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The Role of Dendritic Cells in the Development of Acute Dextran Sulfate Sodium Colitis

Bradford E. Berndt,* Min Zhang,* Gwo-Hsiao Chen,† Gary B. Huffnagle,‡ and John Y. Kao2*†

Dendritic cells (DCs) are essential mediators of the host immune response to surrounding microbes. In this study, we investigate the role of DCs in the pathogenesis of a widely used colitis model, dextran sulfate sodium-induced colitis. The effect of dextran sulfate sodium on the production of proinflammatory cytokines and chemokines by bone marrow-derived DCs (BM-DCs) was analyzed. BM-DCs were adoptively transferred into C57BL/6 mice or DCs were ablated using transgenic CD11c-DTR/GFP mice before treatment with 5% dextran sulfate sodium in drinking water. We found that dextran sulfate sodium induced production of proinflammatory cytokines (IL-12 and TNF-α) and chemokines (KC, MIP-1α, MIP-2, and MCP-1) by DCs. Adoptive transfer of BM-DCs exacerbated dextran sulfate sodium colitis while ablation of DCs attenuated the colitis. We conclude that DCs are critical in the development of acute dextran sulfate sodium colitis and may serve a key role in immune balance of the gut mucosa.

Dendritic cells (DCs) are critical in maintaining immune balance between tolerance and rejection through their role in both the adaptive immune response as professional APCs, and in the innate response via direct contributions to the local cytokine milieu. DCs have been shown to release proinflammatory cytokines and chemokines through activation of TLRs, which induce local polymorphonuclear neutrophil (PMN) infiltration and activate other innate immune cells. Thus, DCs play a key role in mounting a local inflammatory response. In the gut, dysregulation of the immune response to commensal microorganisms may lead to low-grade immune activation resulting in inflammation of the intestine. The recently confirmed association of Crohn’s disease with mutations in the pathogen recognition molecule, Nod2, suggests that DCs may be directly involved in this immune dysregulation. Moreover, DCs isolated from the lamina propria of colitic tissue have been shown to express higher levels of CD40 than those isolated from healthy tissue. Although these studies suggest that DCs play a role in the induction of colitis, their role in the inflammatory cascade leading to intestinal inflammation has not been firmly established.

In experimental colitis involving the use of dextran sulfate sodium (DSS) as the inducing agent, susceptible mice treated with DSS in their drinking water develop colitis within 7 days. Acute DSS colitis is characterized by neutrophilic infiltration and the development of ulcerations and bloody diarrhea. Because this model appears to be independent of a T cell response, as colitis can be induced in mice with severe combined immunodeficiency lacking T and B cells, it is an appropriate tool to study the innate mechanisms of colitis. The mechanism of inflammation is believed to involve the direct toxic effect of DSS on colonic epithelium leading to the direct activation of intestinal macrophages by DSS. However, the role of DCs in the induction of DSS colitis has not been directly demonstrated.

It has been difficult to study the role of DCs in vivo due to the lack of DC-null mice. Jung et al. generated a transgenic (Tg) mouse in which CD11cΔDCs are transiently ablated without affecting peripheral macrophages or B cells. The transgene renders CD11cΔDCs susceptible to diphtheria toxin (DT) via the expression of CD11c promoter-controlled DT receptor (DTR) gene, which mice normally lack. This model may help us gain new insight into the roles of DCs in mucosal immunity. Other investigators have used this model to further the understanding of DC involvement in Mycobacterium tuberculosis (16) and HSV-type 1 infections (17).

In this study, we examined the involvement of DCs in the innate immune response during induction of acute DSS colitis. We found that direct stimulation of bone marrow-derived DCs (BM-DCs) with DSS increases the release of several cytokines and chemokines including those specific for neutrophil recruitment. Adoptive transfer of BM-DCs led to a worsening of DSS colitis while ablation of CD11cΔDCs significantly reduced the severity of the disease. We speculate that activation of DCs is the prerequisite for the induction of acute colitis; hence strategies to target DCs may be effective in the treatment of acute intestinal inflammation.

