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Expression of Blimp-1 in Dendritic Cells Modulates the Innate Inflammatory Response in Dextran Sodium Sulfate–Induced Colitis

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A single nucleotide polymorphism of *PRDM1*, the gene encoding Blimp-1, is strongly associated with inflammatory bowel disease. Here, we demonstrate that Blimp-1 in CD103$^+$ dendritic cells (DCs) critically contributes to the regulation of macrophage homeostasis in the colon. Dextran sodium sulfate (DSS)-exposed Blimp-1^{cko} mice with a deletion of Blimp-1 in CD103$^+$ DCs and CD11c^{hi} macrophages exhibited severe inflammatory symptoms, pronounced weight loss, high mortality, robust infiltration of neutrophils in epithelial regions of the colon, an increased expression of proinflammatory cytokines and a significant decrease in CD103$^+$ DCs in the colon compared with DSS exposed wild-type (WT) mice. Purified colonic macrophages from Blimp-1^{cko} mice expressed increased levels of matrix metalloproteinase 8, 9 and 12 mRNA. WT macrophages cocultured with colonic DCs but not bone marrow–derived DCs from Blimp-1^{cko} produced increased matrix metalloproteinases in an interleukin (IL)-1β– and IL-6–dependent manner. Treatment of Blimp-1^{cko} mice with anti–IL-1β and anti–IL-6 abrogated the exaggerated clinical response. Overall, these data demonstrate that Blimp-1 expression in DCs can alter an innate inflammatory response by modulating the activation of myeloid cells. This is a novel mechanism of contribution of Blimp-1 for the pathogenesis of inflammatory bowel diseases, implicating another therapeutic target for the development of inflammatory bowel disease.

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INTRODUCTION

Inflammatory bowel disease (IBD) has a complex pathogenesis dependent on several factors, including genetic susceptibility of the host, commensal bacteria and the host immune system (1). Genome-wide association studies (GWASs) have significantly advanced our understanding of the genetic contribution to IBD, and metaanalyses of GWASs have established >150 susceptibility loci (2–4). These studies confirmed pathways already identified and also discovered previously unappreciated pathways, raising novel hypotheses about disease pathogenesis (5). However, for most GWAS loci, functional alterations underlying disease susceptibility remain unidentified.

The importance of intestinal microbial flora in disease development in experimental models is being increasingly appreciated. Microbiome-dependent disease development was noted in studies of rodents raised in germ-free conditions or various specific pathogen-free conditions (6,7). Furthermore, different microbiomes can lead to different disease pathogenesis in mouse models of IBD, emphasizing the key role of commensal bacteria in IBD pathogenesis (8). The gut microbiome affects homeostasis or activation of immune cells in the intestine through engagement of pattern recognition receptors, and abnormal expression or activation of pattern recognition receptors can lead to an inflammatory response in the intestine (9–12). Pattern recognition receptor activation by pathogenic gut mi-

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crobes often leads to the expression of proinflammatory cytokines, which drive intestinal inflammation (13,14).

Tissue-resident dendritic cells (DCs) and macrophages are key players in controlling intestinal immune responses (15). The complexity of intestinal macrophage and DC subsets is increasingly appreciated; however, the contribution of these populations to intestinal inflammatory conditions is still unclear (16). In addition to the traditional DC markers CD11c and CD103, DCs can be further subdivided into Batf3-dependent $CD11b^{lo}$ DCs and Batf3-independent $CD11b^{+}$ DCs (17). Recently, an additional population of Batf3-independent $CD11c^{+}CD11b^{+}$ DCs that lack CD103 and CD64 but express chemokine (C-X3-C motif) receptor 1 (CX3CR1) has also been described (18,19). $CD103^{-}CX3CR1^{int}$ DCs are reported to have an immunogenic phenotype (20,21) and to induce the differentiation of TH17 cells (18). Tissue-resident nonmigratory macrophages include a $CD11c^{lo}$ and a $CD11c^{+}$ population, both of which express high levels of F4/80, CD64 and the CX3CR1 chemokine receptor. In the steady state, intestinal DCs and macrophages have been shown to contribute to gut homeostasis through production of interleukin (IL)-10 and induction of regulatory T cells (22,23).

DCs showed the strongest alterations in gene expression among other immune cells, suggesting a role of genetic alterations in IBD (4). *PRDM1*, the gene encoding B lymphocyte–induced maturation protein-1 (Blimp-1), has been demonstrated in GWASs to have IBD susceptibility single nucleotide polymorphisms (SNPs). Moreover, a recent exome sequencing study identified *PRDM1* rare variants that are associated with IBD (24).

In this study, we investigated a pathologic function of Blimp-1 in DCs by using a CD11c-driven Blimp-1 knockout ($Blimp\text{-}1^{cko}$). $Blimp\text{-}1^{cko}$ mice exhibited an exacerbated phenotype with a high mortality after dextran sodium sulfate (DSS)-induced colitis. After DSS exposure, colonic $Blimp\text{-}1^{ko}$ DCs exhibited an increased production of IL-1β and IL-6, and colonic macrophages exhibited a higher expression of matrix metalloproteinases (MMPs). Increased expression of IL-1β and IL-6 by $Blimp\text{-}1^{ko}$ DCs was responsible for the MMP induction in macrophages. Blockade of both IL-1β and IL-6 during DSS exposure mitigated the exacerbated IBD phenotype and mortality in $Blimp\text{-}1^{cko}$ mice. Finally, blockade of MMP by an MMP inhibitor reduced colitis development, supporting the hypothesis that increased expression of MMP is responsible for the exacerbated colitis in $DCBlimp\text{-}1^{ko}$ mice.

MATERIAL AND METHODS

Human Samples Preparation and *In Vitro* Differentiation of Monocyte-Derived DCs

Healthy *PRDM1* IBD SNP rs6911490 risk allele carriers and nonrisk allele–carrying controls were identified from the genotype and phenotype (GaP) registry at The Feinstein Institute for Medical Research (Manhasset, NY, USA). Fresh blood was collected and total peripheral blood mononuclear cells were purified by gradient centrifugation with Ficoll-Paque (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) at 260*g* for 20 min without break. Peripheral blood mononuclear cells were collected from the middle layer and washed with Hanks balanced salt solution for three times. $CD14^{+}$ monocytes were purified with an EasySep Kit (Stem Cell Technologies, Vancouver, Canada), and the purity of $CD14^{+}$ cells was confirmed by flow cytometry. Purified monocytes were cultured in RPMI medium with 10% fetal bovine serum, penicillin-streptomycin, L-glutamine, 10^{6} U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peprotech, Rocky Hill, NJ, USA) and 200 U/mL IL-4 (Peprotech) for 7 d to obtain monocyte-derived DCs (Mo-DCs). These studies were performed according to an institutional review board (IRB)-approved protocol.

Mice and Cell Lines

$Blimp\text{-}1^{cko}$ mice on a BALB/C background for over 10 generations were bred in The Feinstein Institute for Medical Research animal facility and wild-type (WT) BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). To generate CX3CR1-GFP on $Blimp\text{-}1^{cko}$ background, $Blimp\text{-}1^{cko}$ mice were bred with CX3CR1-GFP (The Jackson Laboratory). All mice strains were maintained in a specific pathogen-free facility at The Feinstein Institute for Medical Research.

