Elevated lipopolysaccharide in the colon evokes intestinal inflammation, aggravated in immune modulator-impaired mice

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Im E, Riegler FM, Pothoulakis C, Rhee SH. Elevated lipopolysaccharide in the colon evokes intestinal inflammation, aggravated in immune modulator-impaired mice. Am J Physiol Gastrointest Liver Physiol 303: G490–G497, 2012. First published June 21, 2012; doi:10.1152/ajpgi.00120.2012.—Frequency of gram-negative bacteria is markedly enhanced in inflamed gut, leading to augmented LPS in the intestine. Although LPS in the intestine is considered harmless and, rather, provides protective effects against epithelial injury, it has been suggested that LPS causes intestinal inflammation, such as necrotizing enterocolitis. Therefore, direct effects of LPS in the intestine remain to be studied. In this study, we examine the effect of LPS in the colon of mice instilled with LPS by rectal enema. We found that augmented LPS on the luminal side of the colon elicited inflammation in the small intestine remotely, not in the colon; this inflammation was characterized by body weight loss, increased fluid secretion, enhanced inflammatory cytokine production, and epithelial damage. In contrast to the inflamed small intestine induced by colonic LPS, the colonic epithelium did not exhibit histological tissue damage or inflammatory lesions, although intracolonic LPS treatment elicited inflammatory cytokine gene expression in the colon tissues. Moreover, we found that intracolonic LPS treatment substantially decreased the frequency of immune-suppressive regulatory T cells (CD4+CD25+ and Foxp3+). We were intrigued to find that LPS-promoted intestinal inflammation is exacerbated in immune modulator-impaired IL-10−/− and Rag-1−/− mice. In conclusion, our results provide evidence that elevated LPS in the colon is able to cause intestinal inflammation and, therefore, suggest a physiological explanation for the importance of maintaining the balance between gram-negative and gram-positive bacteria in the intestine to maintain homeostasis in the gut.

Toll-like receptor; microbial recognition; interleukin-10; regulatory T cells

LIPOLYSACCHARIDE (LPS) is the major constituent of the outer membrane of gram-negative bacteria and a specific ligand of Toll-like receptor 4 (TLR4) to produce pleiotropic inflammatory mediators. Given that the human gut harbors a large collection of commensal bacteria, the intestinal (especially colonic) lumen serves as a reservoir of LPS (~50 μg/ml in the colon) (18, 34), which is constantly produced by gram-negative bacteria.

The intestinal microbial composition varies among individuals and also during different stages in the life of an individual. A balance between gram-negative and gram-positive bacteria is, however, maintained in the healthy human gut with a predominance of gram-positive bacteria (7). Gram-negative bacteria are remarkably increased in inflammatory bowel disease (IBD) patients (19, 32, 37) and accumulate at high concentrations in an inflamed lesion of the intestine (14). Many factors, such as medication (e.g., antibiotic use), infection, diet, and ingestion of toxic chemicals, can dramatically alter gut microbial composition. Enhanced gram-negative bacteria in the gut can result in elevated LPS level in the intestine, which subsequently contributes to the development and progress of intestinal inflammation. Similarly, TLR4 was suggested to be strongly upregulated in IBD patients (2), and a TLR4 antagonist was shown to ameliorate mouse experimental colitis (12). In addition, LPS was suggested to have a critical impact on the pathophysiology of necrotizing enterocolitis (NEC) (18). On the basis of these considerations, LPS-TLR4 engagement is believed to be involved in the pathophysiology of intestinal inflammatory diseases. On the other hand, LPS normally present in the gut is believed to be harmless, whereas systemic LPS exposure causes systemic sepsis. To explain why LPS is harmless in the healthy intestine, it has been suggested that the normal intestinal epithelium, which is constantly exposed to gut microbes, might be hyporesponsive to luminal LPS (1, 25). Any direct effect of LPS inside the colon, however, remains to be elucidated.

Accordingly, we examined whether elevated LPS in the colon could induce inflammatory responses. To avoid the systemic effect from oral, intraperitoneal, or intravenous administration of LPS and to study direct effects of LPS in the colon, mice were instilled with LPS by rectal enema. We found that elevated LPS inside the colon elicits transient inflammation in the small intestine, not in the colon, of the normal mouse, and such intestinal inflammation is substantially exacerbated in immune modulator-impaired, such as IL-10−/− or Rag-1−/−, mice. Our results provide evidence that elevated LPS in the colon has the potential to initiate inflammatory responses in the intestine and, therefore, suggest a mechanistic explanation for the importance of maintaining the balance between gram-negative and gram-positive bacteria in the intestine to maintain gut homeostasis.