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§Abbreviations used in this paper: DC, dendritic cell; PMN, polymorphic neutrophil; DSS, dextran sulfate sodium; DT, diphtheria toxin; BM, bone marrow; WT, wild type; CM, complete medium; PMB, polymyxin B; KO, knockout; Tg, transgenic.

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Materials and Methods

Mice

Specific pathogen-free female C57BL/6 mice aged 8 wk, hemizygous B6.FVB-Tg(IAgk-DTR/EGFP)57Lan/J (Tg-CD11c-DTR/GFP) knockout (KO) mice backcrossed to C57BL/6 mice for at least five generations, and wild-type (WT) C57BL/6 littermates aged 8 wk were purchased from The Jackson Laboratory and maintained by the Unit for Laboratory Animal Medicine at the University of Michigan Health System. Experiments were conducted on mice between the ages of 10 and 14 wk. The University of Michigan Committee on Use and Care of Animals approved all animal experiments.

Media, cytokines, and neutralizing Abs

Complete medium (CM) consisted of RPMI 1640 medium with 10% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The recombiant cytokines mouse GM-CSF (10 ng/ml) and mouse IL-4 (10 ng/ml; R&D Systems) were diluted in CM. Neutrophils were depleted by i.p. injection of 0.25 mg of anti-Gr1 (RB6-8C5) (18). IL-12 was neutralized by i.p. injection of 0.25 mg of anti-IL-12 (C17.15) (19).

Generation of BM-derived DCs

Erythrocyte-depleted murine BM cells were cultured in CM with 10 ng/ml GM-CSF and 10 ng/ml IL-4 at 10^6 cells/ml as previously described (20). On day 6, nonadherent BM-DCs were harvested by vigorous pipetting and enriched by gradient centrifugation using OptiPrep density solution (Sigma-Aldrich) according to the manufacturer's instructions. The low-density interface containing DCs was collected by gentle aspiration. The recovered BM-DCs were washed twice with RPMI 1640 and cultured in CM with GM-CSF (10 ng/ml) and IL-4 (10 ng/ml).

Animal studies

In adoptive transfer experiments, mice were injected with 10^6 BM-DCs. To induce colitis, the mice received 5% (w/v) DSS (m.w. 36,000–50,000; ICN Biomedicals) in their drinking water ad libitum for 7 days (21). Weight measurements and Hemoccult (Beckman Coulter) fecal occult blood test measurements were performed daily. The mice were sacrificed on day 7 and the entire colon was exposed by inverting the tissue and thrice washed in PBS followed by LPS restimulation. TNF-α after DSS stimulation by washing DCs poststimulation with PBS followed by LPS followed by PBS was examined by ELISA. The concentration of TNF-α was measured.

Flow cytometric analysis

BM-DCs were washed twice with ice-cold PBS containing 0.5% BSA and sodium azide. After a 30-min incubation with Fc Block (1 μg/100 μl; BD Biosciences/BD Pharamingen), the cells were incubated with FITC, PE, or PerCP-conjugated Abs or isotype control Abs (1/100 dilution). These cells were washed, resuspended in ice-cold 2% paraformaldehyde, and analyzed using a Coulter XL Flow Cytometer. Dot plots and histograms were obtained using FlowJo version 4.3 (Tree Star).

Measurement of CD11c+ DCs in mouse spleen and mesenteric lymph nodes

Single-cell suspensions of mouse spleen and mesenteric lymph nodes were obtained by digesting minced tissue using 1 mg/ml collagenase A (Roche) and 800 μg/ml DNase I (Sigma-Aldrich) in 5% FCS RPMI 1640 for 45 min. Cells were then passed through a 40-μm nylon strainer (BD Biosciences) and washed with RPMI 1640. To measure DC numbers, total leukocytes from mouse spleen and mesenteric lymph nodes were stained with PerCP-conjugated CD3 and CD19 Abs and PE-conjugated CD11c Abs. The CD3/CD19-negative population was gated and the numbers of CD11c+ and GFP+ DCs were calculated. All conjugated Abs and their isotype controls were purchased from BD Biosciences/BD Pharamingen.

