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IL-27 Regulates Homeostasis of the Intestinal CD4+ Effector T Cell Pool and Limits Intestinal Inflammation in a Murine Model of Colitis

Amy E. Troy,* Colby Zaph,2* Yurong Du,* Betsy C. Taylor,* Katherine J. Guild,* Christopher A. Hunter,* Christiaan J. M. Saris,† and David Artis3*

IL-27 limits CD4+ T cell development in vitro and during inflammatory responses in the CNS. However, whether IL-27-IL-27R interactions regulate the homeostasis or function of CD4+ T cell populations in the intestine is unknown. To test this, we examined CD4+ T cell populations in the intestine of wild-type and IL-27R−/− mice. Naïve IL-27R−/− mice exhibited a selective decrease in the frequency of IFN-γ producing CD4+ T,17 cells and an increase in the frequency of T,17 cells in gut-associated lymphoid tissues. Associated with elevated expression of IL-17A, IL-27R−/− mice were more susceptible than Rag−/− mice to development of dextran sodium sulfate-induced intestinal inflammation, indicating an additional role for IL-27-IL-27R in the regulation of innate immune cell function. Consistent with this, IL-27 inhibited proinflammatory cytokine production by activated neutrophils. Collectively, these data identify a role for IL-27-IL-27R interaction in controlling the homeostasis of the intestinal T cell pool and in limiting intestinal inflammation through regulation of innate and adaptive immune cell function. The Journal of Immunology, 2009, 183: 2037–2044.

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Recent studies have described the ability of the cytokine IL-27 to regulate both the T,17 and T,17 cell lineages (25). IL-27 is a heterodimeric cytokine consisting of p28 and EBI3 subunits that bind to a unique receptor formed by the WSX-1/T cell cytokine receptor and gp130 proteins (26–28). Activated macrophages and dendritic cells secrete IL-27, while the IL-27 is expressed on naive CD4+ T cells, NK cells, mast cells, macrophages, and neutrophils (27–32). Initially IL-27 was identified as a T,17-promoting cytokine, as it can synergize with IL-12 to increase IFN-γ production from naive CD4+ T cells through STAT1 signaling (27, 33). However, more recent work has implicated IL-27 as an anti-inflammatory cytokine, due to its ability to suppress cytokine production by CD4+ effector T cells, including T,17 cells (34–36). Consistent with this, IL-27R−/− mice exhibit increased frequencies of T,17 cells in the CNS following Toxoplasma gondii infection or induction of experimental autoimmune encephalomyelitis (37, 38). Mechanistically, IL-27 directly inhibits expression of IL-17A in CD4+ T cells via a STAT1-dependent mechanism (37, 38). Despite growing evidence of multiple roles for IL-27 in CD4+ T cell-mediated inflammatory responses, whether IL-27 can influence intestinal immune homeostasis is unknown (39).

The role of IL-27 in innate immune cell activation is less characterized than its effects on T cells. IL-27, acting on macrophages, has been reported to both increase proinflammatory cytokine production through STAT1 and to suppress cytokine production through STAT3 (31, 40). In addition, neutrophils incubated with IL-27 exhibit decreased production of reactive oxygen species following LPS stimulation (32), but whether IL-27 can influence production of inflammatory cytokines by neutrophils is unknown.

In this report, we demonstrate that IL-27-IL-27R interactions are critical in promoting the T,17, while limiting the T,17 cell pool in

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2 Current address: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC Canada V6T 1Z3.

3 Address correspondence and reprint requests to David Artis, Department of Pathobiology, 314 Hill Pavilion, University of Pennsylvania School of Veterinary Medicine, 380 South University Avenue, Philadelphia, PA 19104-4539. E-mail address: dartis@vet.upenn.edu

4 Abbreviations used in this paper: IBD, inflammatory bowel disease; WT, wild type; mLN, mesenteric lymph node; PEC, peritoneal exudate cell; DSS, dextran sodium sulfate; LTi, lymphoid tissue inducer; LPL, lamina propria lymphocyte; IEL, intraepithelial lymphocyte.
the intestine and the GALT under steady-state conditions. Moreover, in a murine model of acute colitis, IL-27-IL-27R interactions are essential to limit both innate and adaptive immune cell responses that promote fatal intestinal inflammation.

