defect and Developing Post-Infectious Irritable Bowel Syndrome

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著　　者　　名　Satoshi Kotani, Yoshiyuki Mishima, Kenichi Kishimoto, Akihiko Oka, Naoki Oshima, Kousaku Kawashima, Kenjiro Matsumoto, Haruki Usuda, Koichiro Wada, Shunji Ishihara

論 文 内 容 の 要 旨

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits. Its global prevalence is about 10%, and although not life-threatening, IBS markedly impairs quality of life and imposes a substantial socioeconomic burden. The pathogenesis is multifactorial and has been linked to genetic predisposition, diet, low-grade inflammation, brain–gut–microbiota interactions, and psychosocial factors, but no causal treatment is currently available.

A subgroup of patients with previously normal bowel habits develop IBS after acute gastroenteritis, termed postinfectious IBS (PI-IBS), in which diarrhea-predominant IBS is common. Typical triggers are bacterial infections with Campylobacter, Salmonella, Shigella, or Escherichia coli, and young age, female sex, psychological distress, and severe initial inflammation are recognized risk factors. Clinical and experimental data suggest that PI-IBS develops when environmental insults act on genetically susceptible hosts.

In a follow-up study of a large waterborne gastroenteritis outbreak in Walkerton, Canada, single nucleotide polymorphisms in Toll-like receptor (TLR) 9, interleukin-6, and cadherin-1 were identified as genetic risk factors for PI-IBS. How these variants, especially in TLR9, contribute to IBS pathogenesis is still unclear. TLR9 is an innate immune receptor that recognizes unmethylated cytosine–phosphate–guanosine (CpG) DNA derived from bacteria and viruses. Recognition of microbial CpG DNA is crucial for host defense, whereas dysregulated responses to self-DNA may promote autoimmune diseases. Detailed analyses of TLR9 signaling in functional bowel disorders are lacking. The present study was designed to clarify the role of

TLR9 signaling in the pathogenesis of PI-IBS and to provide a basis for new therapeutic strategies.

MATERIALS AND METHODS

C57BL/6J wild-type (WT) mice and TLR2-, TLR4-, or TLR9-deficient knockout (KO) mice were maintained under specific pathogen-free conditions and used at 8–9 weeks of age. Infectious colitis was induced by oral administration of 1.0×10^9 colony-forming units of Citrobacter rodentium; control mice received phosphate-buffered saline. Two weeks after infection, acute colitis was evaluated by body weight change, colon length, histology, and colonic cytokine mRNA expression.

Visceral sensitivity was assessed six weeks after infection, when macroscopic colitis had resolved. Visceromotor responses to colorectal distention were quantified with a barostat-based system. Under general anesthesia, electrodes were implanted into the abdominal wall, and electromyographic activity during graded distention was recorded as an index of visceral pain. To examine the contribution of bradykinin signaling, the bradykinin B1 receptor antagonist R715 and bradykinin B2 receptor antagonist HOE 140 were administered, and their effects on visceral hyperalgesia were evaluated.

Histological damage scores, crypt length, and inflammatory cell infiltration in the distal colon were assessed by hematoxylin–eosin staining. Colonic expression of inflammatory cytokines and bradykinin receptors was quantified by real-time reverse transcription polymerase chain reaction. To explore pathways associated with TLR9 deficiency, DNA microarray analysis was performed using distal colon tissue from infected WT and TLR9 KO mice, followed by validation with quantitative PCR. Immunohistochemistry was used to localize BDKRB1 and BDKRB2 in the colonic mucosa.

Intestinal permeability was evaluated by oral administration of fluorescein isothiocyanate (FITC)–dextran and measurement of plasma fluorescence. Fecal microbiota composition was analyzed by 16S rRNA gene sequencing, and fecal microbiota transplantation from infected TLR9 KO donors into antibiotic-treated recipient mice was performed to examine whether infection-induced dysbiosis contributed to visceral hyperalgesia. Statistical analyses were carried out using Student's t test or analysis of variance with appropriate post hoc tests, and p values <0.05 were considered statistically significant. All animal experiments were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

C. rodentium infection induced acute colitis in all mouse strains, as shown by body weight loss, colon shortening, and histological evidence of epithelial damage with inflammatory cell infiltration. TLR9 KO mice, however, showed relatively mild body weight loss and macroscopic inflammation compared with WT and TLR2 KO mice, despite similar increases in colonic proinflammatory cytokine expression during the acute phase. Intestinal permeability assessed by FITC–dextran did not differ significantly among the groups, suggesting that epithelial barrier

disruption was not a major determinant of subsequent symptom development.

At six weeks after infection, macroscopic and microscopic inflammation had largely resolved in all groups, and colonic cytokine expression had returned to near-baseline levels. Nevertheless, barostat-based assessment revealed that only *C. rodentium*-infected TLR9 KO mice developed persistent visceral hyperalgesia, whereas infected WT, TLR2 KO, and TLR4 KO mice did not show enhanced visceral sensitivity. These findings indicate that a defect in TLR9 signaling specifically predisposes to PI-IBS-like visceral pain, independently of the severity of acute colitis or persistent low-grade inflammation.

To clarify the mechanisms, microarray and quantitative PCR analyses were performed using distal colon tissues. Among the genes differentially expressed between infected WT and TLR9 KO mice, those related to peptide G protein–coupled receptors were enriched, and the bradykinin B1 and B2 receptors (Bdkrb1 and Bdkrb2) were markedly upregulated in infected TLR9 KO mice. Immunohistochemistry localized BDKRB1 and BDKRB2 mainly to the colonic epithelium rather than the enteric nervous system, implying that epithelial changes may contribute to altered sensory signaling leading to visceral hypersensitivity.

Microbiota analysis showed that *C. rodentium* infection induced compositional changes of gut microbiota and that these alterations differed between WT and TLR9 KO mice. Fecal microbiota transplantation from infected TLR9 KO donors into microbiota-depleted recipient mice led to a tendency toward increased visceral sensitivity, but the effect was partial and did not fully reproduce the phenotype of donor mice. Thus, infection-induced alterations of gut microbiota are suggested to be partly involved in the development or modulation of visceral hyperalgesia in the context of TLR9 deficiency, together with other host factors. Systemic administration of the BDKRB1 antagonist R715 or the BDKRB2 antagonist HOE 140 significantly attenuated visceral hyperalgesia in infected TLR9 KO mice, supporting a causal role of bradykinin receptor signaling in PI-IBS-like pain.

Taken together, these results indicate that a TLR9 signaling defect promotes PI-IBS development mainly through a mechanism involving bradykinin receptor upregulation in the colonic epithelium, with infection-induced alterations of gut microbiota suggested as a partial contributor, rather than persistent mucosal inflammation or increased intestinal permeability.

CONCLUSION

This study demonstrated that TLR9 deficiency predisposes to postinfectious irritable bowel syndrome by inducing persistent visceral hyperalgesia after resolution of infectious colitis. In C. rodentium-infected TLR9 KO mice, upregulation of bradykinin B1 and B2 receptors in the colonic epithelium was closely associated with symptom development, while infection-induced changes in gut microbiota were suggested to contribute in part to the establishment or modulation of visceral hyperalgesia. Pharmacological blockade of BDKRB1 and BDKRB2 effectively ameliorated visceral pain, indicating that bradykinin receptor antagonists may represent a promising therapeutic option for PI-IBS in individuals with impaired TLR9 signaling.