Determination of DC frequency in mouse colon

Whole colon prep of mice was adopted from a method previously described by Niess et al. (24). Briefly, the luminal surface of the entire colon was exposed by inverting the tissue and thrice washed in PBS then incubated on a shaker in 1 mM DTT for 10 min, 30 mM EDTA for 10 min, and then digested with 10 mg/ml collagenase A (Roche) and 800 μg/ml DNase I (Sigma-Aldrich) for 40 min at 37°C. The single cells released from the colonic mucosa were then filtered through a 40-μm strainer and were stained with rat PE-conjugated anti-mouse CD11c or isotype controls (BD Biosciences/BD Pharamingen) and analyzed by FACS analysis.

Blood cell counts

Mouse tail blood was collected and placed in microtainer tubes containing EDTA (BD Biosciences Vacutainer Systems). RBC were lysed with water for 20 s. Concentrations of leukocytes were measured in a hemocytometer. The concentration of neutrophils was determined by differential counting on cytopsin slides.

Statistical analysis

Statistical significance was determined by unpaired Student t test and survival analysis using commercially available software (PRISM; GraphPad). Values of p < 0.05 were considered significant. All data are presented as mean ± SEM.

Results

DSS induces production of chemokines and inflammatory cytokines by BM-DCs

To determine whether DCs contribute to the development of DSS colitis, we analyzed the response of BM-DCs to DSS stimulation. Surface markers of day 6 BM-DCs suggested a semimature phenotype: CD11c^high, MHC class II^high, CD54^high, CD40^int, CD80^high, and CD86^high (Fig. 1A). Because commercial chemicals are occasionally contaminated with LPS, we first checked for the presence of contaminating LPS in DSS using a purpald-based colormetric test kit (CHEMetrics). We found that 0.1% DSS solution contained ~80 ng/ml LPS. To assess the ability of DSS to stimulate BM-DCs independent of LPS in vitro, polymyxin B (PMB; Sigma-Aldrich) was used to neutralize LPS. We first showed that PMB was effective in neutralizing E. coli LPS (Sigma-Aldrich; 100 ng/ml) by measuring the IL-12 release by LPS-stimulated BM-DCs and the optimal concentration appeared to be 10 U/ml (Fig. 1B). Next, the efficacy of PMB to neutralize contaminating LPS in DSS was assessed by stimulating BM-DCs with 0.1% DSS in the presence of PMB. We found only a slight reduction of IL-12 release by PMB by PMB, suggesting DSS could directly activate DCs independent of LPS (Fig. 1C). The effects of contaminating LPS were adequately neutralized using 10 U/ml PMB, and this dose was used in subsequent in vitro experiments.

Because acute DSS colitis is characterized by a predominant PMN infiltration (25–27), we measured the levels of several chemotactants that are responsible for leukocyte recruitment, including KC, MIP-2, MCP-1, and MIP-1α (28), along with the inflammatory cytokines TNF-α and IL-12. BM-DCs produced increasing levels of IL-12 when stimulated by DSS (Fig. 1D). DSS stimulation induced BM-DC release of the neutrophil chemokines KC and MIP-2, the leukocyte attractant MCP-1, and TNF-α following DSS stimulation (Fig. 1E). A modest increase in MIP-1α production was also observed. No detectable level of IL-4, IL-10, or IFN-γ produce by DCs was found with DSS stimulation (data not shown).