Ethics

All the experiments conducted in this study followed the guidelines in the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (25). The protocol was approved by the committee on the Ethics of Animal Welfare of The Feinstein Institute for Medical Research (protocol number 2009-048). For the survival study, we monitored mice twice a day throughout the experiments to measure weight and monitor development of sickness. We euthanized mice when the weight loss reached >25% of the original weight by CO_2. Detailed experimental design and protocol can be found in Supplementary Data.

DSS-Induced Colitis and Preparation of Intestinal Cell Suspensions

The 7- to 10-wk-old mice were administered 4% DSS (36,000–50,000 molecular weight; MP Biomedical LLC, Solon, OH, USA) in drinking water for 7 consecutive days. After 7 d, DSS water was replaced with fresh water to allow the mice to recover. To measure colitis, mice were weighed and stool was collected every other day; the stool and rectum of the mice were also examined for blood. For inhibition assays, anti–IL-1β and IL-6 (100 μg each) together or separately (eBioscience, San Diego, CA, USA), anti–tumor necrosis factor (TNF)α (100 μg) (eBioscience), doxycycline (50 μg) (Sigma-Aldrich, St. Louis, MO, USA) or control IgG (100 μg) were administered by intraperitoneal injection on d 0, 3 and 5 of DSS treatment. To calculate disease activity index (DAI), colitogenic phenotype was investigated and scored: weight loss

(0: 0% loss, 1: 1–5% loss, 2: 5–10% loss, 3: 10–20% loss, and 4: >20% loss from baseline); stool consistency (0: normal, 2: pasty/semiformed stool that did not adhere to the anus, and 4: liquid); and bleeding (0: negative hemoccult test, 2: positive hemoccult test, and 4: gross bleeding); DAI = scores of (weight loss + stool consistency + bleeding)/3.

On d 3 or 7 of DSS treatment, mice were sacrificed and their mesenteric lymph nodes (mLNs), small intestines and colons were removed. Colon length and weight was measured. Peyer patches were removed from the intestine. Intestines were cut open longitudinally along their entire length, cleaned and washed by vortexing three times with complete medium (2% fetal bovine serum in Hanks balanced salt solution), and incubated in complete medium containing 1 mmol/L DL-dithiothreitol solution (Sigma-Aldrich) for 20 min while shaking at 37°C and then incubated in 5 mmol/L ethylenediaminetetraacetic acid (Sigma-Aldrich) in complete medium for 50 min while shaking at 37°C. Afterward, the intestines were washed once with complete medium and incubated in 0.3 mg/mL collagenase (C5138; Sigma-Aldrich) in complete medium for 45 min at 37°C.

Antibodies and Flow Cytometry Analysis of Colon Myeloid Subsets

Anti-mouse F4/80-FITC, CD103-PE, CD11b-PE-Cy7, CD11c-APC, CD45-APC-Cy7, MHCII-PB, GR-1-PE-Cy5 and Siglec-H-efluor450 were all purchased from eBioscience. Anti-mouse CD4-FITC was purchased from BD Biosciences (San Jose, CA, USA).

The colonic, small intestine and mLN cells were stained and analyzed by flow cytometry (LSRII; BD Biosciences) or sorted on a cell sorter (FACSAria; BD Biosciences) to look at different populations of myeloid cells as well as isolate cells for RNA extraction and quantitative polymerase chain reaction (qPCR).

TH17 Cell Differentiation

A 48-well plate was coated with 5 μg/mL anti-CD3ε antibody (145-2C11). The next day, total $CD4^+$ T cells ($CD45^+TCR\beta^+CD4^+$) were purified from colon and incubated under TH17 differentiation conditions; 2 μg/mL anti-CD28 (37.51), 10 μg/mL anti-CD4, 10 μg/mL anti–IL-2, 10 μg/mL anti–interferon (IFN)-γ, 5 ng/mL transforming growth factor (TGF)-β and 20 ng/mL IL-6. All the antibodies were purchased from BD Biosciences and recombinant cytokines were purchased from Peprotech. On d 4, cells were restimulated with 100 ng/mL phorbol myristate acetate, 1 μg/mL ionomycin and 20 μg/mL brefeldin A for 6 h.

Coculture Assays

DCs were prepared either from bone marrow–derived DCs (BM-DCs) differentiated with Flt3L (Peprotech) or purified colonic DCs and stimulated with lipopolysaccharide (LPS) (100 ng/mL) or muramyl dipeptide (MDP) (100 ng/mL) for 24 h. After stimulation, DCs were washed thoroughly and cocultured with macrophages at a 1:5 (DC:macrophage) ratio for 3 d. After coculture, macrophages were purified by using F4/80 staining. A total of 10 μg/mL anti–IL-1β and anti–IL-6 or control IgG were used for the cytokine-blocking experiments.

Gene Expression Analysis

Total RNA was extracted by an RNeasy kit (Qiagen, Valencia, CA, USA), and total RNA was quantified by a Nanodrop™ spectrophotometer (Thermo Scientific [Thermo Fisher Scientific Inc., Waltham, MA, USA]). cDNA was synthesized with iScript™ (Bio-Rad, Hercules, CA, USA). Amplification was performed with a LightCycler 480 (Roche, Indianapolis, IN, USA). Gene-specific primers were purchased from TaqMan gene expression assay (Invitrogen [Thermo Fisher Scientific]), and relative expression of each gene was quantified by using HPRT or Polr2a for normalization.

Cytokine Measurements

Cytokines secreted from either human Mo-DCs or mouse colonic DCs were measured by multiplex panel designed and purchased from Meso Scale Discovery (MSD, Rockville, MD, USA) or by enzyme-linked assay (ELISA) (BD Biosciences).

Western Blotting

To measure secreted MMP protein, colon macrophages were purified by cell sorter and 2×10^5 cells were cultured in total 200 μL DC-conditioned medium for 72 h. Supernatant was collected and macrophage cell lysate was prepared in 40 μL RIPA buffer with complete protease inhibitor (Roche). Antibodies for MMP-12 (ab25897) and MMP-8 (ab81286) (Abcam, Cambridge, MA, USA) was diluted in 0.5% milk in Tris-buffered saline with 0.05% Tween 20 (TBST) and applied to the membrane for overnight rocking at 4°C. The next day, the membrane was washed with TBST three times, and horseradish peroxidase–conjugated goat anti-rabbit antibody was applied. Chemiluminescent light was exposed to X-ray film and developed.

Statistical Analysis

One-way analysis of variance was applied to determine statistics, and p values <0.05 were considered significant.

All supplementary materials are available online at www.molmed.org.

