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Endotoxicity of Lipopolysaccharide as a Determinant of T-Cell—Mediated Colitis Induction in Mice

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BACKGROUND & AIMS: The intestinal microbiota is an important determinant of the mucosal response. In patients with inflammatory bowel diseases, the mucosal immune system has inappropriate interactions with the intestinal microbiota. We investigated how the composition of the intestinal microbiota affects its endotoxicity and development of colitis in mice. **METHODS:** Germ-free C57BL/6J-Rag^{1tm1Mom} (*Rag1*$^{-/-}$) mice were colonized with 2 different types of complex intestinal microbiota. Colitis was induced in *Rag1*$^{-/-}$ mice by transfer of CD4^{+}CD62L^{+} T cells from C57BL/6J mice. Colonic tissues were collected and used for histologic analysis and cell isolation. Activation of lamina propria dendritic cells and T cells was analyzed by flow cytometry. **RESULTS:** After transfer of CD4^{+}CD62L^{+} T cells, mice with intestinal Endo^{lo} microbiota (a low proportion of *Enterobacteriaceae*, high proportion of *Bacteroidetes*, and low endotoxicity) maintained mucosal immune homeostasis, and mice with highly endotoxic Endo^{hi} microbiota (a high proportion of *Enterobacteriaceae* and low proportion of *Bacteroidetes*) developed colitis. To determine whether the effects of Endo^{hi} microbiota were related to the higher endotoxic activity of lipopolysaccharide (LPS), we compared LPS from *Enterobacteriaceae* with that of *Bacteroidetes*. Administration of *Escherichia coli* JM83 (wild-type LPS) to the mice exacerbated colitis, and *Escherichia coli* JM83 + htrBPG (mutated LPS, with lower endotoxicity, similar to that of *Bacteroidetes*) prevented development of colitis after transfer of the T cells to mice. **CONCLUSIONS:** The endotoxicity of LPS produced by the intestinal microbiota is a determinant of whether mice develop colitis after transfer of CD4^{+}CD62L^{+} T cells. This finding might aid the design of novel biologics or probiotics to treat inflammatory bowel disease.

Keywords: Mouse Model; IBD; Prevention; Bacteria.

The mammalian gastrointestinal tract harbors a dense and diverse community of an estimated 10—100 trillion micro-organisms[1–3] consisting of 500—1000 different species, of which the vast majority are bacteria.[2,4] It is well accepted that in inflammatory bowel disease (IBD), the mucosal immune system reacts inappropriately toward the commensal microbiota.[5] No particular microbial species has been consistently linked to IBD pathogenesis or prevention; however, some symbiotic bacterial species have been shown to prevent inflammatory host responses.[2,6–9]

Numerous animal models have been generated to experimentally investigate human IBD,[10] including erosive models of acute colitis (eg, dextran sodium sulfate [DSS]-induced colitis), spontaneous models of chronic colonic, and/or small bowel inflammation induced by targeted gene deletion (eg, interleukin [*IL*]*10*$^{-/-}$ mice) or induction by disruption of T-cell homeostasis (eg, *Rag1*$^{-/-}$ mice).[10] As chronic colitis results from a dysregulated immune response to components of the normal intestinal microbiota, it is reasonable to suggest that the T-cell—dependent models are significantly more relevant to human disease than are the erosive models of acute colitis, if one wishes to investigate the immunologic mechanisms inducing, perpetuating, or preventing chronic colitis.[10] Microbe-associated molecular pattern, such as lipopolysaccharide (LPS) or flagellins, are recognized by different pattern recognition receptors. However, there is a dichotomic role for Toll-like receptor (TLR) in intestinal inflammation.[11] For example, *IL2*$^{-/-}$ *MyD88*$^{-/-}$ mice develop colitis independent of TLR signaling, and *IL10*$^{-/-}$ *MyD88*$^{-/-}$ mice remain healthy,

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Abbreviations used in this paper: **clp, colonic lamina propria; DC, dendritic cell; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MUT, mutant; rDNA, ribosomal DNA; TLR, Toll-like receptor; WT, wild type.**

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indicating an inflammation promoting effect of MyD88-dependent TLR.[12] Additionally, in the DSS model of acute inflammation, which is based on disruption of tight junction proteins and severe disturbance of the intestinal barrier, the depletion of the intestinal microbiota through antibiotics resulted in aggravation of acute colitis, which was ameliorated by feeding of LPS.[13] In the Crohn's disease–like murine T-cell–induced chronic colitis, the roles of LPS and of pattern recognition receptor signaling remain unclear.

The goal of our study was to show the influence of qualitatively different TLR4 signals on development of chronic T-cell–driven colitis. We demonstrate that the endotoxicity of the intestinal microbiota given by the composition of the intestinal bacterial communities determines either the maintenance of intestinal homeostasis or the induction of colitis in genetically predisposed hosts. Importantly, T-cell–transferred *Rag1*$^{-/-}$ mice, with low endotoxic microbiota due to a high number of bacteria of the anaerobic *Bacteroidetes* group, were protected from induction of transfer colitis, and *Rag1*$^{-/-}$ mice, with high endotoxic microbiota due to a high number of commensal *Enterobacteriaceae*, develop colitis. The low endotoxic *Escherichia coli* JM83 +*htrB*$_{Pg}$ strain (*E coli*$_{MUT}$) with alterations in the acylation pattern promoted intestinal homeostasis, and feeding with the high endotoxic *E coli* JM83 K-12 wild-type (*E coli*$_{WT}$) stain resulted in severe intestinal inflammation. This was, in particular, supported by feeding experiments with isolated LPS from both the WT and mutant (MUT) strain.