MATERIALS AND METHODS

Mice. C57BL/6, CD-1, C3H/HeJ, C3H/HeOuJ, IL-10−/− (C57BL/6 background), and Rag-1−/− mice (8 wk old) were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were housed in a specific pathogen-free facility of the Division of Laboratory Animal Medicine at the University of California Los Angeles (UCLA). The Institutional Animal Care and Use Committee of UCLA approved all animal procedures.

Reagents. Ultrapure LPS (purified from Escherichia coli O111:B4) and peptidoglycan (PGN) were purchased from InvivoGen (San Diego, CA). Recombinant mouse IL-10 was purchased from Biosource International (Camarillo, CA). Limulus amebocyte lysate test

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kit and purified flagellin were obtained from Lonza (Basel, Switzerland).

**Intracolonic administration of LPS.** As previously described (4, 17, 30), 150 µl of LPS (25 or 50 µg/mouse) or saline (as a vehicle) were administered via an 18-gauge, 2.5-mm-diameter feeding tube that was advanced through the rectum into the colon until the tip was 3 cm proximal to the anus (midcolon). We ensured that there was no LPS leakage after the procedure.

**Body weight loss and morbidity evaluation.** Body weight loss (%) was evaluated as previously described (4, 17). General signs of morbidity were calculated by assignment of well-established scores for intestinal inflammation parameters (30, 33) based on changes in stool consistency and rectal bleeding. Briefly, stool consistency was scored as follows: 0 = normal well-formed pellets, 2 = pasty and semiformal pellets that stick to the anus, 4 = liquid stools that stick to the anus (watery diarrhea). Rectal bleeding was determined as follows: 0 = no bleeding, 2 = slight visible bleeding, 4 = gross bleeding. The resulting scores from an individual mouse were added and divided by 2, resulting in a total morbidity score ranging from 0 (healthy) to 4 (maximal severity).

**Histology.** The entire mouse small intestine and colon were excised, and 1-cm segments of the transverse tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The histological severity of inflammation was graded in a “blinded” fashion on a scale of 0–4 for ulceration and neutrophil infiltration, as previously described (17, 30).

**Measuring cytokine production.** An ELISA was performed according to the manufacturer’s instructions to measure cytokine expression. We used the appropriate kits from Biosource International and R & D Systems (Minneapolis, MN). Myeloperoxidase (MPO) ELISA kit was obtained from Hycult Biotech (Plymouth Meeting, PA). All assays were performed in triplicate, and values are means ± SD.

**Regulatory T cell flow cytometry.** CD-1 mice (n = 4/group) were intracolonically treated with LPS or saline for 2 days. Spleens were processed to prepare splenocytes. The cells were allowed to pass through a 26-gauge needle and preseparation filters (Miltenyi Biotec, Bergisch Gladbach, Germany) to remove clumps or debris. Red blood cells were removed by addition of RBC-Lysing Buffer (Sigma-Aldrich), and a Dead Cell Removal kit (Miltenyi Biotech) was used to remove dead cells. CD4+ T cells were then isolated using a CD4+ T cell isolation kit.
cell isolation kit (Miltenyi Biotec) according to the manufacturer’s instruction. A Mouse Treg Flow Kit (BioLegend, San Diego, CA) was used to double-stain the collected and enriched CD4⁺ T cells with fluorescence-conjugated antibodies (CD25-phycoerythrin and CD4- allophycocyanin); then the cells were stained with Alexa Fluor 488-conjugated FoxP3 antibody. Cells were analyzed by flow cytometry of CD4⁺/CD25⁺ or CD4⁺/Foxp3⁺ regulatory T (Treg) cells.

Statistical analysis. Statistical analyses were conducted with GraphPad Prism (GraphPad Software, La Jolla, CA).

