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Cutting Edge: IL-36 Receptor Promotes Resolution of Intestinal Damage

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IL-1 family members are central mediators of host defense. In this article, we show that the novel IL-1 family member IL-36γ was expressed during experimental colitis and human inflammatory bowel disease. Germ-free mice failed to induce IL-36γ in response to dextran sodium sulfate (DSS)-induced damage, suggesting that gut microbiota are involved in its induction. Surprisingly, IL-36R−deficient (Il1rl2−/−) mice exhibited defective recovery following DSS-induced damage and impaired closure of colonic mucosal biopsy wounds, which coincided with impaired neutrophil accumulation in the wound bed. Failure of Il1rl2−/− mice to recover from DSS-induced damage was associated with a profound reduction in IL-22 expression, particularly by colonic neutrophils. Defective recovery of Il1rl2−/− mice could be rescued by an aryl hydrocarbon receptor agonist, which was sufficient to restore IL-22 expression and promote full recovery from DSS-induced damage. These findings implicate the IL-36/IL-36R axis in the resolution of intestinal mucosal wounds. *The Journal of Immunology, 2016, 196: 34–38.

Crohn’s disease (CD) and ulcerative colitis (UC), the two major clinical phenotypes of inflammatory bowel disease (IBD), are associated with dysregulated innate and adaptive immune responses toward gut microbiota (1). Members of the IL-1 family of cytokines are upregulated in the inflamed mucosa during experimental colitis and human IBD and contribute to intestinal inflammation (2). Interestingly, however, mice deficient in IL-1β or IL-18, or components of their processing, are more susceptible to dextran sodium sulfate (DSS)-induced colitis (3–6), and polymorphisms leading to decreased Nlrp3 expression in humans are associated with increased risk for developing CD (7). Thus, IL-1 family members may contribute to proinflammatory responses, as well as to resolution of inflammation (2, 8, 9).

Recently, information on the role of IL-36 family members, which are members of the IL-1 superfamily, has begun to emerge (10, 11). However, little data exist about the function of the IL-36/IL-36R axis in the intestine. In this article, we demonstrate that IL-36γ was induced during intestinal injury/infammation and that IL-36R influenced neutrophil (NP) accumulation, IL-22 production, and repair of intestinal damage following injury. Treatment with an aryl hydrocarbon receptor (AhR) agonist was sufficient to induce intestinal IL-22 expression and promote recovery in Il1rl2−/− mice. Collectively, these findings support a novel contribution of the IL-36/IL-36R axis to the repair of damaged intestinal mucosa.

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Abbreviations used in this article: AhR, aryl hydrocarbon receptor; CD, Crohn’s disease; cLP, colonic lamina propria; CNV, conventionally housed; DAI, disease activity index; DC, dendritic cell; DSS, dextran sodium sulfate; FICZ, 6-formylindolo (3,2-b) carbazole; GF, germ free; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IEL, intestinal epithelial lymphocyte; II.C, innate lymphoid cell; MΦ, macrophage; NΦ, neutrophil; UC, ulcerative colitis; WT, wild-type.

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Materials and Methods

Mice

Wild-type (WT) C57BL/6 and Rag1<sup>−/−</sup> mice were from The Jackson Laboratory. Il1f9<sup>−/−</sup> mice were from Amgen. Germ-free (GF) mice were maintained as previously described (12). Protocols were approved by the Georgia State University and Emory University Institutional Animal Care and Use Committees.

Reagents

Abs were from eBioscience, with the exception of CD45, CD103, and CD4 (Becton Dickinson). Dead cells were identified using the Aqua Dead Cell Stain Kit (Invitrogen). Murine IL-36γ was from R&D Systems. ELISAs for IL-36γ (antibodies-online.com) and IL-22, CXCL1, and CXCL2 (eBioscience) were performed following the manufacturers’ instructions.