RESULTS

Low Blimp-1 Expression and Increased Proinflammatory Cytokine Expression in Mo-DCs from *PRDM1* IBD SNP Carriers

PRDM1 has been identified as a gene contributing to colitis pathogenesis by GWASs (3,24). To understand the function of the SNP associated with Blimp-1 in DCs, Mo-DCs were generated from the SNP rs6911490 risk allele (TT) carriers and nonrisk allele (CC) controls. All individuals had no evidence of autoimmune disease and therefore gene expression in Mo-DCs was not altered by disease or by immunosuppressive therapy. Blimp-1 expression was significantly lower in Mo-DCs from risk allele carriers compared with control individuals (Figure 1A). When Mo-DCs were stimulated

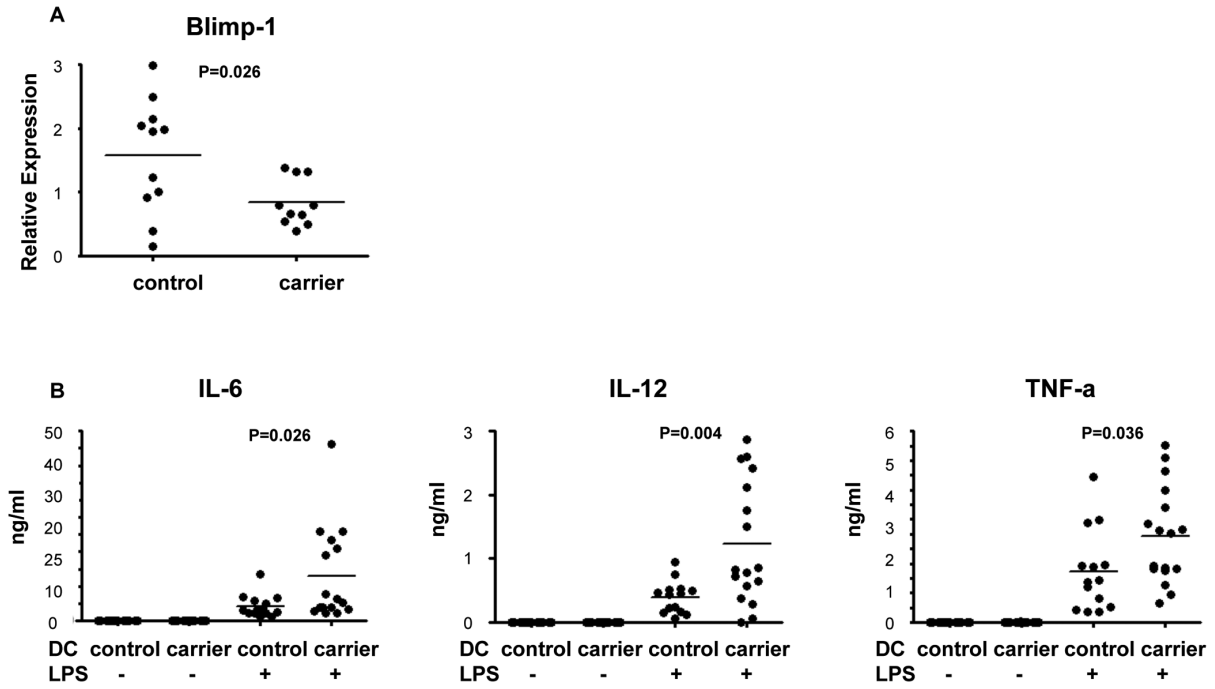

Figure 1. Mo-DCs derived from *PRDM1* IBD risk allele carrier have low levels of Blimp-1 and increased proinflammatory cytokine expression. (A) Blimp-1 expression was measured by qPCR of Mo-DCs differentiated from monocytes carrying CC nonrisk (control) or TT risk (carrier) of rs6911490. Each dot represents an individual person and the bar represents the mean (n = 7). (B) Mo-DCs differentiated from either control or carrier monocytes as indicated in (A) were incubated with or without 1 μg/mL LPS for 18 h. After stimulation, supernatant was harvested and the levels of cytokines were measured with a human 9-flex multiplex cytokine kit from MSD. Each dot represents an individual person and the bar represents the mean (n = 7).

with LPS, production of the proinflammatory cytokines IL-6, IL-12 and TNFα was higher in *PRDM1* IBD carrier than in controls (Figure 1B). We did not identify significant differences in induction of other cytokines, such as IL-10, IFNγ, IL-1β and IL-8 (data not shown).

Increased Susceptibility of Blimp-1^{cko} Mice to DSS-Induced Acute Colitis

The development of SLE-like phenotypes of Blimp-1^{cko} mice on a C57BL/6 background, obtained by mating CD11c-cre mice to floxed Blimp-1 mice, has been described previously (26). Because *PRDM1* IBD risk allele carriers exhibit a low level of Blimp-1 and a proinflammatory phenotype in Mo-DCs, we decided to investigate whether a Blimp-1 deficiency in DCs might alter innate immune homeostasis in the intestine. We examined Blimp-1 deletion in DC subsets and macrophages in the colon of BALB/c mice. The majority of macrophages (CD103-F4/80$^+$CD64$^+$) expressed moderate levels of CD11c, and about 10% of macrophages were CD11c^{hi} with a mean fluorescence intensity equivalent to DCs (Figure 2A). We isolated CD103$^+$ DCs, CD11c^{hi} macrophages and CD11c^{low} macrophages and measured Blimp-1 expression. Although there was no change in the level of Blimp-1 mRNA in total macrophages (CD11c$^+$ and CD11c$^-$), CD11c^{hi} macrophages isolated from Blimp-1^{cko} mice showed significantly reduced levels of Blimp-1 (Figure 2B). The lack of reduction of Blimp-1 expression in the whole macrophage population is likely due to the relatively small proportion of macrophages that are CD11c^{hi}. In contrast, DCs (CD103$^+$CD64$^-$) showed a strong decrease in the level of Blimp-1 mRNA. Thus, Blimp-1 deletion occurred in CD103$^+$ DC and CD11c^{hi} macrophages in these mice. We also determined the expression of Blimp-1 in CD103$^-$CD11b$^+$CX3CR1^{int}CD64$^-$ DCs, a recently identified intestinal DC subset (18). The level of Blimp-1 expression was low in this DC subset (~10-fold less compared with CD103$^+$CX3CR1$^-$ DCs), and no significant difference was observed between WT and Blimp-1^{cko} mice (Figure 2C).

We compared the inflammatory response to DSS-induced colitis in Blimp-1^{cko} and WT mice. WT mice showed a significant but mild response to DSS and fully recovered. Surprisingly, administration of the same concentration of DSS to Blimp-1^{cko} mice led to a faster onset and more severe inflammatory response with high mortality on both BALB/c background (Figure 3A) and C57BL/6 background (data not shown). We decided to use Blimp-1^{cko} on BALB/c for further studies. Pathology of colon samples from both WT and Blimp-1^{cko} mice was compared on d 3 and 7 of DSS treatment. At the early time point (d 3), there