Materials and Methods

Mice

Wild-type (WT) C57BL/6 and mice lacking the WSX-1 subunit of the IL-27R (IL-27R−/− mice) were obtained from Taconic Farms (courtesy of Christiaan Saris, Amgen). Rag−/− and Rag−/−/IL-27R−/− mice were bred and maintained at the University of Pennsylvania. Mice were sex- and age-matched for all experiments. All mice were maintained in a specific pathogen-free facility at the University of Pennsylvania School of Veterinary Medicine and all experiments were performed with the permission and guidance of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Isolation of lymphoid cells for analysis

At necropsy, mesenteric lymph nodes (mLN) were harvested and the large and small intestines removed for visual inspection. The cecal patch was removed before the large intestine was opened, cut into 1-cm pieces, and harvested in ice cold DMEM containing 5% FBS for isolation of intraepithelial and lamina propria lymphocytes. mLN were mechanically disrupted and large and small intestines were striped of their epithelium (for isolation of intraepithelial lymphocyte (IEL) then digested with collagenase/dispase (for isolation of lamina propria lymphocyte (LPL) as previously described (41) to obtain single cell suspensions for analysis.

Cytokine assays

Single cell suspensions were prepared in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, 25 mM HEPES, and 5 × 10−3 M 2-ME. Cells were plated in medium alone or in the presence of anti-CD3 and anti-CD28 (eBioscience; 1 µg/ml each). Cell-free supernatants were harvested after 48 h, and analyzed for cytokine secretion by ELISA. For organ culture, 1 cm sections of large intestine were rinsed briefly in PBS before being placed in 48-well plates containing 0.5 ml of cell medium for 12 h. Supernatants were removed for ELISA as above and the tissue was weighed. Cytokines were then normalized per mg of tissue weight. Intracellular staining for IL-17A and IFN-γ was performed following stimulation for 4 h with 50 ng/ml PMA (Sigma-Aldrich) and 500 ng/ml ionomycin (Sigma-Aldrich) in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Cells were stained with the fluorochrome-conjugated Abs PerCP-CD4 (BD Bio-

Histology of intestines

A 1-cm section of large intestine proximal to the cecum was removed and flushed with PBS before being fixed in 4% paraformaldehyde. Tissues were embedded in paraffin and cut using standard histological techniques. Five micron tissue sections were stained with H&E.

Neutrophil and macrophage cytokine production

Peritoneal macrophages and neutrophils were obtained following i.p. injection of C57BL/6 mice with 1 ml of 4% thioglycollate (Sigma-Aldrich). Mice being used for macrophage collection were injected with anti-Gr1 (RB6-8C5; gift of Dr. David Abraham, Thomas Jefferson University, Philadelphia, PA.) to deplete neutrophils 6 h before and 2 days after thiogly-
collate injection and were sacrificed 3 days postthioglycollate injection. For neutrophil collection, mice were sacrificed 4 h postthioglycollate injection. Peritoneal exudate cells (PECs) were obtained by flushing the peritoneum with 10 ml of ice-cold PBS. RBC in PEC suspensions were lysed with ammonium chloride buffer before cells were counted and plated at a final density of 5 × 106 cells/ml in neutrophil medium (DMEM containing 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, 1 mM sodium pyruvate, and 5 mM 2-ME). Cells were cultured with CpG (Coley Pharmaceuticals; 10 µg/ml), LPS (Sigma-Aldrich; 10 ng/ml), rIL-10 (eBioscience; 10 ng/ml), and rIL-27 (Amgen; 100 ng/ml), rIL-10 (eBioscience; 10 ng/ml), and rIL-27 (Amgen; 100 ng/ml), where indicated. After incubation for 2, 4, or 8 h RNA isolated from cells was used for real-time PCR as indicated below.

RNA isolation and real-time PCR

RNA was isolated from cultured cells using RNeasy Spin columns (Qiagen). RNA was reverse transcribed to cDNA using Superscript RT (Invitrogen). Quantitative real-time PCR was performed on cDNA with primer sets using SYBR Green chemistry (Qiagen). All reactions were run on an ABI 7500 Fast Real-time PCR System (Applied Biosystems) with β-actin as the endogenous control. Samples were normalized to β-actin and to experimental controls using the ΔΔCt method.