A possible explanation for the increase in DC proinflammatory cytokine/chemokine production was toxic injury caused by DSS on DCs, resulting in the release of intracellular cytokines. To investigate this further, we assessed the viability of DCs after DSS stimulation by washing DCs poststimulation with PBS followed by LPS restimulation. TNF-α secretion, a marker of cell viability, was assessed with LPS restimulation. We found that DSS-treated DCs were viable morphologically and were capable of producing high levels of TNF-α following LPS...
restimulation (Fig. 1F). Therefore, our data showed no evidence of DSS-induced toxicity on DCs and the mechanism of stimulation may involve phagocytosis of DSS as reported in DSS stimulation of macrophage (12). Thus, direct DSS stimulation of BM-DCs induces production of inflammatory mediators that are the hallmarks of DSS colitis.
Adoptive transfer of BM-DCs worsens acute DSS colitis

Because we observed that DSS stimulates production of neutrophil chemoattractants and Th1 cytokines, we determined whether increased numbers of DCs in vivo would exacerbate DSS colitis. We first demonstrated that adoptively transferred CFSE-labeled BM-DCs migrated to inflamed colonic tissue (Fig. 2A). Next, we compared the bleeding scores between the two treatment groups of mice and found mice adoptively transferred with BM-DCs developed rectal bleeding earlier than mice receiving PBS alone (Fig. 2B), the significance of the early bleeding was unclear since the overall bleeding scores over a 7-day period was not statistically different. This may be due to the high dose of DSS used which caused significant rectal bleeding in both treatment groups. Although we found no significant differences in weight loss between the two treatment groups (Fig. 2C), a trend of increased mortality was observed in the DC adoptive transfer group (Fig. 2D).

Quantitative histological analysis of adoptive transfer mice based on leukocyte infiltration, crypt destruction, edema, and hemorrhage revealed evidence of a significant increase in disease severity (31.00 ± 2.11) compared with controls (19.36 ± 0.95) (Fig. 2, E and F). Because these data suggest that an increase in the number of DCs is associated with more severe colitis, the frequency of DCs residing in the colon should increase following transfer of

FIGURE 2. Adoptive transfer of BM-DCs worsens acute DSS colitis. To confirm transferred BM-DCs migrate to inflamed colonic tissue, mice were injected with either CFSE-stained BM-DCs or saline control and treated with 5% DSS for 3 days. A, Colon sections stained with 4',6'-diamidino-2-phenylindole (DAPI) to visualize cell nuclei were analyzed for the presence of fluorescein-positive (green) cellular infiltrate. Next, mice were injected with PBS (n = 11) or BM-DCs (n = 10) and were treated with DSS for 7 days. B, Blood scores, C, body weight, and D, mortality were recorded daily. E, Micrographs of H&E paraffin sections (swiss-roll cut) of mouse colon were taken at ×40 (left panels) and the inset at ×100 magnification. F, histological scores were determined based on the colitis index (22). Adoptive transfer of BM-DCs resulted in a significant increase in colitis severity. Data shown are representative of three independent experiments (DC Txf, adoptive transfer). G, Determination of DC frequency in mouse colon. Single cells released from whole colon digest of mice (n = 2/group) were stained with PE-conjugated CD11c or isotype control Abs and analyzed by FACS. Representative CD11c vs forward scatter dot plots gated on the side-scatter high population are shown. CD11c+ region (box with percent of gated cells) indicate an increase in DC frequency in colon of DC Txf mice compared with PBS control mice.
BM-DCs. We performed whole colon digest to determine the frequency of colonic DCs with DSS stimulation and found that there is an increase in the frequency of CD11c<sup>+</sup> colonic DCs in DC<sup>tf</sup> mice compared with the frequency of colonic DCs in PBS mice (Fig. 2G). These data further indicate greater numbers of DCs may cause increased susceptibility to DSS colitis. Thus, adoptive transfer of DCs leads to a worsening of DSS colitis, supporting a proinflammatory role for DCs in acute experimental colitis.