The current results shed new light on the previously unrecognized role of LPS toxicity in the maintenance of intestinal immune homeostasis and suggest novel treatment options to shape mucosal immunity in patients with IBD.

Material and Methods

Mice

For the experiments, inbred C57BL/6J mice and C57BL/6J-Rag^{1tm1Mom} (*Rag1*$^{-/-}$)[14] mice were used. Germ-free mice were colonized with different complex intestinal microbiota by co-housing with Endo^{lo} or Endo^{hi} colonized C57BL/6 mice bred and kept in isolated ventilated cages. Mice were free of *Helicobacter hepaticus*, norovirus, and rotavirus. Endo^{lo} mice harbor microbiota with a high proportion of *Bacteroidetes* and low proportion of *Enterobacteriaceae*, and Endo^{hi} *Rag1*$^{-/-}$ mice harbor a high proportion of *Enterobacteriaceae* and low proportion of *Bacteroidetes*. *Rag1*$^{-/-}$ mice were transplanted with 5×10^5 splenic CD4$^+$CD62L$^+$ T cells at 8–10 weeks of age.[15–20] Endo^{hi} *Rag1*$^{-/-}$ mice were analyzed after manifestation of colitis 4–6 weeks after T-cell transfer, Endo^{lo} *Rag1*$^{-/-}$ mice 6 weeks after T-cell transfer. All animal experiments were reviewed and approved by the responsible Institutional Review Board.

Growth of Bacteria, Isolation of LPS, and Analytical Methods

E coli strains (*E coli* JM83 [*E coli*$_{WT}$] and *E coli* JM83 +*htrB*$_{Pg}$ [*E coli*$_{MUT}$][21]) were grown in Luria Bertani medium until log phase. Where indicated, 100 μg/mL ampicillin and isopropyl-β-D-thiogalactopyranoside (1 mM) was added. The LPS were extracted according to Galanos et al,[22] in the yields of 2.6% (WT) and 2.9% (MUT). Fatty acid analyses[23] and high-resolution electrospray ionization Fourier transform ion cyclotron mass spectrometry[24] were performed as published. Mice were challenged with 10^8 viable bacteria or with 160 μg purified LPS (6.4 μg/g mouse).

See the Supplementary Materials for information on cell culture experiments, analysis of fecal samples by culture methods and quantitative polymerase chain reaction, isolation of DNA from fecal samples, preparation of amplicon pool and 454-sequencing, bioinformatic analysis, isolation of lp dendritic (DC) and T cells, intracellular cytokine staining, flow cytometry, histology, and statistics.

Results

Low Endotoxicity of the Intestinal Microbiota in Rag1$^{-/-}$ Mice Is Associated With Mucosal Immune Homeostasis

In a model of IBD, we investigated whether the endotoxicity of complex intestinal microbiota influenced the incidence or severity of colitis. Therefore, *Rag1*$^{-/-}$ mice, raised in a germ-free facility, were colonized with 2 types of complex intestinal microbiota with different endotoxicity. We used microbiota with a low TLR4-activating effect (Endo^{lo}) (Figure 1*A*) characterized by low numbers of *Enterobacteriaceae* (including *E coli*) and high numbers of *Bacteroidetes* (including *Bacteroides vulgatus* or *Porphyromonas sp*) (Figure 1*B* and *C*) and, in addition, a high TLR4-activating microbiota (Endo^{hi}) (Figure 1*A*) characterized by high numbers of *Enterobacteriaceae* and low numbers of *Bacteroidetes* as revealed by culture techniques (Figure 1*B*) and quantitative polymerase chain reaction (Figure 1*C*). Analysis of the intestinal microbiota by 454 sequencing of the 16S ribosomal DNA (rDNA) amplicons containing the variable regions V3–V6 revealed 70.3% of *Bacteroidetes* and 22.94% of *Firmicutes* in the Endo^{lo} *Rag1*$^{-/-}$ mice. Proteobacterial (including *E coli*) 16S rDNA amplicons were below the detection limit. In Endo^{hi} *Rag1*$^{-/-}$ mice, 0.19% of the analyzed 16S rDNA amplicons belonged to *Proteobacteria* (*Enterobacteriaceae*, including *E coli*, are a family within this phylum), 32.42% to *Bacteroidetes* and 61.84% to *Firmicutes* (including, eg, the classes *Bacilli* with the order of *Bacillales* and *Lactobacillales*, *Clostridia*, or *Erysipelotrichia*) (Figure 1*D*). All mice described in this study were raised in these colonies to assure early perinatal colonization with the complex microbiota defined here.

On transfer of T cells from healthy specific pathogen-free C57BL/6 mice the Endo^{hi} *Rag1*$^{-/-}$ mice developed severe colitis within 6 weeks, lost significant amounts of weight, and exhibited pronounced inflammation of colonic mucosa and submucosa. In contrast, T-cell–transferred Endo^{lo} *Rag1*$^{-/-}$ mice remained healthy for 6 weeks, as indicated by both weight gain during the course of the experiment and missing histologic signs of inflammation (Figure 2*A*–*C*).

DCs in the colonic lamina propria (clp) of T-cell–transferred Endo^{hi} *Rag1*$^{-/-}$ mice showed significantly higher expression of major histocompatibility complex (MHC) class II and CD40 as compared with the lp DC