Statistics

Unless otherwise noted, groups of animals were compared using Student’s unpaired t test. p values <0.05 were considered significant (*).
Results

IL-27-IL-27R interactions regulate homeostasis of the TH17 cell pool in the intestine

IL-27 enhances IFN-γ production by activated CD4+ T cells, though under strongly polarizing TH1 conditions, it can suppress IFN-γ production (26, 35). IL-27 is also known to suppress IL-17A production in vitro and in models of CNS inflammation (37, 38, 42). However whether IL-27-IL-27R interactions can influence the homeostasis of CD4+ T cells in the intestine is unknown. To test whether IL-27 could influence production of proinflammatory cytokines by colonic CD4+ T cells, single cell suspensions were isolated from the mLN that drain the intestine and from the lamina propria of the colon (LPLs) of naive WT and IL-27R−/− mice and stained ex vivo for intracellular cytokines. The frequency of IFN-γ producing CD4+ T cells in the mLN and the colon of IL-27R−/− mice was reduced between 3- and 6-fold relative to WT C57BL/6 mice (Fig. 1, A and B). In contrast, frequencies of IL-17A-producing CD4+ T cells were increased between 2- and 3-fold in IL-27R−/− mice compared with WT controls (Fig. 1, C and D), leading to a significant increase in the ratio of TH17: TH1 cells in the colon and draining lymph node of IL-27R−/− mice relative to WT (Fig. 1E). These data suggest that IL-27-IL-27R interactions regulate the in vivo frequency of TH1 and TH17 cells in the intestinal microenvironment and GALT.

IL-27-IL-27R interactions govern expression of IFN-γ and IL-17A in a murine model of intestinal inflammation

Despite dysregulation of intestinal TH1 and TH17 cell homeostasis, IL-27R−/− mice do not develop spontaneous intestinal inflammation (data not shown), indicating that IL-27R-independent regulatory mechanisms exist under steady-state conditions. To determine whether altered frequencies of CD4+ T cells in the intestine influenced susceptibility to intestinal inflammation, we used a high-dose model of dextran sodium sulfate (DSS) model of colitis. High-dose DSS is a model of acute intestinal inflammation; DSS is toxic to intestinal epithelial cells and results in exposure of subepithelial immune cells to commensal bacteria, recruitment of additional innate and adaptive immune cells, over-production of proinflammatory cytokines, and development of focal ulcerations in the large intestine (43). WT and IL-27R−/− mice were exposed to high dose DSS in drinking water; 3 days later, cells were isolated from the mLN and the colon (IEL and LPLs) and the expression of proinflammatory cytokines was examined by flow cytometry. Consistent with ex vivo analysis of naive mice, mLN, IEL, and LPL cells isolated from DSS-treated IL-27R−/− mice contained significantly decreased frequencies of IFN-γ CD4+ T cells relative to WT (Fig. 2, A and B). The absolute number of IFN-γ+ CD4+ T cells in the mLN of DSS-treated IL-27R−/− mice was also significantly decreased relative to WT controls (Fig. 2C). In contrast, cells isolated from the mLN and colon of DSS-treated IL-27R−/− mice contained significantly higher frequencies of TH17 cells relative to WT mice (Fig. 2, D and E), and the absolute number of TH17 cells in the mLN was significantly increased relative to WT (Fig. 2F). When mLN cells from DSS-treated IL-27R−/− mice were polyclonally stimulated, they produced significantly increased amounts of IL-17A protein relative to WT controls (Fig. 2G). As a result of these differences, the ratio of TH17 to TH1 cells following DSS treatment was significantly increased in IL-27R−/− relative to DSS-exposed WT mice (Fig. 2H).

Associated with altered frequencies and numbers of colonic TH1 and TH17 cells, DSS-treated IL-27R−/− mice developed more rapid and severe intestinal inflammation compared with WT controls. Although DSS-treated WT mice began to lose weight between days 3 and 4, IL-27R−/− mice lost significantly more weight than WT controls as early as day 1 postexposure (Fig. 3A). In addition, diarrhea, intestinal bleeding, and weight loss were consistently elevated in DSS-treated IL-27R−/− mice relative to WT mice, leading to significantly increased morbidity at days 2 and 3 post-DSS (Fig. 3B). DSS-induced intestinal inflammation was also significantly increased at the spleen and mesenteric lymph nodes of IL-27R−/− mice relative to WT mice (Fig. 3C).