Treatment with DT effectively ablates CD11c<sup>+</sup> DCs in the spleen, mesenteric lymph nodes, and colonic mucosa of Tg-CD11c-DTR/GFP mice

To further examine the role of DCs in DSS colitis, we used Tg-CD11c-DTR/GFP (Tg) mice to selectively ablate DCs in vivo (15). This Tg construct takes advantage of the fact that mice normally lack the gene that codes for DTRs. The CD11c<sup>+</sup> cells that express DTRs in this Tg mouse are thus sensitive to DT, which allows selective ablation on DT injection. Although low-to-intermediate expression of CD11c has been reported in macrophages, detailed analyses of the effect of DT on immune cells have revealed that the toxin selectively ablates DCs but not F4/80<sup>+</sup> macrophages (15, 29). To confirm whether DT treatment in Tg mice depleted DCs, we compared the number of DCs in spleen, mesenteric lymph nodes, and colonic tissue of DSS-treated Tg mice injected with DT vs PBS. The numbers of DCs in the spleen and mesenteric lymph nodes were significantly reduced in the DT-treated Tg mice (Fig. 3, A and B). Of note, percent totals of T cells and B cells were similar in both groups and appeared to be unaffected by DT treatment (upper panels, Fig. 3, A and B). Because the transgene also

FIGURE 3. Treatment with DT effectively ablates CD11c<sup>+</sup> DCs in the spleen, mesenteric lymph nodes, and colonic mucosa of Tg-CD11c-DTR/GFP mice. A and B. Tg-CD11c-DTR/GFP-DTR (Tg) mice were injected with DT or PBS (n = 2/group) and treated with DSS for 7 days. Single-cell suspensions of splenocytes and mesenteric lymph nodes were stained with PerCP-conjugated CD3/CD19 and PE-conjugated CD11c for FACS analysis. The CD<sup>3</sup>/CD19<sup>+</sup> population (non-T, non-B) was gated and the CD11c<sup>+</sup>/GFP<sup>+</sup> population of DT-injected Tg mice was compared with PBS-injected Tg mice (numbers indicated on plots represent percent of gated cells). DT treatment induced a 67% reduction in CD11c<sup>+</sup> population in both the spleen and lymph nodes. Of note, total numbers of T and B cells did not decrease after DT injection. Data shown are representative of one of three separate experiments. C. Ablation of DCs in colonic tissue. Tg-CD11c-GFP-DTR mice (n = 3) were treated with DSS for 5 days to induce colitis. Mice were then injected with DT and sacrificed 24 h postinjection. DC involvement in inflamed gut mucosa was demonstrated by the presence of GFP-positive using fluorescent microscopy. Cell nuclei were visualized with DAPI stain. CD11c-GFP<sup>+</sup> (green) cells were observed in areas of inflammatory infiltrate (arrows), but not in DT-treated Tg mice. Representative H&E micrographs were also shown (lower panels). Data shown are representative of one of three separate experiments (mLN, mesenteric lymph node).
expresses GFP, we determined the presence of DCs in colonic tissue by detecting GFP\(^+/\)H11001 cells with fluorescent microscopy. GFP\(^+/\)H11001 DCs were detected in DSS-induced inflammatory infiltrates in colonic tissue of PBS-injected Tg mice (left panel, Fig. 3C), whereas GFP\(^+/\)H11001 cells were absent from similar inflammatory infiltrates in DT-treated Tg mice (right panel, Fig. 3C). These findings indicate that DT successfully reduced the number of CD11c\(^+/\)H11001 DCs, both in peripheral immune compartments and at the site of colonic inflammation.

**Treatment of Tg mice with DT did not deplete neutrophils**

A potential confounding factor in this model is the possibility that DT may affect PMN function, as PMNs express low-to-intermediate levels of CD11c (30). To determine whether DT treatment depleted PMN numbers, we compared the peripheral blood neutrophil counts of both groups. The diseased portions of intestine from both groups showed the presence of PMNs (Fig. 4, A and B). Moreover, equal proportions of PMNs were observed in peripheral smears of both groups, suggesting that DT injection did not deplete neutrophil numbers (Fig. 4C). Thus, this model offers a unique opportunity to examine the specific role of DCs in the development of colitis.