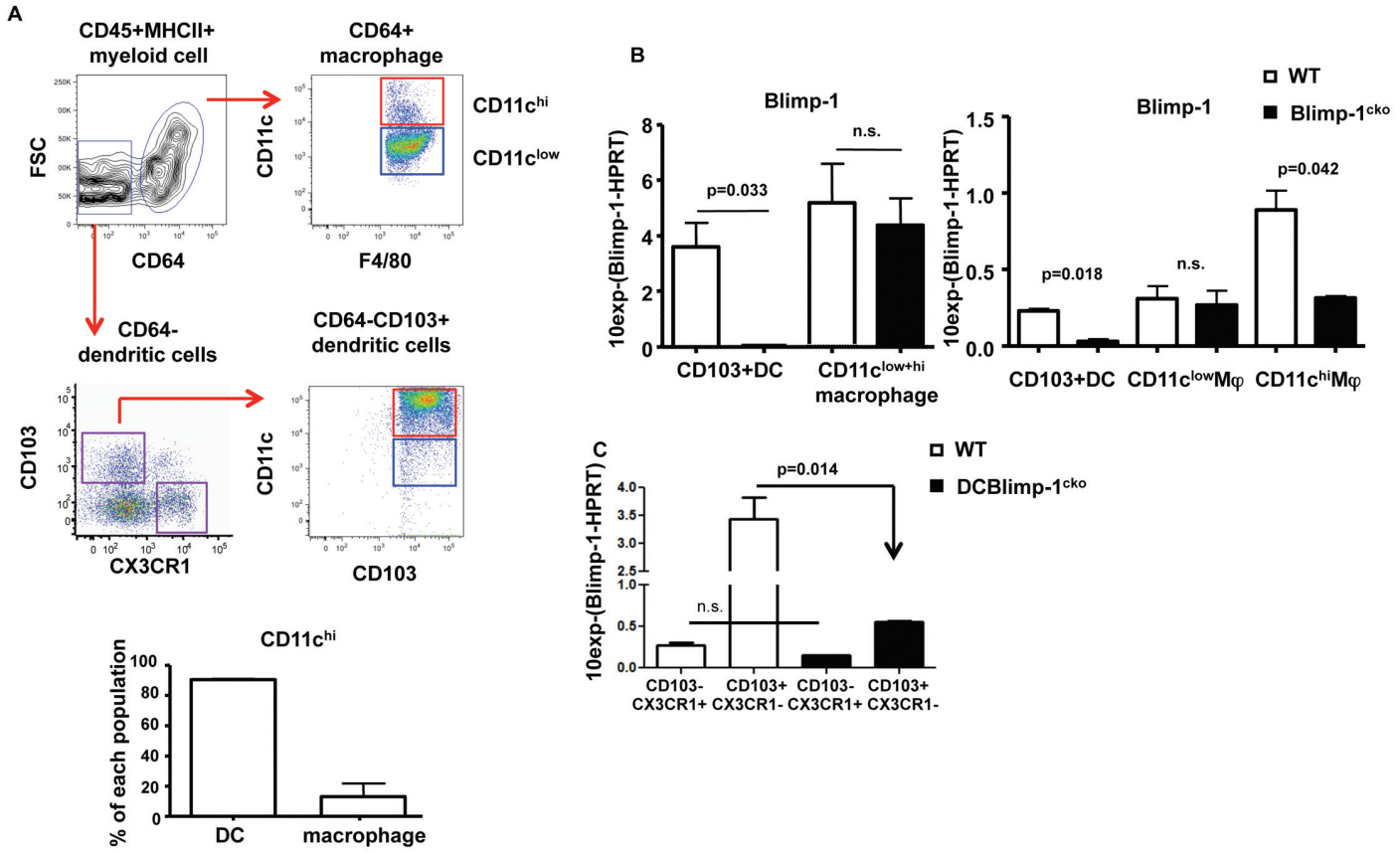

Figure 2. Blimp-1 expression in DCs and macrophages. (A) Flow cytometry strategy of identifying colonic DC and macrophages. Graph represents percentage of CD11c^{hi} cells in DCs and macrophages. Mean ± standard error of the mean (SE) are shown (n = 5). (B) DCs and total CD64^{+}F4/80^{+} macrophages (including CD11c^{int} and CD11c^{hi}) (left graph) and CD11c^{low} or CD11c^{hi} macrophages (right) were sorted as depicted in (A), and the level of Blimp-1 was quantified by qPCR. Mean ± SE are shown (n = 3). (C) Blimp-1 expression was measured by qPCR in CD103^{+} and CD103^{-} colonic DCs from WT and Blimp-1^{cko} mice. The graph presents the mean ± SE of three independent experiments.

was no clear difference observed in the colon pathology (data not shown). In accordance with the greater weight loss and mortality, colons harvested at d 7 of DSS treatment of Blimp-1^{cko} mice exhibited highly inflamed phenotypes (Figure 3B), increased weight with a shorter length of colon and an increased histology score, compared with WT mice (Figure 3C). There were significant architectural changes including severe crypt damage in the colons from Blimp-1^{cko} mice. In summary, these observations demonstrate that there is an exacerbated IBD pathology in mice with an absence of Blimp-1 expression in both CD103^{+} DCs and a small population of CD11c^{hi} macrophages.

DSS-Induced Mortality Is Not due to Bacterial Spreading or a Different Microbial Community

Appropriate induction of IgA and IgM in mucosal lymphoid tissue is critical for controlling bacterial spreading, and defects can lead to a high mortality in response to DSS administration, as demonstrated in a MyD88^{ko} mouse (27). Therefore, we compared fecal immunoglobulin (Ig) levels and commensal bacterial spreading to peripheral organs in WT and Blimp-1^{cko} mice. There was no significant difference in the level of IgG, IgM or IgA in fecal samples on d 7 of DSS administration (data not shown). In addition to the normal immunoglobulin level, no bacteria were detected in peripheral tissues, such as liver, mLNs and blood of either strain (data not shown). We also wanted to investigate whether a difference in the commensal bacterial community between WT and Blimp-1^{cko} mice might account for the difference in colitis severity. It is known that the bacterial community can affect the development of colitis in this model (8). Fecal pellets were collected from individual WT and Blimp-1^{cko} mice, and the composition of the bacterial community was analyzed by 16s RNA sequencing. There was no significant difference between WT and Blimp-1^{cko} mice. Both groups of mice exhibit one dominant genus, *Clostridium* (~80% of classified bacteria), followed by others, *Corprococ-*