DSS-treated IL-27R−/− mice also developed significantly more severe inflammation in the large intestine (Fig. 3D). DSS-induced intestinal inflammation was significantly increased in the large intestine of DSS-treated IL-27R−/− mice relative to WT mice (Fig. 3E). These data suggest that IL-27-IL-27R interactions play an important role in regulating the development of intestinal inflammation and that these effects are mediated, at least in part, by the regulation of TH17 cell homeostasis.
inflammation was rapidly fatal in the absence of IL-27-IL-27R interactions, suggesting that these changes in disease index were biologically relevant. Although WT mice began to die 9 days following exposure to DSS, mortality of IL-27R−/− mice was >75% by day 9 post-DSS (Fig. 3C). Indeed, IL-27R−/− mice succumbed to inflammation beginning as early as day 2 of DSS treatment (Fig. 3C). Histological examination of the colons of DSS-treated IL-27R−/− mice as early as day 3 post-exposure revealed loss of crypt architecture, epithelial damage, and the presence of more numerous foci of inflammation in the lamina propria relative to WT mice (Fig. 3D). These results indicate that IL-27-IL-27R interactions are critical for the limitation of IL-17A expression and for prevention of clinical disease and associated mortality following acute intestinal damage.

**IL-27-IL-27R interactions control innate immune cell responses in the intestine**

Although CD4+ T cells play a role in the pathogenesis of DSS-induced colitis, innate immune cells can also contribute to disease (44). The rapid onset of DSS-induced colitis in IL-27R−/− mice suggested that in addition to influencing the intestinal CD4+ T cell pool, IL-27-IL-27R interactions might affect innate immune cell recruitment or function. Consistent with this hypothesis, flow cytometric analysis of lamina propria cells revealed increased frequencies of F4/80+CD11b+ macrophages and CD11b+Gr1+ neutrophils in the colons of DSS-treated IL-27R−/− mice relative to WT mice (Fig. 4). IL-27-IL-27R interactions could therefore, in addition to influencing CD4+ T cell responses, directly regulate innate immune cell function following DSS exposure. To test this, Rag−/− and Rag−/−/IL-27R−/− mice were exposed to 5% DSS. DSS-treated Rag−/− mice exhibited gradual weight loss and development of clinical disease (Fig. 5A). However Rag−/−/IL-27R−/− mice exhibited more rapid weight loss and elevated disease scores compared with DSS-treated Rag−/− mice (Fig. 5B). Consistent with this increased morbidity, exposure to DSS was fatal for 100% of Rag−/−/IL-27R−/− mice exposed to 5% DSS for 9 days, while no Rag−/− mice had died at this time point (Fig. 5C). Histologic examination of intestines from Rag−/−/IL-27R−/− mice at day 5 post-DSS treatment revealed increased crypt hyperplasia, epithelial cell loss, and more severe ulcerations of the colon compared with DSS-treated Rag−/− mice (Fig. 5D).

Flow cytometric analysis revealed a modest increase in the frequency of total CD11b+ monocytes (1.5-fold; 0.9 to 1.4%) and Gr1+ neutrophils (1.2-fold; 0.7 to 0.9%) in the lamina propria of Rag−/− mice following DSS exposure (Fig. 6A). However, the frequency of CD11b+ monocytes and CD11b+Gr1+ neutrophils present in the lamina propria of Rag−/−/IL-27R−/− mice was dramatically increased following DSS treatment, with >45-fold elevation in the frequency of CD11b+ cells (from 0.2 to 9.1%) and a 73-fold increase in CD11b+Gr1+ neutrophils (from 0.1 to 7.3%). On day 4, colonic explants from DSS-treated mice were cultured for 24 h and supernatants were assayed for expression of proinflammatory cytokines. Colonic explants from Rag−/−/IL-27R−/− mice exposed to DSS for 3 days produced almost 500-fold more IL-6 and 50-fold greater IL-17A per gram of tissue than those

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**FIGURE 3.** IL-27-IL-27R interactions are required to prevent rapid and severe disease in a model of acute intestinal inflammation. Daily weight loss (A) and pathology scores (B, see Materials and Methods for disease parameters) of WT (+/+ ) and IL-27R−/− (−/− ) mice exposed to high-dose DSS. Data are representative of four experiments, n = 3–4 mice per group. C, Kaplan-Meyer survival plot for WT (+/+ ) and IL-27R−/− (−/− ) mice exposed to 5% DSS. Data are pooled from five independent experiments (n = 18 for both WT and IL-27R−/−). Curves are significantly different by the logrank test; *, p < 0.05; ***, p < 0.001. D, H&E stained colonic sections from naive and 3 day DSS-exposed WT and IL-27R−/− mice. Bar = 100 μm.

