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Postoperative Ileus Involves Interleukin-1 Receptor Signaling in Enteric Glia

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BACKGROUND & AIMS: Postoperative ileus (POI) is a common consequence of abdominal surgery that increases the risk of postoperative complications and morbidity. We investigated the cellular mechanisms and immune responses involved in the pathogenesis of POI. **METHODS:** We studied a mouse model of POI in which intestinal manipulation leads to inflammation of the muscularis externa and disrupts motility. We used C57BL/6 (control) mice as well as mice deficient in Toll-like receptors (TLRs) and cytokine signaling components (TLR-2$^{-/-}$, TLR-4$^{-/-}$, TLR-2/4$^{-/-}$, MyD88$^{-/-}$, MyD88/TLR adaptor molecule 1$^{-/-}$, interleukin-1 receptor [IL-1R1]$^{-/-}$, and interleukin (IL)-18$^{-/-}$ mice). Bone marrow transplantation experiments were performed to determine which cytokine receptors and cell types are involved in the pathogenesis of POI. **RESULTS:** Development of POI did not require TLRs 2, 4, or 9 or MyD88/TLR adaptor molecule 2 but did require MyD88, indicating a role for IL-1R1. IL-1R1$^{-/-}$ mice did not develop POI; however, mice deficient in IL-18, which also signals via MyD88, developed POI. Mice given injections of an IL-1 receptor antagonist (anakinra) or antibodies to deplete IL-1α and IL-1β before intestinal manipulation were protected from POI. Induction of POI activated the inflammasome in muscularis externa tissues of C57BL6 mice, and IL-1α and IL-1β were released in ex vivo organ bath cultures. In bone marrow transplantation experiments, the development of POI required activation of IL-1 receptor in nonhematopoietic cells. IL-1R1 was expressed by enteric glial cells in the myenteric plexus layer, and cultured primary enteric glia cells expressed IL-6 and the chemokine monocyte chemotactic protein 1 in response to IL-1β stimulation. Immunohistochemical analysis of human small bowel tissue samples confirmed expression of IL-1R1 in the ganglia of the myenteric plexus. **CONCLUSIONS:** IL-1 signaling, via IL-1R1 and MyD88, is required for development of POI after intestinal manipulation in mice. Agents that interfere with the IL-1 signaling pathway are likely to be effective in the treatment of POI.

Keywords: Inflammatory Response; Gastrointestinal Dysmotility; Pathogen Recognition; Innate Immunity.

Postoperative ileus (POI) is an iatrogenic impairment of propulsive gastrointestinal (GI) motility that frequently occurs after abdominal but also extra-abdominal surgery.[1] Previous work indicates that its pathophysiology comprises a response to an intestinal manipulation (IM) triggering a complex orchestrated immune response, leading to activation of resident macrophages[2]; activation of transcription factors such as nuclear factor κB, signal transducer and activator of transcription 3 (STAT3), and early growth response protein 1 (EGR-1); and production of various proinflammatory and chemoattractive mediators such as interleukin (IL)-6, IL-1β, chemokine (C-C motif) ligand 2 (also called monocyte chemotactic protein 1 [MCP-1]), and integrins and cell adhesion molecules (intercellular adhesion molecule 1 [ICAM-1] and lymphocyte function–associated antigen 1 [LFA-1]).[3] The subsequent inflammatory infiltrate includes cells that release factors such as prostaglandins and nitric oxide,[4,5] which are suggested to directly affect gut motility by acting on enteric neurons.

The molecular responses to bowel manipulation that are a causal factor in the motility disturbances that characterize POI remain unclear. Bacterial overgrowth and translocation due to intestinal obstruction is a well-documented feature in surgical patients,[6] and translocation of luminal bacteria to mesenteric lymph and mucosa has been shown in animal models of POI.[7,8] Hence, pattern recognition receptors such as Toll-like receptors (TLRs) may be implicated in the cellular inflammation that characterizes POI. Resident muscularis externa (ME) macrophages are highly responsive to bacterial cell wall molecules such as lipopolysaccharide and express TLR-4.[9] On the other hand, surgical manipulation is associated with tissue trauma that can lead to a sterile innate immune response via pattern recognition receptors that are capable of sensing host-derived endogenous damage-associated molecular patterns.[10]

Abbreviations used in this paper: **GFAP, glial fibrillary acidic protein; GI, gastrointestinal; Ig, immunoglobulin; IL, interleukin; IL-1R, interleukin 1 receptor; IL-1R1, interleukin-1 receptor type I; IL-1ra, IL-1 receptor antagonist; IL-18R, interleukin-18 receptor; IM, intestinal manipulation; MCP-1, monocyte chemotactic protein 1; ME, muscularis externa; MPO, myeloperoxidase; mRNA, messenger RNA; POI, postoperative ileus; TLR, Toll-like receptor; WT, wild-type.**

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In the present study, we investigated if and how TLRs as well as interleukin-1 receptor (IL-1R)–dependent pathways contribute to the development of POI. TLRs signal via the intracellular adaptor molecules MyD88 or TRIF, of which MyD88 is also a central molecule in the signaling pathways of interleukin-1 receptor type I (IL-1R1) and IL-18 receptor (IL-18R). Interestingly, we found that the IL-1R1 ligands IL-1α and IL-1β, rather than TLR or IL-18 signaling, selectively play a significant role in different phases of POI genesis. Our data indicate that in the affected bowel wall, enteric glia IL-1R1 are a prominent target of IL-1 released after surgery. Hence, endogenous as well as exogenous inhibitory mechanisms of IL-1R1 signaling may emerge as new options in the treatment of POI.