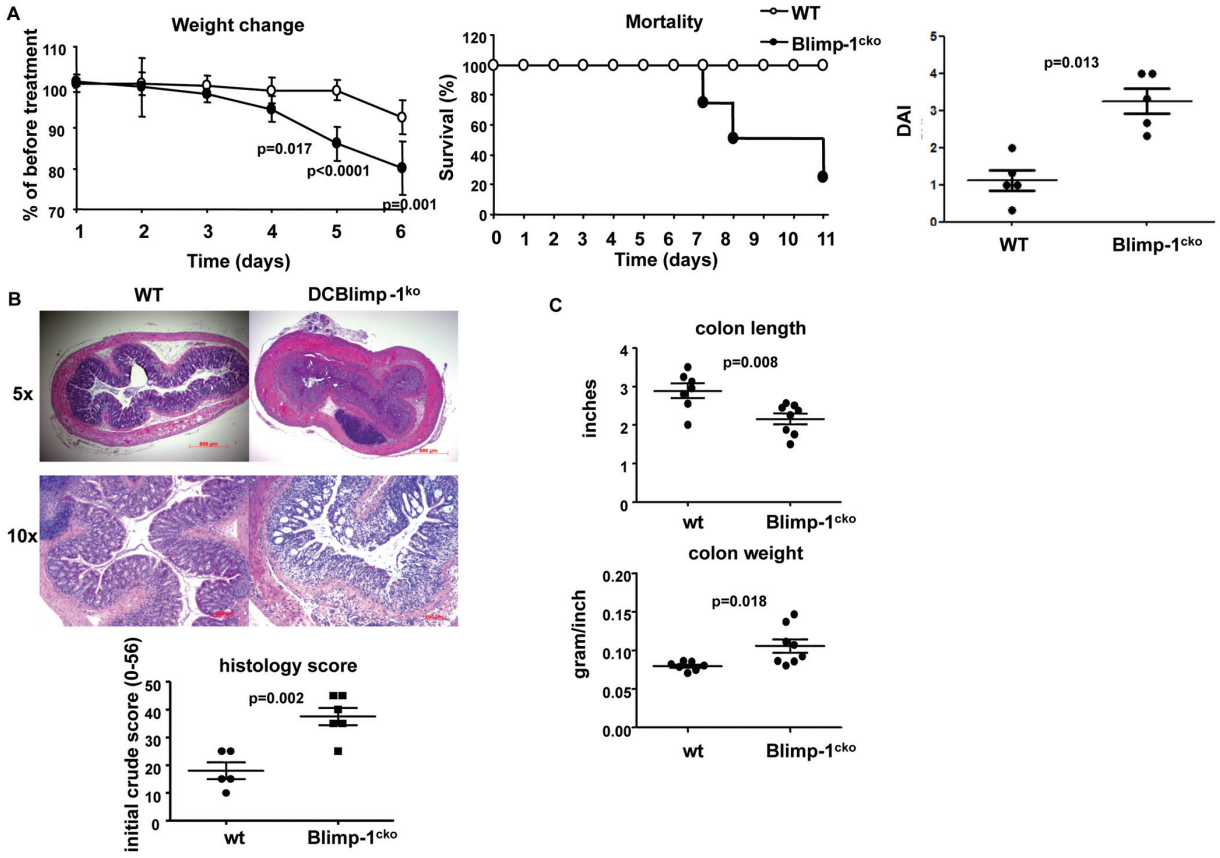

Figure 3. Increased pathological phenotypes by DSS-induced colitis in Blimp-1^{cko} mice. (A) Weight change and mortality was monitored during DSS treatment and calculated based on d 0. DAI was determined at d 7 of DSS treatment. Mean ± SE are shown (n = 4). (B) Histology of colon from d 7 DSS-treated WT and Blimp-1^{cko} mice. The distal colon was removed and fixed with 4% PFA. Hematoxylin and eosin (H&E) staining was performed, and sections were imaged with a Xeiss camera at 5× and 10× magnification. Histology score was evaluated based on the level of damage in tissue and infiltration of mononuclear cells. (C) Colon length and weight were measured from DSS-treated WT or Blimp-1^{cko} mice. Each dot represents an individual mouse and the bar represents the mean ± SE (n = 5).

cus and *Dehalobacterium* (~5 and 3% each). These data suggest that DSS-induced mortality in DCBlimp-1^{ko} mice is due to increased inflammation in the intestine and irreversible tissue damage rather than systemic bacteremia or a difference in the composition of commensal bacteria.

Increased Expression of Inflammatory Cytokines in Blimp-1^{cko} Mice during DSS Colitis

It is well known that an aberrant production of proinflammatory cytokines plays a critical role in the pathogenesis of IBD (28,29). There were very low levels of expression of proinflammatory cytokines in entire colons and in isolated myeloid cells (CD45^{+}MHCII^{+}CD11c^{+}and CD11b^{+}) from both WT and Blimp-1^{cko} mice before DSS administration. However, DSS exposure significantly induced proinflammatory cytokine expression, especially IL-1β, IL-6 and IL-23, in purified colonic myeloid cells with a significantly greater expression in myeloid cells from Blimp-1^{cko} mice (Figure 4A). We also observed increased expression of IL-6 and IL-23 in myeloid cells from the small intestine of Blimp-1^{cko} mice (data not shown). The increased IL-23 expression in myeloid cells suggested that the increased pathology might be due to an increase in TH17 cells in Blimp-1^{cko} mice. However, there was no difference in the percent of TH17 cells in the colon between WT and Blimp-1^{cko} mice before or after DSS treatment (Figure 4B), ruling out the contribution of TH17-mediated pathogenesis in this model. We also measured IL-10, a cytokine known to have an important role in immune homeostasis in the intestine, but there were no differences in expression between WT and Blimp-1^{cko} mice (Figure 4A).

To identify which myeloid cell population was responsible for the increased proinflammatory cytokine expression, CD103^{+} colonic DCs and macrophages were isolated on d 3 of DSS treatment and cytokine expression was measured. The difference between WT and Blimp-1^{cko} mice was attributable to DCs rather than macrophages (Figure 4C).