**FIGURE 4.** IL-27-IL-27R interactions suppress accumulation of innate immune cells in a model of acute intestinal inflammation. Flow cytometry of lamina propria leukocytes isolated from 1 day high dose DSS exposed WT and IL-27R−/− mice. All plots are gated on live cells; numbers in plots represent the frequency of gated cells. Data are taken from one experiment of three showing similar results.
isolated from DSS-exposed Rag^{-/-} mice (Fig. 6B). Collectively, these data indicate that IL-27-IL-27R interactions, in addition to regulating the T_{H}1 and T_{H}17 cell pools, can limit innate cell recruitment and production of IL-6 and IL-17A, which together promote intestinal inflammation.

**rIL-27 inhibits production of proinflammatory cytokines by activated neutrophils**

The increased severity of colitis in Rag^{-/-}/IL-27R^{-/-} mice indicates that IL-27-IL-27R interactions can suppress intestinal inflammation independently of their effects on T cells. We therefore hypothesized that innate immune cells expressing the IL-27R could also be functionally regulated by IL-27-IL-27R interactions. To test this, peritoneal macrophages and neutrophils were stimulated with TLR ligands in the presence or absence of rIL-27 and cytokine expression analyzed by real-time PCR. To obtain macrophages, mice were treated with anti-Gr-1 mAb before harvesting PECs at 3 days postthioglycollate injection. PECs harvested from neutrophil-depleted mice were 45% F4/80^{+}CD11b^{+} macrophages and only 1–2% Gr-1^{+} (Fig. 7A).

**FIGURE 5.** IL-27-IL-27R interactions on innate immune cells suppress intestinal inflammation in high-dose DSS colitis. Daily weight loss (A) and disease score (B) of Rag^{-/-} and Rag^{-/-}/IL-27R^{-/-} mice exposed to high dose DSS. Data are representative of three experiments, n = 3 mice per group. C, Kaplan-Meyer survival plot for Rag^{-/-}/IL-27R^{-/-} and Rag^{-/-}/IL-27R^{-/-} mice exposed to DSS. Data are pooled from three independent experiments, n = 8 mice. Survival curves were analyzed by the logrank test. D, Large intestine sections were obtained from naive mice or 5 day DSS-exposed mice stained with H&E. Right panels are magnified from indicated areas. Bar = 100 μm. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

**FIGURE 6.** IL-27-IL-27R interactions on innate immune cells limit accumulation and proinflammatory cytokine production following high-dose DSS colitis. A, Flow cytometry of lamina propria leukocytes isolated from day 5 DSS-treated Rag^{-/-} and Rag/IL-27R^{-/-} mice. All plots are gated on live cells; numbers in plots represent the frequency of gated cells. Data are taken from one experiment of three showing similar results. B, Picograms/ml of cytokine produced during 24 h organ culture normalized per gram of organ weight for DSS-exposed mouse colon ± SD. Data represent one experiment with n = 3 mice per group. BLD, below the limit of detection. *, p < 0.05.

**FIGURE 7.** rIL-27 decreases production of proinflammatory cytokines by neutrophils responding to bacterial products. A, Flow cytometry plot of 3 day PECs from anti-Gr-1-treated mice. B and C, PECs were incubated in complete medium with LPS and cytokines where indicated and RNA extracted 4 h poststimulation. Bar graphs represent cytokine expression by real-time PCR relative to that of cells incubated in medium alone. ***, p < 0.001.
lowing exposure to LPS, PEC cultures depleted of Gr-1+ cells produced the proinflammatory cytokines IL-6 (Fig. 7B) and IL-12/IL-23p40 (Fig. 7C). Treatment of cultures with rIL-10 suppressed cytokine production, as previously reported (45). However, rIL-27 treatment of macrophage cultures did not significantly affect production of IL-6 or of IL-12/23p40 in response to LPS (Fig. 7, B and C).

To test whether IL-27 could modulate neutrophil function, early PECs were elicited with thioglycollate. Of these cells, 95% were CD11b+ myeloid cells, and 75% of CD11b+ cells coexpressed Gr1 (Fig. 7D). Stimulation of PECs with CpG led to significant increases in IL-6 (Fig. 7E) and IL-12/23p40 mRNA levels (Fig. 7F). Coincubation of cells with rIL-10 inhibited CpG-mediated transcription of these proinflammatory cytokines. Strikingly, coincubation of PECs with rIL-27 significantly inhibited IL-6 (Fig. 7E) and IL-12/IL-23p40 cytokine mRNA levels (Fig. 7F). These data provide the first indication that IL-27-IL-27R interactions, in addition to inhibiting accumulation of innate immune cells in vivo, can limit proinflammatory cytokine expression by TLR ligand-activated neutrophils.