RESULTS

Intracolonic LPS treatment causes transient mucosal damage in the small intestine, not in the colon. Aberrant TLR4 activation by LPS in the intestine was suggested to be associated with NEC (3, 18), suggesting that TLR4 activation by LPS may be linked with the pathophysiology of intestinal inflammation. Similarly, the frequency of gram-negative bacteria is dramatically increased in the inflamed intestine (19, 32, 37). On the basis of these considerations, we studied whether elevated LPS on the luminal side of the colon could cause intestinal inflammatory responses. To avoid the systemic effect from oral or intraperitoneal administration of LPS and to study direct LPS effects in the intestine, mice were intracolonically treated with an LPS enema. In CD-1 mice, LPS enema resulted in a remarkable weight loss of 15% in 2–3 days; in mice treated with vehicle, weight loss was not observed (Fig. 1 A). Interestingly, the weight loss was less evident in 4–5 days after initiation of LPS administration and completely restored in 10 days. Consistent with the body weight loss, LPS-treated mice also showed increased morbidity in 2–3 days after initiation of LPS treatment, and the morbidity was not discernible in 5 days, whereas vehicle-treated mice were completely normal throughout the experimental period.

Since C3H/HeJ mice have a point mutation at the cytoplasmic Toll/IL-1 receptor (TIR) domain of TLR4, rendering the mouse unresponsive to LPS (26, 29), we used C3H/HeJ mice and their control (C3H/HeOuJ) to confirm whether this transient LPS response is specifically mediated by TLR4. Similar to the results from CD-1 mice, LPS enema in C3H/HeOuJ mice induced a pronounced weight loss of 15% and increased morbidity in 2–3 days. As expected, these mice completely recovered from the inflammation in 9–10 days (Fig. 1B). However, LPS-defective C3H/HeJ mice did not lose weight, nor did they develop intestinal inflammation upon LPS treatment (Fig. 1C). These results indicate that LPS-induced intestinal inflammation is specifically mediated by TLR4.

We next examined whether intracolonic LPS treatment results in histopathological changes in the intestinal mucosa. In the small intestine (represented by the jejunum) of the mouse subjected to LPS enema for 2 days, we observed severe mucosal damage and inflammation characterized by crypt atrophy, ulcer and erosive lesions, and intense infiltration of inflammatory cells (Fig. 1, D and E). In these mice, intestinal...
villi structure was substantially degenerated and the crypt regions were severely damaged compared with the crypts in vehicle-treated mice. However, the small intestine from mice treated with LPS for 5 days showed regenerated villi and crypts in the epithelium, reflecting the transient nature of the intestinal inflammation in response to LPS.

In contrast to the small intestine, there was no histological evidence of colonic inflammation in the mouse subjected to LPS enema (Fig. 1, F and G), and the colonic epithelia from the LPS-treated mice were intact and their microscopic histology was comparable to that of vehicle-treated mice. These results indicate that elevated LPS on the luminal side of the colon has deleterious effects in the small intestine remotely, but we were surprised to find that it does not affect the integrity of the colonic epithelium.

**Intracolonic LPS administration increases intestinal fluid secretion and alters inflammatory cytokine production.** Given that fluid secretion is frequently associated with intestinal inflammation, we evaluated fluid secreted in the small intestine in response to LPS. We found that intracolonic LPS administration significantly increased intestinal fluid secretion 2 and 5 days after initiation of LPS treatment (Fig. 2A). We next examined inflammation-related cytokine production in the secreted intestinal fluid. LPS exposure for 2 or 5 days substantially increased TNFα production, but the extent of the induction was reduced 5 days after the LPS treatment was initiated (Fig. 2B). In contrast to the proinflammatory cytokine TNFα, the anti-inflammatory cytokine IL-10 was significantly reduced 2 days after initiation of LPS treatment (Fig. 2C). We were intrigued to find that reduced IL-10 production at 2 days after initiation of LPS treatment rebounded at 5 days (Fig. 2C). These data indicate that intracolonic LPS reciprocally alters TNFα and IL-10 production in the small intestine. Since TNFα and IL-10 are representative pro- and anti-inflammatory cytokines, respectively, augmented TNFα and diminished IL-10 levels 2 days after LPS administration appear to be associated with the pathophysiology of LPS-driven intestinal inflammation. Collectively, the differential expression of TNFα and IL-10 represents the transient nature of LPS-driven intestinal inflammation.

Next, we tested whether LPS on the luminal side of the colon also enhances inflammatory cytokine production in the intestinal tissue, including the small intestine and colon. We found that MPO, IL-6, and KC were substantially upregulated in the small intestine of mice subjected to intracolonic LPS treatment compared with vehicle-treated mice (Fig. 2D). In addition, LPS treatment also resulted in enhanced inflammatory cytokine (IL-6, KC, macrophage inflammatory protein 3α, and TNFα) production in the colon (Fig. 2E).