Isolation of colonic lamina propria cells and flow cytometry

Isolation of colonic lamina propria (cLP) cells, staining, and analyses were performed as described (13). cLP cells were defined as CD45<sup>+</sup>Ab<sub>2</sub>CD11b<sup>+</sup>F4/80<sup>+</sup>CD103<sup>+</sup> macrophages (MΦs), CD45<sup>+</sup>Ab<sub>2</sub>CD11c<sup>+</sup>F4/80<sup>+</sup>CD103<sup>+</sup> dendritic cells (DCs), Lin<sup>−</sup>CD19<sup>−</sup>ROG<sup>−</sup>NKp46<sup>−</sup>CD117<sup>−</sup> immune cells (ILCs), and CD45<sup>+</sup>CD11b<sup>-</sup>Ly6C<sup>-</sup>Ly6G<sup>+</sup> cells (NΦs).

Microarray analysis

Microarray processing was done by the Vanderbilt Microarray Shared Resource using Murine Genome 4.0 2.0 microarray chips (Affymetrix). The Gene Expression Omnibus accession number is GSE68269 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=olgvmwanflgd&acc=GSE68269).

Real-time PCR

RNA isolation and first-strand cDNA synthesis were performed as previously described (13). Primers were as described previously (14), with the exception of mIl19 (F, 5′-TTGACTTGGACACGAGGTTG-3′; R, 5′-GGGTTACTTGGATAGGGATAG-3′) and hIl19 (F, 5′-GGCTATGATTGGTTAAACC-3′; R, 5′-ATCTTCTGCTCTTATTGCTGCAAT-3′). cDNA from human IBD tissues were obtained in plate arrays (OriGene).

In vitro stimulation

Bone marrow–derived MΦs (1 × 10<sup>6</sup> cells/ml) were stimulated with TLR agonists for 12 h.

DSS-induced colitis

Mice were provided 3% (w/v) DSS (MP Biomedicals) in the drinking water for 5 d and then switched to normal drinking water for recovery. Daily clinical assessment of DSS-treated animals was performed as described previously (13).

CD4<sup>+</sup>CD45RB<sup>hi</sup>-induced colitis

Pure WT CD4<sup>+</sup>CD45RB<sup>hi</sup>T cells (4 × 10<sup>5</sup>) were injected i.p. into Rag1<sup>−/−</sup> recipients. Colonos were harvested when clinical signs of chronic colitis were evident (4 wk).

Helicobacter hepaticus–induced colitis

H. hepaticus (1 × 10<sup>6</sup> CFU strain 51449; American Type Culture Collection) was gavaged to mice on days 0, 2, and 4. Mice also received 1 mg anti–IL-10R (Bio X Cell; 1B1.2) Ab via i.p. injection on days 0, 7, 14, and 21 after H. hepaticus infection (15).

Wound-healing assays

Mucosal wound-healing assays were performed as previously described (16).

In vivo Ahr treatment

6-Formylindolo (3, 2-b) carbazole (FICZ; Enzo Life Sciences; 1 µg/mouse) was injected i.p. on day 3 of DSS treatment for 5 d.

Histology

Colons were fixed in 10% formalin. Paraffin embedding, sectioning, H&E staining, and slide scanning was performed at Emory’s Winship Cancer Institute-Pathology Core.