Discussion

This study identifies a previously unrecognized role for IL-27-IL-27R interactions in controlling the balance of proinflammatory cytokine production by the intestinal CD4+ T cell pool under steady-state conditions. IL-27R−/− mice exhibited altered frequencies of CD4+ T cells; Th1 cell frequencies were decreased and Th17 frequencies increased relative to WT mice in both the mLN and the colon. Following exposure to DSS, these differences were maintained in IL-27R−/− mice. The accumulation of Th17 cells in the intestines of IL-27R−/− mice was associated with earlier onset of clinical disease, increased intestinal inflammation, and earlier death compared with WT mice. Our data also indicate that IL-27 suppresses intestinal inflammation in this model through its interaction with innate immune cells. Rag-/-/IL-27R−/− mice exposed to DSS exhibited increased disease severity and increased accumulation of neutrophils and monocytes relative to DSS-treated Rag-/- mice, and disease in these mice was accompanied by elevated intestinal IL-6 and IL-17A. In addition to limiting innate cell accumulation in the lamina propria, rIL-27 decreased proinflammatory cytokine production by neutrophils in vitro. Together, the effects of IL-27-IL-27R interaction on CD4+ T cells and neutrophils contribute to limit disease in a murine model of acute intestinal inflammation.

As IL-27 can directly induce T-bet expression by naïve CD4+ T cells (33), decreased steady-state frequencies of IFN-γ producing CD4+ T cells in the IL-27R−/− intestine are consistent with a defect in de novo Th1 cell differentiation. However, when cells from IL-27R−/− mice were polyclonally restimulated, IFN-γ protein levels were not significantly different between WT and IL-27R−/− (data not shown), which is in keeping with the inability of IL-27-IL-27R signaling to further enhance IFN-γ production in activated T cells (46). In contrast, naïve IL-27R−/− mice exhibit increased frequencies of Th17 cells in the GALT relative to WT mice. Increased frequencies of Th17 cells observed in the absence of IL-27-IL-27R interaction may represent increased differentiation, survival, or retention of these cells, which are constitutively present in the intestinal microenvironment (1). Because naïve IL-27R−/− mice do not exhibit elevated levels of Th17-promoting cytokines such as IL-6 or IL-23 (data not shown), it is likely that IL-27 acts directly on intestinal CD4+ T cells. Consistent with this, IL-27 has been shown in vitro and in inflammatory settings to suppress IL-17A production by activated CD4+ T cells (37, 38, 47). In addition, because IFN-γ can inhibit IL-17A production in vitro (48, 49), decreased IFN-γ production by CD4 T cells in the intestine of IL-27R−/− mice could contribute to increased Th17 frequencies.

Our IL-27R−/− mice lack WSX-1 but continue to express the gp130 receptor subunit. However, IL-27 has not been shown to signal through gp130 alone or through other receptors, and only IL-27 has been shown to signal through WSX-1. It therefore seems likely that the effects we observe are due to blockade of IL-27 signaling alone. IL-27 can signal in CD4+ T cells through both STAT1 and STAT3, and differential signaling may be responsible for the multiple modes of IL-27 action (46). Activation of STAT1 by IL-27 leads to T-bet expression and Th1 cell differentiation while suppressing Th17 cell differentiation (33, 37, 38). STAT1 and STAT3 are activated in models of colitis driven by Th1 and Th17 cells and in colon biopsies from IBD patients (50–52), suggesting that these pathways might be relevant to IL-27R signaling in the intestine following exposure to DSS, as well as in human IBD.

The close proximity of commensal flora and dietary Ag in the intestinal lumen makes precise control of cytokine production from intestinal CD4+ T cells essential. Commensal bacteria can induce Th1 and Th17 cytokine responses following experimental colonization, and the intestines of healthy humans contain commensal-responsive Th1 and Th17 cells (3, 4, 53, 54). In addition, our laboratory recently reported that IL-25 produced in response to commensal bacteria suppresses Th17 cell development by decreasing IL-23 levels (2). Our present study suggests that IL-27 production is another mechanism by which regulation of intestinal Th1 and Th17 cells that accumulate in response to commensal-dependent signaling may be achieved.