Collectively, these data suggest that elevated LPS levels in the colon are able to elicit inflammatory responses in the small intestine and colon, while epithelial tissue damage was observed in the small intestine, not in the colon.

**Intracolonic LPS treatment reduces Treg cell population.** Treg cells (5–10% of peripheral CD4⁺ T cells) are the key factor for peripheral tolerance; they actively inhibit inflammation and are involved in maintaining the immune balance in the normal gut by inhibiting T cell activation and proliferation against bacterial molecules (31). Since our data showed that intracolonic LPS elicited the inflammatory responses in the intestine and, furthermore, studies suggested that intestinal inflammatory diseases are directly associated with excessive T cell activation (6), we next investigated whether T cell activation would be involved in LPS-induced intestinal inflammation. We observed that spleens of mice treated with intracolonic LPS were enlarged compared with those of vehicle-treated mice (Fig. 3A). Given that enhanced LPS level in the colon evolves to intestinal inflammation with upregulated pro-inflammatory cytokine production and epithelial damage in the small intestine, an enlarged spleen appears to result from those inflammatory responses.

From these spleens harvested from intracolonically LPS-treated mice, we isolated CD4⁺ T cells. Using flow cytometry, we found a substantial reduction in the population of immunosuppressive Treg cells (CD4⁺/CD25⁺ or CD4⁺/FoxP3⁺) in mice intracolonically treated with LPS compared with vehicle-treated mice: 6.14% and 1.60% in vehicle- and LPS-treated mice, respectively, for CD4⁺/CD25⁺ and 6.28% and 1.80% in vehicle- and LPS-treated mice for CD4⁺/FoxP3⁺ (Fig. 3B).

These data demonstrate that augmented LPS in the colon is able to reduce the population of Treg cells, which are critical for suppressing inflammatory responses, resulting in a predisposition to intestinal inflammation.
LPS-induced intestinal inflammation is dramatically enhanced in IL-10<sup>−/−</sup> mice. IL-10<sup>−/−</sup> mice are predisposed to develop spontaneous intestinal inflammation when housed in conventional conditions but do not show the inflammation in a germ-free environment. The inflammation in IL-10<sup>−/−</sup> mice is, therefore, considered to be commensal microflora-dependent (27), and microbial pattern molecules are able to trigger the development and progress of the intestinal inflammation. Moreover, our data show that intracolonic LPS treatment results in reduced IL-10 production in the small intestine (Fig. 2). Accordingly, we examined the impact of elevated colonic LPS in the absence of IL-10. We administered LPS intracolonically to normal IL-10<sup>−/−</sup> and IL-10<sup>+/+</sup> mice. We were surprised to find that intracolonic LPS treatment caused severe inflammation in the small intestine and colon, characterized by marked fluid secretion, red and swollen intestine, and shrunken cecum, compared with vehicle-treated IL-10<sup>−/−</sup> mice (Fig. 4A). In addition, the stomach was larger in LPS- than vehicle-treated IL-10<sup>−/−</sup> mice and appeared to be inflamed (Fig. 4A). As a reasonable explanation for this observation, we speculate that severe inflammation in the intestine might be transmitted to the directly adjacent organ, resulting in an inflamed stomach.

Furthermore, all the LPS-exposed IL-10<sup>−/−</sup> mice revealed severe signs of intestinal inflammation and died within 3 days after initiation of intracolonic LPS administration, while LPS treatment did not evoke mortality in IL-10<sup>+/+</sup> mice (Fig. 4, B and C). In contrast to LPS treatment, intracolonic administration of other microbial factors, such as PGN, did not result in clinical signs of inflammation in IL-10<sup>−/−</sup> and IL-10<sup>+/+</sup> mice.

Since it was suggested that administration of recombinant IL-10 protein ameliorates colonic inflammation in mouse colitis models (36), we tested whether reconstitution of IL-10