Results and Discussion

IL-36γ is expressed during experimental colitis and human IBD

Resident MΦs (Cx3cr1<sup>hi</sup>Ly6C<sup>−</sup>) are abundant in the healthy cLP, whereas a distinct subset of Cx3cr1<sup>low/−</sup>Ly6C<sup>−</sup> cells, which are predominantly inflammatory MΦs, infiltrates the colon after DSS treatment (17) (Supplemental Fig. 1A). To identify genes that are preferentially expressed by these inflammatory MΦs, we conducted microarray analysis between FACS-sorted CD45<sup>+</sup>Ab<sub>2</sub>CD11b<sup>+</sup>F4/80<sup>+</sup> cLP MΦs isolated from healthy mice (>80% Cx3cr1<sup>hi</sup>Ly6C<sup>−</sup> “resi-dent”) or mice treated for 5 d with DSS (>50% Cx3cr1<sup>low/−</sup>Ly6C<sup>−</sup> “infiltrating”). Using hierarchical clustering, 820 genes were differentially expressed >2-fold (log2 scale) between cLP MΦs isolated from healthy control or inflamed (DSS) colon (Supplemental Fig. 1B, middle panel). Among these genes, the gene ontology processes enriched by DAVID showed “Immune Response” as the top-ranked process. Of the 62 genes in this category, the top 10 (fold change) are shown. Interestingly, the novel IL-1 family member Il1f9 (renamed IL-36γ) was the top-ranked gene that was most preferentially expressed by cLP MΦs isolated from DSS-treated mice (5-fold increase log2 scale), whereas other members of the IL-1 family only showed modest increases or remained unchanged (data not shown). Validation of these observations by quantitative PCR confirmed that IL-36γ mRNA, but not IL-36α or IL-36β mRNA, was highly expressed in the inflamed colon of DSS-treated mice (Fig. 1A); correspondingly, protein levels were also increased as detected by Western blot and ELISA (Fig. 1B). This increased IL-36γ expression (~9-fold) in the inflamed colon following DSS treatment was recapitated in additional models of colitis, including the CD45RB<sup>hi</sup> transfer model (~13-fold) and the H. hepaticus model (15) (~5-fold) (Fig. 1C). Importantly, IL-36γ mRNA expression was also increased in the human colonic mucosa from individuals with IBD compared with healthy controls, and no significant differences were noted between UC and CD samples (Fig. 1D).

To investigate cellular sources of IL-36γ in the inflamed intestine, IL-36γ mRNA expression was analyzed among cLP DCs, MΦs, intestinal epithelial lymphocytes (IELs), and intestinal epithelial cells (IECs). cLP MΦs and IECs expressed ~600- and ~85-fold higher levels of IL-36γ, respectively, compared with DCs or IELs (Fig. 1E). Because DSS treatment disrupts the epithelial barrier and exposes immune cells to microbes, the role of microbiota in IL-36γ induction was explored. Conventionally housed (CNV) or GF mice were treated with DSS for 5 d, and IL-36γ mRNA expression was assessed. As shown in Fig. 1F, colonic tissue from GF mice expressed ~25-fold lower levels of IL-36γ mRNA than did CNV mice. Consistent with this observation, stimulation of bone marrow–derived MΦs with LPS or CpG significantly induced IL-36γ mRNA expression (Supplemental Fig. 1C).

IL-36R contributes to colonic wound healing

To evaluate the biological functions of IL-36γ in vivo, Il1r1<sup>−/−</sup> mice were treated for 5 d, and the se-
verity of colonic inflammation was assessed. Consistent with a proinflammatory role for the IL-36 pathway (10), we observed impaired wound healing in Il1rl2+/− mice compared with WT (Fig. 2A). Although Il1rl2+/− mice eventually recovered from colitis, the IL-36 pathway may regulate IL-22 production following DSS-induced damage, the role of IL-36R in promoting wound healing of damaged intestinal mucosa.

**IL-36R contributes to DSS-induced IL-22 production**

IL-22 is a barrier-protective cytokine that stimulates epithelial proliferation and restitution, the secretion of antimicrobial peptides, and protection from intestinal inflammation (19). Because Il1rl2+/− mice displayed impaired recovery from DSS-induced damage, the role of IL-36R in IL-22–mediated intestinal barrier protection was investigated. Interestingly, upon DSS treatment, IL-36γ expression preceded that of IL-22, with IL-36γ peaking on day 3, followed by IL-22 beginning on day 4 and peaking on day 5 (Fig. 3A). These data suggested that IL-36γ may regulate IL-22 production following DSS-