Because IL-27R−/− mice do not develop spontaneous colitis, alterations in Th17 cell populations in naïve mice are not sufficient to result in inflammation. The role of IL-27-IL-27R interactions has been examined in other models of colitis. Initially, Blumberg and colleagues (55) observed that mice lacking the EBI3 subunit of IL-27 developed clinical disease similar to that of WT mice following exposure to TNBS, which predominantly induces a Th1 response. In addition, we have shown that IL-10−/- mice, when crossed onto an IL-27−/− background, exhibit delayed onset of spontaneous colitis that correlates with decreased Th17 cell responses (56). We now report that IL-27-IL-27R interactions in the intestine contribute to limitation of severe injury following high-dose DSS treatment.

IFN-γ production from CD4+ T cells is reduced following high-dose DSS treatment, yet this decrease in Th1 cells is not sufficient to prevent acute intestinal inflammation, as IL-27R−/− mice are more susceptible to this model of colitis. Yoshida and colleagues (57) found that IL-27R−/− mice exhibited reduced pathology following long-term treatment with a low dose of DSS, and we have also observed reduced symptoms of disease in IL-27R−/− mice following exposure to low-dose DSS (data not shown). Low-dose DSS is a model of chronic ulcerative colitis with low mortality. Yoshida et al. (57) cited reduced systemic production of proinflammatory cytokines, including IFN-γ, as central to the resistance of IL-27R−/− mice to this disease model. IL-17A was not measured in IL-27R−/− mice exposed to low-dose DSS, but treatment of WT mice with neutralizing Ab to IL-17A renders them more susceptible, suggesting that IL-17A may play a protective role in low-dose DSS colitis. This could occur through the known ability of IL-17A to promote expression of proteins that increase the barrier function of intestinal epithelial cells (58, 59). The model of high-dose, acute DSS colitis we use in the present study causes severe intestinal injury and is rapidly fatal. In this inflammatory setting, our data specifically support a role for IL-27 in suppression of Th17 cell-associated inflammation following severe damage to the intestinal epithelium. The ability of IL-27 to suppress cytokine
production is known, in strongly inflammatory environments, to supercede its ability to enhance T<sub>TH1</sub> cell responses (35). Taken together, these results indicate that IL-27 can promote or suppress colitis depending on the etiology, severity, and attendant cytokine environment associated with a particular inflammatory event. In addition, our data are in keeping with evidence that colitis can occur through the action of multiple cell types and signaling pathways. This experimental evidence includes the differential requirement for MyD88-dependent TLR signaling in development of spontaneous colitis, as MyD88 is required for colitis in IL-10<sup>−/−</sup> but not IL-2<sup>−/−</sup> mice (60).

These data also identify a role for IL-27-IL-27R interaction in early recruitment or expansion of innate immune cells in the lamina propria following DSS exposure. IL-27-IL-27R interactions influence cytokine production by the cell populations responding to intestinal damage. For example, while IL-17A levels were below the limit of detection in DSS-treated Rag<sup>−/−</sup> mice, IL-17A protein was detectable in intestinal organ cultures from DSS-treated IL-27R<sup>−/−</sup>/Rag<sup>−/−</sup> mice. FACS staining of Rag<sup>−/−</sup> and IL-27R<sup>−/−</sup>/Rag<sup>−/−</sup> mice exposed to DSS revealed that the dominant source of IL-17A was not a CD11b<sup>+</sup> myeloid cell (data not shown). O’Shea and colleagues (61) have reported that lymphoid tissue inducer (LTi) cells, which do not express most lineage markers, are capable of producing IL-17A in response to zymosan stimulation through the IL-23-IL-17A axis (19, 44, 63, 64). Our data now illustrate an additional level of regulation of these innate immune cell populations through IL-27-IL-27R interaction.

Taken together, our results indicate that IL-27-IL-27R interactions can, through their effects on innate and adaptive immunity, promote T<sub>TH1</sub> cytokines in the intestine while aiding in suppression of T<sub>TH17</sub> cell responses and fatal inflammation following intestinal injury. IL-27 is a potential candidate for treatment of chronic inflammatory diseases, including IBD. Its ability to both promote and suppress intestinal cytokine responses suggests, however, that specific inhibition of signaling pathways will be required to harness the anti-inflammatory properties of IL-27.

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Disclosures
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