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**Fig. 4.** LPS-induced intestinal inflammation is exaggerated in IL-10<sup>−/−</sup> mice. A: photographs of the entire gastrointestinal tract of IL-10<sup>−/−</sup> mice on a C57BL/6 background intracolonically treated with vehicle or LPS (25 µg/mouse) for 2 days. B and C: Kaplan-Meier survival plots. B: significant difference in survival rate of IL-10<sup>−/−</sup> mice treated with vehicle (n = 15), LPS (n = 16), LPS + recombinant mouse IL-10 (rIL-10, 0.1 µg/mouse, n = 16), or peptidoglycan (PGN, 25 µg/mouse, n = 12). Significant survival difference was determined by log-rank test followed by Bonferroni’s multiple-comparison method: P < 0.0001 (vehicle vs. LPS); P < 0.0001 (LPS vs. LPS + rIL-10). C: no change in survival of IL-10<sup>+/+</sup> mice treated with vehicle, LPS, LPS + rIL-10, or PGN (n = 12/group). D: body weight change (% 100%) in IL-10<sup>−/−</sup> mice intracolonically treated with vehicle (n = 4), PGN (n = 16), LPS (n = 20), or LPS + rIL-10 (n = 10). No LPS-treated IL-10<sup>−/−</sup> mice survived more than 2 days. Data were compared by 2-way ANOVA followed by Bonferroni’s multiple-comparison t-test: *P < 0.001 vs. vehicle; ***P < 0.001 vs. vehicle. E: volume of intestinal fluid obtained from entire length of the small intestine of IL-10<sup>−/−</sup> mice treated with vehicle, LPS, or LPS + rIL-10 for 2 days. Values are means ± SD (n = 4/group). *P < 0.05 (Mann-Whitney U-test).

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deficiency in IL-10−/− mice with recombinant IL-10 protein would reduce LPS-induced intestinal inflammation. IL-10−/− mice were intracolonically treated with a mixture of LPS and recombinant mouse IL-10 protein (rIL-10). We found that ≥60% of the mice were still alive 7–10 days later, whereas all the IL-10−/− mice with LPS enema died within 3 days (Fig. 4B). As shown in IL-10+/+ mice treated with LPS alone, treatment with LPS + rIL-10 did not induce mortality in IL-10+/+ mice (Fig. 4C). In IL-10−/− mice, body weight was reduced up to 20% by 4 days after initiation of LPS + rIL-10 treatment and was subsequently restored. In contrast, vehicle- and PGN-treated IL-10−/− mice showed the normal pattern of weight gain, although it was somewhat slower in PGN- than vehicle-treated mice (Fig. 4D). Consistent with these results, in IL-10−/− mice, treatment with LPS + rIL-10 substantially reduced fluid secretion in the intestine compared with mice treated with LPS alone (Fig. 4E). These results demonstrate that elevated colonic LPS elicits severe intestinal inflammation in the absence of IL-10.

Rag-1−/− mice did not recover from LPS-induced weight loss. Our data show that mice intracolonically instilled with LPS exhibit intestinal inflammation in 2–3 days and then develop a tolerance to prolonged LPS exposure (Fig. 1). Moreover, excessive T cell activation is believed to cause intestinal inflammation, and immune-suppressive Treg cells are responsible for peripheral tolerance to LPS. On the basis of these considerations, we hypothesized that T cells could be involved in evoking tolerance to extended LPS exposure.

Given that Rag-1−/− mice produce no mature T cells (35), we exploited Rag-1−/− mice to demonstrate whether T cells are implicated in the pathophysiology of LPS-induced intestinal inflammation. Similar to LPS-treated control (C57BL/6) mice, in Rag-1−/− mice, intracolonic LPS administration resulted in significant weight loss of 15.8% over 4 days of LPS treatment. We were surprised to find that these mice failed to recover from the weight loss over 10 days (11.6% weight loss at day 10). In contrast, control wild-type mice exhibited marked weight loss of 14.2% over 2 days of LPS treatment; after 2 days, they began to recover the lost weight and were fully recovered within 10 days (Fig. 5). These data suggest that T cell activation is involved in the pathophysiology of LPS-induced intestinal inflammation and appears to be necessary for tolerance to extended LPS exposure.

**DISCUSSION**

In the diverse and complex microbial milieu of the gut, an increase in gram-negative bacteria has been monitored in intestinal inflammatory diseases, including IBD (19, 32, 37), implying that elevated LPS from increased gram-negative bacteria may contribute to the development and progress of the intestinal inflammation. Indeed, this notion appears to be supported by a report that intracolonic administration of LPS elicited colitis in rabbits (16). Moreover, instillation of LPS into the lumen of the intestine was suggested to cause scattered microvascular lesions, which are commonly observed in the rectal mucosa of adults with acute diarrhea (20). On the other hand, intestinal LPS has been considered nonharmful, because LPS is well tolerated by the colonic epithelium and even provides some beneficial effects. Indeed, luminal LPS exposure in the intestinal mucosa does not affect intestinal epithelial barrier function (24). Moreover, LPS-TLR4 engagement promotes intestinal epithelial cell proliferation and, thereby, protects the mucosa against dextran sulfate sodium-induced mouse colitis (28).