**Ablation of CD11c-expressing DCs attenuated the severity of DSS colitis**

Because increasing DC numbers led to a worsening of DSS colitis (Fig. 2), ablation of DCs before DSS administration should attenuate colitis. We observed a significantly reduced incidence of rectal bleeding in Tg mice compared with DT-treated WT littermates as the DSS administration progressed (Fig. 5A), but no significant difference in weight loss (Fig. 5B). Differences in disease severity were confirmed by histological examination (Fig. 5, C and D), which showed that Tg mice had a significantly lower colitis index (7.00 ± 1.03) than the controls (20.14 ± 1.12). Of note, two DT-injected Tg mice died without signs of colitis. This has been reported with repetitive DT injection in these mice (15). These results indicate that DCs are critical in the development of acute colitis.

**Interventions targeting events downstream of DC activation did not significantly impact the development of DSS colitis**

Anticytokine therapies are currently being used in the management of chronic inflammatory conditions. We speculate that DC targeting therapy may be more effective than blockade of downstream events. We interrupted PMN recruitment by blocking Gr1, or blocking IL-12 signaling using neutralizing Ab against IL-12 and found no significant reduction of colitis score compared with PBS control (Fig. 6). Thus, anti-DC therapy appeared to be more effective in controlling inflammation than targeting further downstream events.

**Discussion**

DCs have been implicated as the critical APCs able to sample luminal microbes and orchestrate appropriate host response to maintain mucosal homeostasis (31). Mutations in their sensing mechanisms (e.g., NOD2) is associated with inflammatory bowel disease (6–8). The role of DCs in the development of colitis has been inferred by several studies (32–35); however, because there is no currently available CD11c KO mouse model, the involvement of DCs remains speculative. In this study, the role of DCs were shown by adoptive transfer of BM-DCs and transient ablation of...
DCs in vivo. But first, we showed that, in vitro, DSS-stimulated BM-DCs produced more proinflammatory cytokines and chemotactants than PBS controls (Fig. 1). Adoptive transfer of BM-DCs led to more severe colitis (Fig. 2) and ablation of DCs significantly attenuated the severity of the disease (Fig. 5). We speculate that during DSS-induced gut injury, DCs are critical in the regulation of mucosal inflammation.

Our study is the first to provide direct evidence that DCs are involved in the development of acute DSS colitis. The DT-Tg mouse model allows for selective ablation of CD11c<sup>+</sup> cells, most of which are DCs (15). Although low-to-intermediate expression of CD11c has been reported in neutrophils and some macrophages, detailed analyses of how DT affects immune cells have revealed that the toxin selectively ablates DCs, but not T cells, B cells, or F4/80<sup>+</sup> macrophages (15, 29). Concerns have been raised by the evidence that DT treatment in Tg mice leads to the depletion of two small subsets of macrophages, marginal zone macrophages, and metallophilic macrophages, leaving the dominant proportion of macrophages unaffected (29). Recognizing that the effect of DT in the Tg mice is largely due to the reduction of CD11c<sup>+</sup> DCs, and given the lack of a CD11c-null mouse model, this Tg strategy represents the best system currently available for studying the role of DCs in vivo.

Our results support the findings of previous studies demonstrating the possible involvement of DCs in experimental colitis. Depleting both macrophages and DCs ameliorated IL-10 KO colitis (32), but exacerbated DSS colitis (33) inferring that these phagocytic cells are involved in various models of colitis; however, the individual roles each cell plays during the initiation of inflammation were not elucidated. In a T cell transfer model of colitis, increased numbers of CD134L-expressing DCs were observed in mesenteric lymph nodes, and anti-CD134L treatment blocked colitis development, suggesting that DCs may be involved (34). Moreover, in the Stat3-deficient colitis model, increased numbers of CD11c<sup>+</sup> DCs were observed in inflamed tissue and were found to produce the proinflammatory Th1 cytokine, IL-12 (35). Our data showed that adoptive transfer of DCs worsened the severity of DSS colitis, further indicating a critical role for DCs in the development of colonic inflammation.