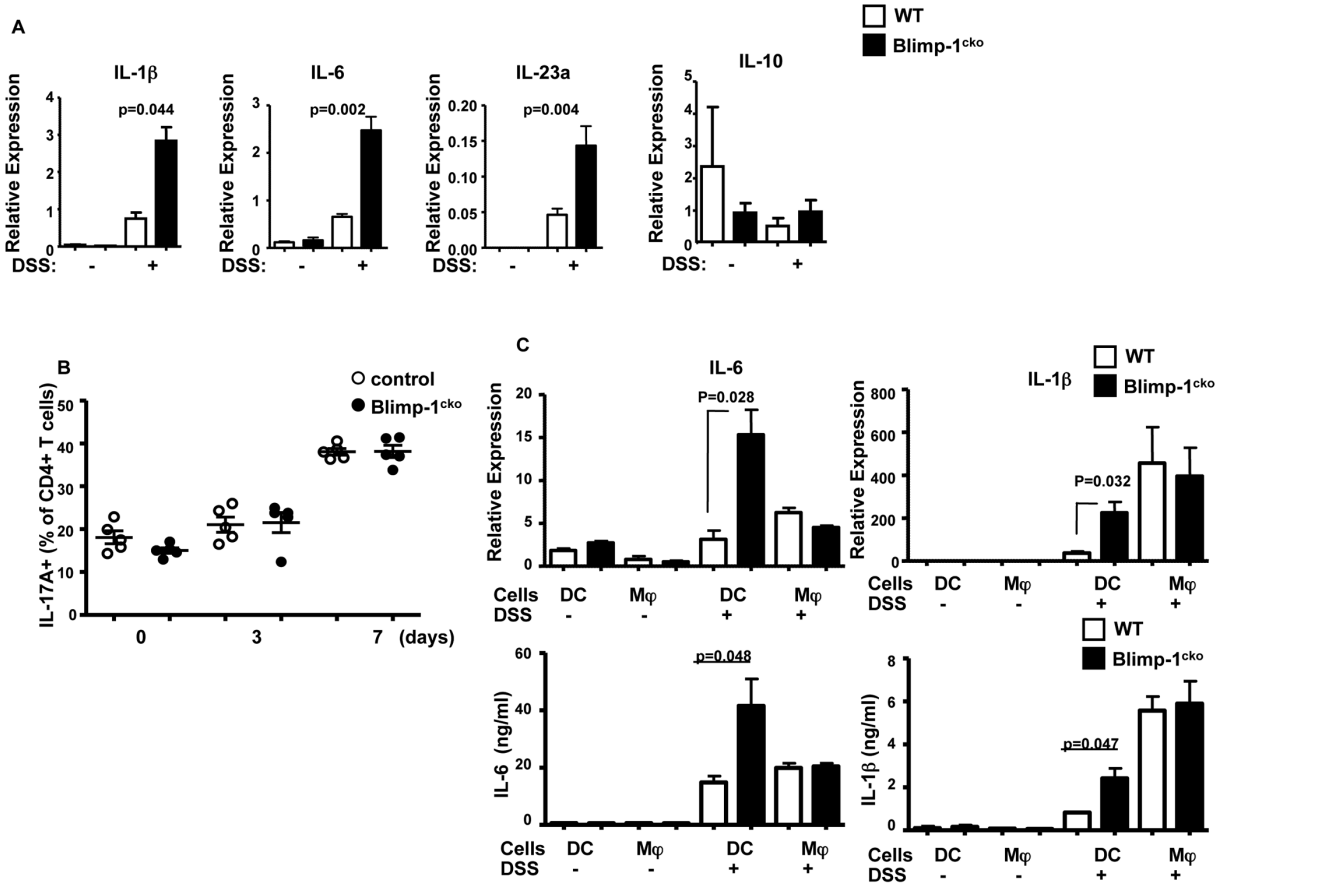

Figure 4. Increased expression of proinflammatory cytokines in colonic DCs from Blimp-1^{cko} mice. (A) Pro-/antiinflammatory cytokines were measured in total myeloid cells in the colon. Each bar represents the mean ± SE (n = 4). (B) CD4$^+$ T cells were purified from the colon of d 0, 3, and 7 DSS-treated WT and Blimp-1^{cko} mice and cultured in TH17 differentiation culture media for 4 d. The percentage of IL-17A–positive cells was calculated from CD4$^+$ T cells (mean ± SE) (n = 5). (C) DCs and Mφ (macrophages) isolated from the colon with or without DSS treatment in WT and Blimp-1^{cko} mice. Cytokine expression was measured by qPCR (upper panels) and by ELISA (lower panels). Each bar represents the mean ± SE (n = 4).

Alterations in Myeloid Cell Populations in Blimp-1^{cko} Mice during DSS-Induced Colitis

An increased expression of proinflammatory cytokines can be due either to their increased expression at the single cell level or to the expansion of cytokine-producing DCs in Blimp-1^{cko} mice. The frequency of DCs and macrophages was calculated as the number of DCs or macrophages normalized to the number of total myeloid cells. No significant difference was observed in either the basal DC or macrophage populations between WT mice and Blimp-1^{cko} mice (data not shown). However, at d 3 of DSS exposure, there was a decrease in DCs and an increase in macrophages in the colon (Supplementary Figure S1A) and an increase in macrophages in mLNs (Supplementary Figure S1B) of Blimp-1^{cko} mice compared with WT mice. This difference was no longer observed at d 7 of DSS treatment (Supplementary Figure S1C). Thus, alteration in infiltrating cells was present only at an early time point of DSS treatment and was an intestine-restricted phenotype. The increased percentage of macrophages might be due to recruitment from the periphery or proliferation of colonic macrophages in response to inflammatory factors presumably derived from DCs.

Together with an expansion of macrophages, we observed a strong infiltration of polymorphonuclear neutrophils (CD11b^{hi}Ly-6G$^+$) in the colon of Blimp-1^{cko} mice compared with WT mice (Figure 5). This infiltration was significant on d 3, consistent with increased inflammation at that early time point.

Increased Expression of MMPs by Macrophages in the Colon

To understand the enhanced inflammation and severe mortality in Blimp-1^{cko} mice, we asked whether there might be enhanced tissue destruction by the expanded macrophage population. Macrophages are known to be pathogenic in the intestine through secretion of various tissue-damaging enzymes, including MMPs

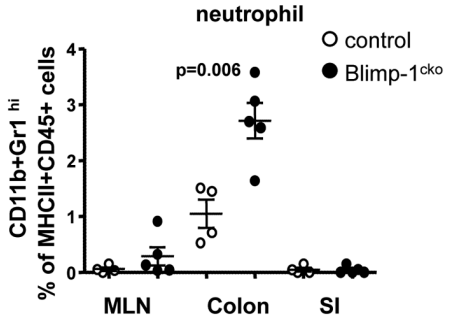

Figure 5. Increased influx of neutrophils into the colon in DSS-treated Blimp-1^{cko} mice. Frequency of neutrophils was calculated within CD45^{+} MHCII^{hi} cells in mLN, colon and small intestine (SI) from d 3 DSS-treated WT or Blimp-1^{cko} mice. Each dot represents an individual mouse and the bar represents the mean ± SE (n = 4).

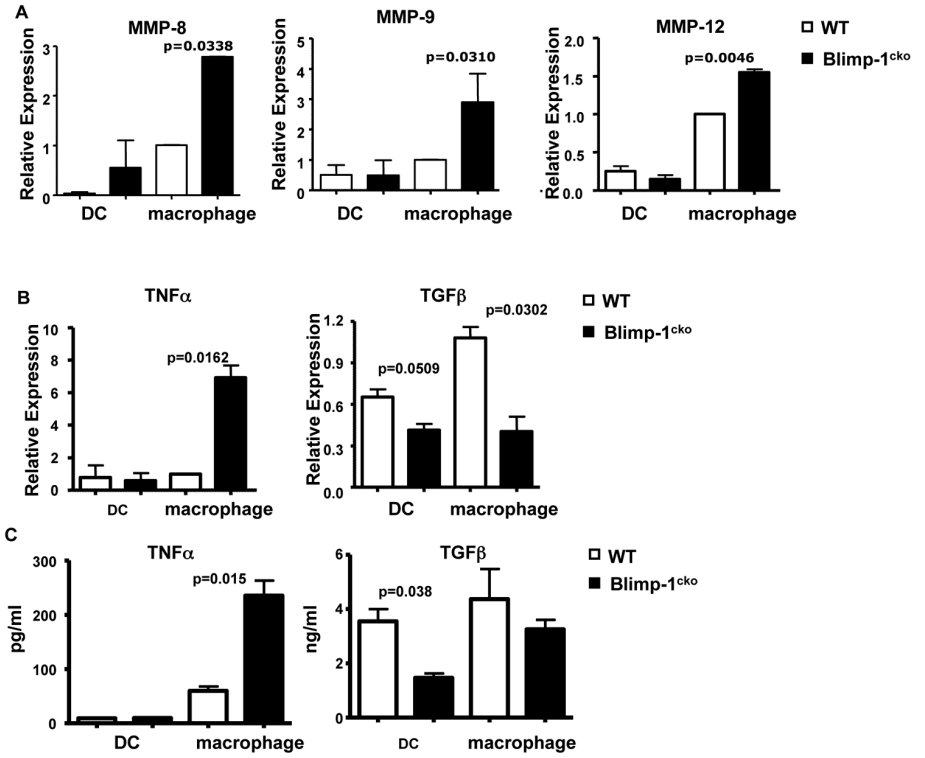

Figure 6. Increased expression of MMPs and proinflammatory cytokine in colonic macrophages from DSS-treated Blimp-1^{cko} mice. At d 3 of DSS treatment, DCs and macrophages were isolated from colons of WT or Blimp-1^{cko} mice, and expression of MMPs (A) and cytokines (B) was measured by qPCR and ELISA (C). Means ± SE are shown (n = 6).

(30,31). We measured MMPs in isolated colonic macrophages during DSS administration. MMP-8, MMP-9 and MMP-12 mRNA was highly increased in colonic macrophages from Blimp-1^{cko} mice compared with WT mice on d 3 of DSS administration (Figure 6A). RNA for other MMPs and protectins, such as MMP-2, Ptsg2, Lta4h and Alox5ap, involved in wound healing, did not differ between control and Blimp-1^{cko} mice (data not shown). We also measured MMP mRNA in DCs from the colon of mice given DSS; the level of MMP expression in DCs was minimal, and there was no detectable difference between Blimp-1^{cko} and WT mice. TNFα mRNA and protein was increased and TGFβ mRNA decreased in colonic macrophages from Blimp-1^{cko} mice compared with WT mice (Figures 6B, C). These data suggest that macrophages from Blimp-1^{cko} mice were skewed toward production of tissue-destructive proteins during DSS-induced colitis.