However, in support of the potential inflammatory effect of LPS inside the intestine, in this study, we have demonstrated that an increase in the amount of LPS in the colon causes severe, but transient, inflammation in the small intestine, but not in the colon. These findings might raise the following questions. 1) How does LPS treatment in the colon remotely provoke severe inflammation in the small intestine, but not in the colon? 2) Why would LPS-driven intestinal inflammation be transient, rather than persistent? In answer to question 1, there are several examples of remotely activated intestinal inflammation. For instance, exposure of the rat jejunal to cholera toxin induces fluid secretion not only locally, but also remotely in the colon, which was not exposed to the toxin (22). In addition, rotavirus infection in the mouse small intestine also remotely causes fluid secretion in the colon by allowing epithelial cells to produce biological mediators that can then activate the enteric nervous system (15). Similarly, our data show that LPS treatment in the colon causes severe inflammation in the small intestine remotely, while it induces proinflammatory cytokine production in the colon without causing adverse effects on the colonic mucosa (Figs. 1 and 2). Regarding the small intestinal inflammation remotely induced by colonic LPS, we speculate that lamina propria cells in the colon might respond to colonic LPS, or epithelial cells in the colon could be able to sense luminal LPS, to induce inflammatory mediators, which will trigger subsequent inflammatory responses in the small intestine.

In answer to question 2, various examples of the protective mechanism against LPS can be proposed. Secretory IgA antibody in the intestine serves as an external barrier to neutralize LPS in the intestine (8). In addition, the colonic epithelium is rich in mucin-producing goblet cells, and the mucus layer can
prevent LPS attachment to the mucosal surface in the colon (21). Although transmural passage of LPS was suggested to be almost imperceptible in the normal colon (23), LPS can be absorbed at a molecular level by a specific cellular transporter, the chylomicron, in the intestinal epithelial cells and, thereby, can induce inflammatory responses (13). In this case, however, immune-suppressive Treg cells and an anti-inflammatory cytokine, such as IL-10, can engage in modulating inflammatory responses (9–11). Moreover, TLR-inhibitory molecules [e.g., Toll-interacting protein (TOLLIP), IL-1 receptor-associated kinase (IRAKM), single immunoglobulin IL-1 receptor-related protein (SIGIRR), spliced variant of myeloid differentiation factor 88 (MyD88s), and TIR domain-containing adaptor-inducing IFN-β (TRIF)] are strongly expressed or can be upregulated by inflammatory responses in intestinal epithelial cells and, subsequently, can disrupt a prolonged inflammatory response (5). Taken together, although colonic LPS is able to elicit inflammatory responses initially, these protective mechanisms may allow the intestine to contain the inflammatory response. Although we appreciate that a clear answer is not straightforward, thanks to some of these protective mechanisms, mice treated with colonic LPS could recover from the intestinal inflammation. This speculation can be supported by our findings that in intestinal inflammation was severe in IL-10−/− and Rag-1−/− mice, and they failed to recover (Fig. 4), and 2) T cell-depleted Rag-1−/− mice could not recover from LPS-induced intestinal inflammation (Fig. 5). We, however, acknowledge that the exact mechanism by which LPS in the colon can induce small intestinal inflammation remotely but does not damage the colonic epithelium locally is still not clear.

In summary, our study demonstrates that elevated LPS from gram-negative bacteria on the luminal side of the colon causes intestinal inflammation, and these inflammatory responses are remarkably enhanced in IL-10−/− and Rag-1−/− mice, in which the immune-modulatory function is impaired. Because overgrowth of gram-negative bacteria is commonly observed in the inflamed intestine (14, 37) and LPS is believed to be one of the causes of NEC (18), our study suggests important evidence to determine the effect of colonic LPS in the development and progress of intestinal inflammatory diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.I. and S.H.R. are responsible for conception and design of the research; E.I., F.M.R., and S.H.R. performed the experiments; E.I. and S.H.R. analyzed the data; E.I. and S.H.R. interpreted the results of the experiments; E.I., F.M.R., and S.H.R. drafted the manuscript; E.I., C.P., and S.H.R. edited and revised the manuscript; E.I., F.M.R., C.P., and S.H.R. approved the final version of the manuscript; S.H.R. prepared the figures.

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