Materials and Methods

Animals

Experiments were performed with wild-type (WT) ~8- to 12-week-old male C57BL/6 mice (Janvier, Saint Berthevin Cedex, France) with a mean body weight of 20 to 25 g. Additionally, TLR-2$^{-/-}$, TLR-4$^{-/-}$, TLR-2/4$^{-/-}$, MyD88$^{-/-}$, and MyD88/TRIF$^{-/-}$ mice were used. IL-1R1– and IL-18–deficient mice, CSF-1 mutant op/op$^{-/-}$ mice (B6C3Fe a/a-Csf1op), and B6.PL-Thy1a/CyJ(CD90.1) mice were obtained from Jackson Laboratories (Charles River, Sulzfeld, Germany). All experiments were performed in accordance with federal law regarding animal protection and were approved by the government and the local animal committee of the university.

Bone marrow transfer (Supplementary Methods) was used to create IL-R1$^{-/-}$ chimera mice in which the genetic deficiency of IL-1R1 was contained to either the circulating cells (IL-1R1$^{-/-}$ > WT chimera) or nonhematopoietic tissue (WT > IL-1R1$^{-/-}$).

Animal Model of POI

POI was induced by a standardized small bowel manipulation procedure as described previously.[11] In some experiments, depleting antibodies against IL-1α, IL-1β, or corresponding control immunoglobulin (Ig) G (200 μg; BioLegend, Fell, Germany) were administered immediately after surgery. In another experiment, recombinant IL-1α, IL-1β, or IL-18 was administered intraperitoneally (2 μg/kg body wt) immediately after surgery or to untreated mice. In yet another experiment, the IL-1 receptor antagonist (IL-1ra) anakinra (Kineret Swedish Orphan Biovitrum AB, Stockholm, Sweden) was administered intraperitoneally (100 mg/kg body wt) 1 hour or 3 hours before IM. All animals underwent indicated analyses 3 or 24 hours postoperatively.

Functional Studies

To determine the effects of IM on gut motility, we measured the GI transit of a nonabsorbable tracer 24 hours after IM as previously described.[11] Further methods for immunohistochemical and immunofluorescence analysis, quantitative polymerase chain reaction, organ culture, and protein expression analysis are available in Supplementary Methods.

Cell Cultures

Enteric glia were isolated from enzymatically digested mice small bowel ME. All culture methods and treatments are described in Supplementary Methods.

Results

POI Develops Independently of TLR-2, TLR-4, and TLR-9 Signaling

To analyze whether TLRs are involved in the pathogenesis of POI, we first determined gene transcription of the proinflammatory mediators IL-6, MCP-1 (Figure 1*A*–*D*), and pro-IL-1β (Supplementary Figure 1*A*), which are critically induced by IM, in WT and TLR-deficient mice. IM led to significant up-regulation of IL-6, MCP-1, and pro-IL-1β transcripts within the postsurgical ME 3 hours after IM. TLR-2$^{-/-}$, TLR-4$^{-/-}$, and TLR-2/4$^{-/-}$ mice displayed protection against the increase in inflammatory genes after IM surgery compared with WT mice. Further, protein release of IL-6 and MCP-1 from ME tissue preparations (Figure 1*C* and *D*) showed no significant changes in IM-induced protein levels among WT and TLR$^{-/-}$ mice. In agreement, TLR deficiency did not affect postoperative extravasation of myeloperoxidase (MPO^{+}) neutrophils to the manipulated ME (Figure 1*E*) and correspondingly did not protect functionally against POI given the postoperative transit time (Figure 1*F*), with the exception that TLR-2/4$^{-/-}$ mice showed normalized GI transit after IM surgery ($P < .001$). Similar to TLR-2– and TLR-4–deficient mice, TLR9$^{-/-}$ mice were not protected against POI in this model (not shown) in any parameter of POI examined.

MyD88 Signaling Mediates POI Independently of TLRs

The majority of TLRs signal via MyD88 adaptor protein, whereas TLR-4 and TLR-3 also signal via TRIF. Therefore, we analyzed the postoperative responses to IM in the same setting in MyD88- and MyD88/TRIF-deficient mice. When we analyzed early expression of inflammatory mediators in muscularis tissue after IM surgery, we observed that MyD88- and MyD88/TRIF-deficient mice were protected against IM-induced inflammatory responses and POI. The up-regulation of IL-6, MCP-1, and pro-IL-1β messenger RNA (mRNA) levels (Figure 2*A* and *B* and Supplementary Figure 1*B*) as well as IL-6 and MCP-1 protein levels (Figure 2*C* and *D*) was significantly attenuated in both groups compared with WT IM controls. Consistently, MPO^{+} cell infiltration (Figure 2*E*) and delay in GI transit (Figure 2*F*) were attenuated in MyD88- and MyD88/TRIF-deficient mice. Hence, a surgery-induced inflammatory response in the bowel wall critically depends on MyD88 signaling rather than TLR- and TRIF-dependent pathways.

IM Induces Mature IL-1α and IL-1β Release in the ME

In addition to TLRs, MyD88 mediates proinflammatory signaling through IL-1R1 as well as IL-18R. Next, we assessed