CD103^{+} Colonic DCs Switch Residential Macrophages into Tissue-Destructive Macrophages

To test whether Blimp-1^{ko} DCs were responsible for the enhanced MMP expression in macrophages, we cultured macrophages with DCs *in vitro*. Macrophages were generated from the BM of WT mice, and DCs were prepared from the colon of either WT or Blimp-1^{cko} mice. To recapitulate the complex bacterial stimulation in the colon, we decided to use the bacterial recognition motifs, LPS and MDP, enabling activation of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–containing protein 2 (NOD2) in DCs. These motifs were chosen, since the importance of TLRs and NOD2 in IBD is well described (32). Colon-derived Blimp-1^{ko} DCs preactivated by MDP significantly enhanced MMP expression in macrophages compared with MDP-preactivated WT DCs (Figure 7A). In contrast, neither LPS-activated Blimp-1^{ko} DCs nor MDP-activated Blimp-1^{ko} BM-DCs enhanced MMP expression in macrophages (Figure 7B). Direct activation of colonic macrophages with MDP did not induce MMP expression in macrophages from WT or Blimp-1^{ko} mice (data not shown), suggesting specific molecules derived from colonic DCs are required for induction of MMPs in colonic macrophages. To determine whether soluble factors secreted by Blimp-1^{ko} DCs were responsible for MMP expression in macrophages, supernatants from MDP-stimulated Blimp-1^{ko} DCs were incubated with WT macrophages. MMP mRNA expression was increased by exposure to secreted products of MDP-activated Blimp-1^{ko} DCs (Figure 7C). To confirm increased protein expression by DC stimulation, MMP-12, which was most highly expressed among MMPs, was measured in culture supernatant. Figure 7D shows MMP-12 expression in the supernatant; only supernatant from MDP-stimulated DCs induced MMP-12 expression in macrophages. Next, we compared cytokine expression between WT and Blimp-1^{ko} DCs stimulated with LPS or MDP to identify molecules responsible for induction of MMPs.

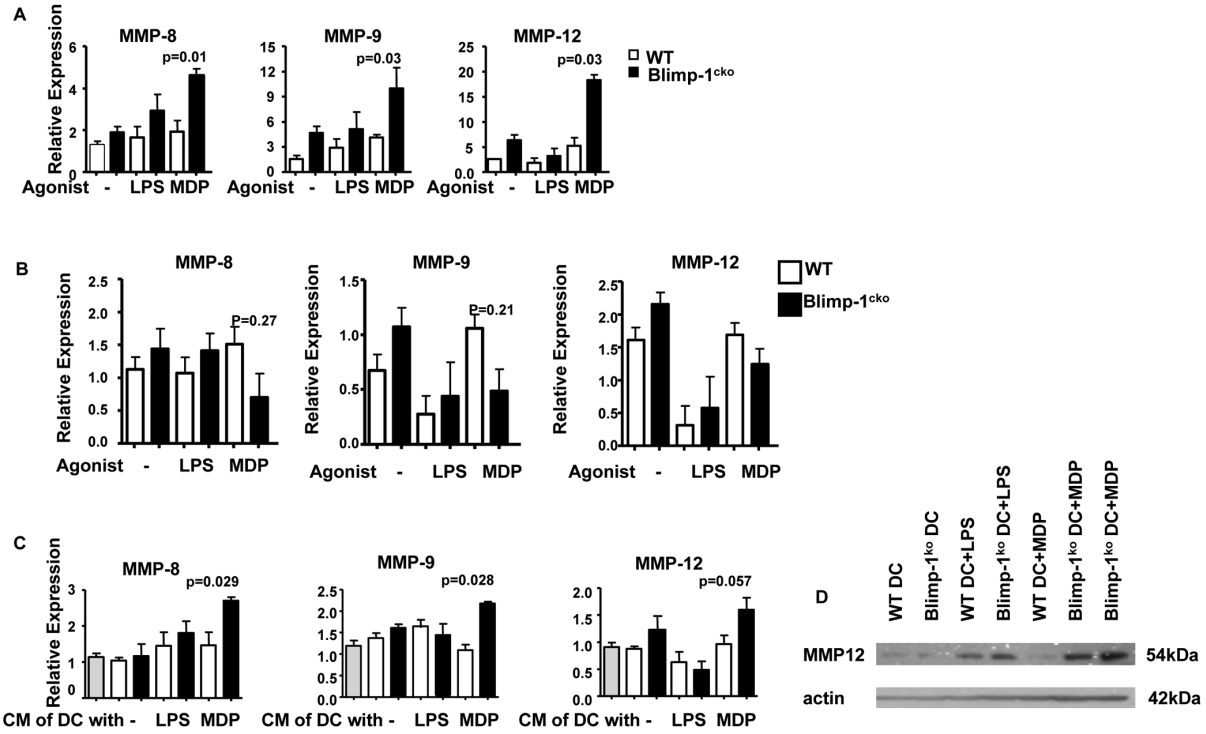

Figure 7. Soluble factor derived from colonic DCs increased MMP expression in macrophages. Macrophages were cocultured with colonic DCs (A) or BM-DCs (B) *in vitro* as described in Material and Methods. Supernatant was harvested and DCs and macrophages were separated by cell sorting to measure MMP by qPCR. The bar represents the mean ± SE (n = 4). (C) BM-macrophages were cultured without (gray bar) or with conditioned medium (CM) prepared from (A), and then MMP expression was measured by qPCR. The bar represents the mean ± SE (n = 4). (D) MMP-12 protein was measured by Western blotting. Supernatant were prepared from experiment (A), and anti–MMP-12 Western blotting was performed. For sample loading control, macrophages from the same experiments were lysed and actin level was measured.

IL-6 and IL-1β were significantly increased in MDP-activated (Figure 8A), but not LPS-activated, colonic DCs (data not shown). We therefore tested whether IL-6 and IL-1β secreted from DCs were responsible for increased MMP expression. Anti–IL-6 and –IL-1β neutralizing antibodies were introduced during the culture of macrophages. Blockade of both cytokines significantly reduced MMP mRNA expression compared with control antibody-treated macrophages, demonstrating that IL-6 and IL-1β are responsible for increased MMP in macrophages (Figure 8B).

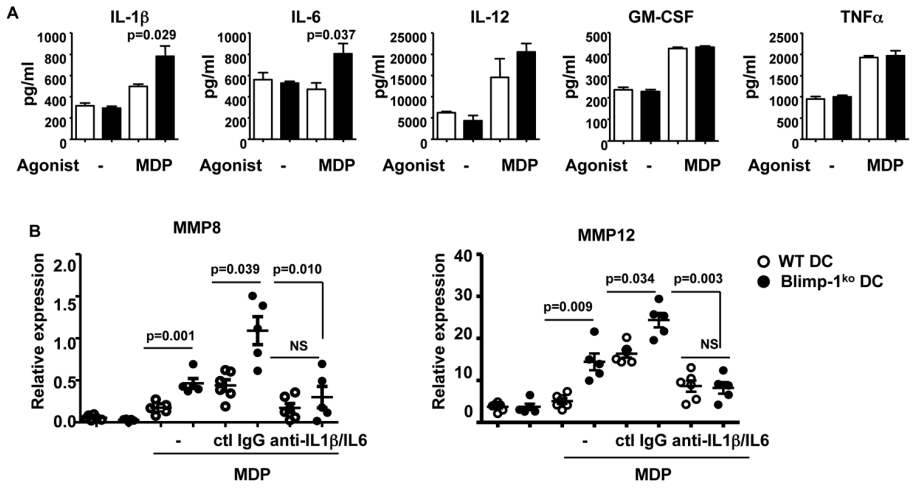

Figure 8. Increased expression of IL-1β and IL-6 is responsible for enhanced MMP expression in macrophages. (A) Cytokine levels were measured by MSD. Supernatants were prepared from colonic DC from WT (open bar) or Blimp-1^{cko} (closed bar) mice with or without stimulation with MDP. The bar represents the mean ± SE (n = 4). (B) Cytokines were blocked during macrophage activation with conditioned medium (CM) of MDP-preactivated DCs. MMP expression was measured by qPCR. Each dot represents an individual mouse and the bar represents the mean ± SE (n = 3).