Our data indicate that BM-DCs are able to produce chemokines and Th1 cytokines when stimulated by DSS. The importance of chemokine and Th1 cytokine production in colitis has been recognized (36, 37) and our data indicate that DCs are important contributors of these factors. MIP-2 (38, 39), along with KC (40), serve as potent neutrophil attractants. Increased MIP-2 secretion has been associated with exacerbated DSS colitis both in an adi- ponectin KO model (41), and when epithelial cells were engineered to secrete MIP-2 (27). Our data indicate that in response to DSS, DCs directly contribute to the distinctive neutrophil involvement of this model of colitis (25). Moreover, DSS-activated DCs also produced Th1 cytokines and other attractants that may further exacerbate gut inflammation. We found that DCs produced MIP-1α in vitro, which has been shown to mobilize DC precursors into circulation (42), thereby possibly increasing the pool of DCs available to participate in inflammatory processes. DSS-activated DCs also appear to secrete MCP-1, a macrophage attractant (43), which also has been shown to be involved with DSS colitis (44). We speculate that DCs also significantly contribute to the IL-12 and TNF-α environment that leads to Th1-mediated chronic colitis (36).

The current paradigm of the pathogenic mechanism of DSS colitis involves two processes. First, DSS has a direct toxic effect on colonic epithelium leading to a leaky tight junction and bacterial translocation (45, 46). Then, DSS, along with the translocated bacteria, activate resident macrophages, which recruit neutrophils, a predominant leukocyte found during acute DSS colitis (11, 12). Recent evidence, however, questions the role of bacteria as a direct stimulator of the inflammatory response during DSS treatment. Blockade of bacterial stimulation, either by TLR-4 KO, antibiotic decontamination, or a germfree environment, resulted in worsening of colitis (46–48). This implicates that DSS colitis does not require the presence of luminal bacteria or the activation of APCs from bacterial translocation to cause injury.

Considering the results of our studies and of others (33), we propose a different paradigm for the pathogenic mechanism of DSS colitis, in which DSS first injures colonic epithelium and subsequently activates DCs leading to production of chemokines and proinflammatory cytokines. We speculate that DCs act upstream of macrophages in this inflammatory process, because ablation of DCs during the development of acute DSS colitis attenuates the disease. These data suggest that direct injury to the colonic mucosa by DSS, along with the presence of luminal bacteria and intestinal macrophages, were not sufficient to trigger a significant inflammatory response. Thus, we speculate that DCs are required to mount an acute innate inflammatory cascade leading to the pathogenesis of DSS colitis. Others have also suggested that DCs, rather than macrophages, can serve as initiators of the innate immune response. Smythies et al. (49) demonstrated that human intestinal macrophages do not express innate response receptors and do not produce proinflammatory cytokines in response to an array of inflammatory stimuli. Pertinent to DSS colitis, Shintani et al. (50) showed that DSS-pulsed macrophages produced undetectable levels of TNF-α, IL-1α, and IL-6. Of note, it has been shown that simultaneous ablation of DCs and macrophages led to a worsening of DSS colitis (33). Combining this data with our finding that DC ablation protected against the development of DSS colitis, one can speculate that intestinal macrophages serve a suppressive role distinct from the apparent inflammatory role of DCs in this model.

Our data also suggest that targeting DCs may offer therapeutic benefits during acute inflammation. We showed that targeting downstream events, such as blockade of PMN recruitment or neutralizing IL-12, led to no significant reduction of colitis severity (Fig. 6). As there is room for improvement with current anticytokine therapies, strategies to target activated DCs during acute exacerbation of diseases, such as chronic colitis, may benefit patients in addition to blockade of neutrophil recruitment or proinflammatory cytokines.

In summary, our study provides direct evidence that DCs are critical mediators of the innate immune response during acute DSS colitis. The results offer new insights into the pathogenic mechanisms of this commonly studied colitis model.

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Disclosures
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