**significant reduction in NFκB accumulation in the wound bed** (Supplemental Fig. 2A, 2B). Because NFκB can aid in wound repair (18), these cells were examined in the wound beds of Il1rl2+/− and Il1rl2+/+ mice following mucosal biopsy. As shown in Supplemental Fig. 2A and Supplemental Fig. 2B, wound beds in Il1rl2+/− mice exhibited a significant reduction in NFκB accumulation compared with Il1rl2+/− mice. Consistent with these observations, treatment of colonic explants or IEC cultures with IL-36γ significantly induced expression of the NFκB chemokines CXCL1 and CXCL2 (Supplemental Fig. 2C, 2D). These data were also supported by evidence showing that IECs constitutively expressed Il1rl2 mRNA during the steady-state and following treatment of mice with DSS (data not shown). Collectively, these results suggest an important role for IL-36R in promoting wound healing of damaged intestinal mucosa.
induced damage. Therefore, IL-22 expression in the colon of Il1rl2−/− and Il1rl2+/− mice following DSS treatment was quantitated. After 5 d of DSS treatment, Il1rl2−/− mice had significantly reduced IL-22 protein expression compared with Il1rl2+/− mice (Fig. 3B). IL-22 protein expression in Il1rl2−/− colons returned to baseline by day 8, because these mice repaired intestinal damage, whereas Il1rl2−/− mice failed to induce IL-22 at day 8, because intestinal damage progressed. Interestingly, robust IL-22 expression in Il1rl2+/+ colons following DSS treatment was abolished in GF mice, similar to IL-36γ expression (data not shown).

These data demonstrate that the IL-36 pathway is involved in DSS-induced IL-22 expression.

In the DSS model, IL-22–producing NΦs provide a major contribution to the resolution of colonic injury (14). Thus, we explored whether there are defects in IL-22–producing NΦs following DSS treatment in the absence of IL-36γ. Indeed, a significant decrease in IL-22 production by NΦs from Il1rl2−/− mice was observed compared with Il1rl2+/+ mice with regard to frequency (Fig. 3C) and absolute cell number (Fig. 3D), whereas T cells and ILCs did not exhibit significant differences. These data are consistent with the observations of Zindl et al. (14), showing that IL-22–producing NΦs are important contributors to the resolution of DSS-induced colonic damage and suggest that IL-36γ is involved in the differentiation of this important cell type. Of note, very low levels of IL-22 were detected in Il1rl2−/− and Il1rl2+/− wound beds following mucosal biopsy (data not shown), suggesting that the extent and type of injury dictate whether the IL-36 pathway may predominantly control NΦ recruitment and/or differentiation into IL-22 producers.

FICZ induces IL-22 and resolution of colonic damage in Il1rl2−/− mice

Because IL-22–producing NΦs were significantly reduced in colons of DSS-treated Il1rl2−/− mice (Fig. 3C, 3D), we next investigated whether boosting IL-22 production from other cellular sources in vivo could complement this defect. The AhR pathway was shown to induce IL-22 production from CD4+ T cells and ILC3s in the intestine (20, 21); therefore, we treated Il1rl2−/− mice with the AhR agonist FICZ during the course of DSS treatment beginning at day 3. Although Il1rl2−/− mice failed to recover from DSS-induced intestinal mucosal damage and had to be euthanized, consistent with Fig. 2A and 2B, FICZ-treated Il1rl2−/− mice showed a significant reduction in DAI (Fig. 4A) and histological damage (Fig. 4B, 4C) and were able to fully recover from intestinal damage, similar to Il1rl2+/− mice or Il1rl2+/+ mice treated with FICZ. Importantly, FICZ treatment enhanced IL-22 mRNA expression in Il1rl2−/− mice during DSS-induced damage (Fig. 4D); however, we do not exclude the possibility that FICZ mediated protective effects independent of IL-22 induction.

Collectively, these data implicate the IL-36/IL-36R axis in the repair of intestinal mucosal wounds. Although the IL-36 pathway plays a pathogenic role in chronic psoriatic disorders (10), it is beneficial during acute intestinal damage. NΦs may play a central role in IL-36–mediated resolution of intestinal damage by phagocytosing and killing bacteria and/or producing IL-22 and other proresolving factors (22) that contribute to epithelial barrier repair and control of inflammation. Future insight into the functions of the IL-36 pathway during acute and chronic mucosal inflammation may contribute to the development of novel therapeutic strategies aimed at manipulating this cytokine axis.

Disclosures

J.E.T. was an employee at Amgen during the time of the studies and owns Amgen stock. The other authors have no financial conflicts of interest.

References