Blocking of IL-1β/IL-6 Reversed the Colitis Phenotypes in Blimp-1^{cko} Mice

To test whether IL-1β and/or IL-6 were responsible for the increased susceptibility to DSS-induced colitis in Blimp-1^{cko} mice, we administered neu-

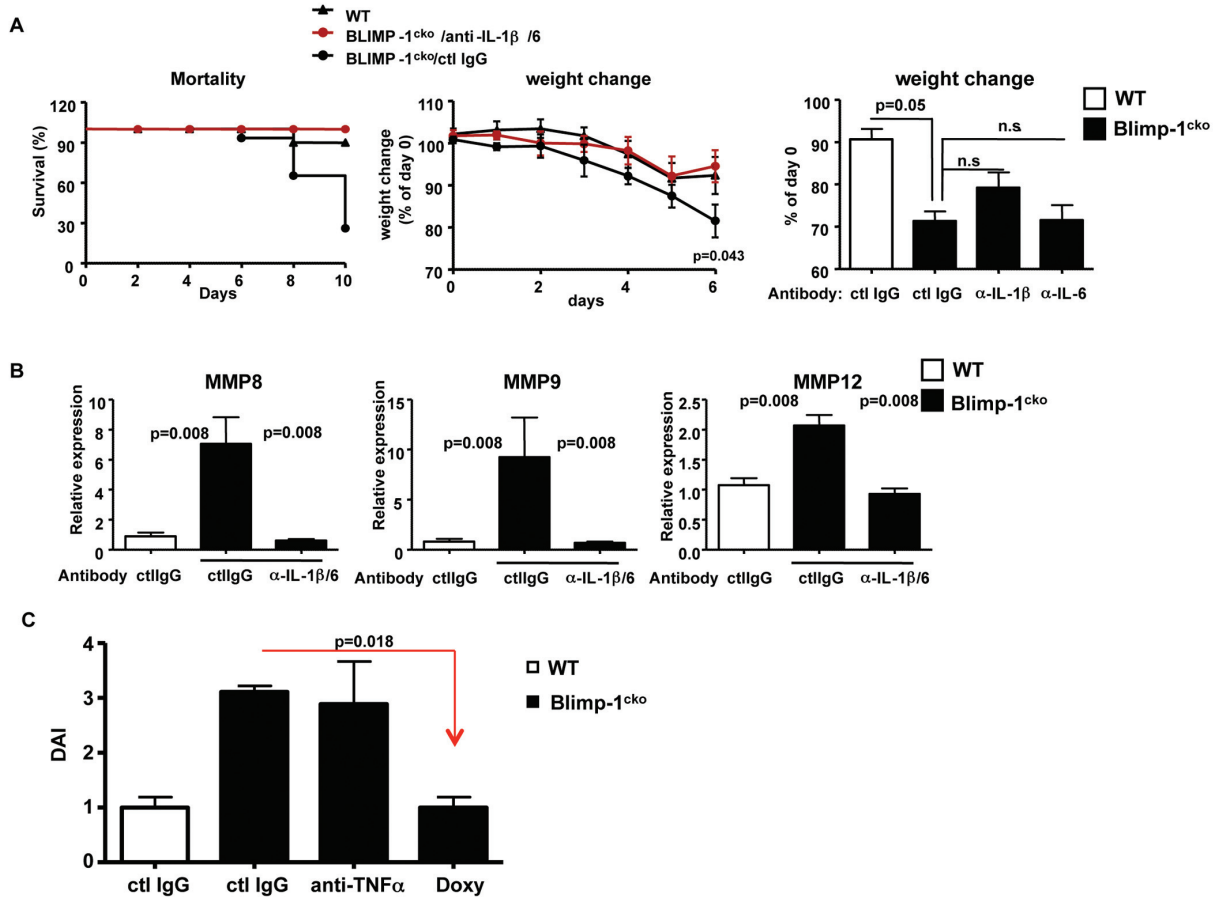

Figure 9. Blocking of IL-1β and IL-6 during the DSS treatment reduces colitis in Blimp-1^{cko} mice. (A) Either control (ctl)-IgG or anti–IL-1β/6 neutralizing antibody was administered intraperitoneally (100 μg each antibody) on d 0, 3 and 5 of DSS treatment in WT and Blimp-1^{cko} mice. Mortality (left) and weight loss (middle) was monitored. On the right panel, weight loss is shown at d 7 DSS treatment with ctl-IgG and either anti–IL-1β or –IL-6. The bar represents the mean ± SE (n = 4). (B) WT and Blimp-1^{cko} mice were treated with DSS for 3 d with either ctl-IgG or anti–IL-1β/6 intraperitoneally, and MMP expression was measured in colonic macrophages by qPCR. Means ± SE of three independent experiments are shown. (C) Ctl-IgG, anti-TNFα neutralizing antibody (100 μg) or doxycycline (50 mg/kg) was administered intraperitoneally on d 0, 3 and 5 of DSS treatment in WT and DCBlimp-1^{ko} mice. DAI was calculated on d 7 of DSS treatment. Data are means ± SE (n = 3). n.s., Not significant.

tralizing antibodies to IL-1β/IL-6 during DSS administration. Administration of neutralizing antibodies to both IL-1β and IL-6, but not control antibody, led to improvements in mortality and weight loss in Blimp-1^{cko} mice (Figure 9A). MMP-8, -9 and -12 mRNA expression was downregulated in macrophages isolated from antibody-treated Blimp-1^{cko} mice (Figure 9B), demonstrating that IL-1β and IL-6 contribute significantly to the increased MMP mRNA expression in macrophages. Blockade of IL-1β or IL-6 individually was not sufficient to improve clinical symptoms in Blimp-1^{cko} mice (Figure 9A, right panel) or to reduce MMP mRNA expression in macrophages (data not shown), suggesting that either IL-1β or IL-6 can enhance colitis susceptibility. Mounting evidence indicates the importance of TNFα in colitis development (32). Because we observed that TNFα secretion was increased in macrophages from Blimp-1^{cko} mice (Figure 6B), we administered anti-TNFα neutralizing antibody during DSS exposure. TNFα blockade did not reduce colitis severity in Blimp-1^{cko} mice (Figure 9C), suggesting that TNFα may not be the major mechanism for tissue injury in this model. To determine whether the increased MMP expression in macrophages was critical to the increased colitis phenotype, we administered doxycycline, a pan-MMP inhibitor, during induction of colitis. This treatment significantly improved the colitis phenotype (Figure 9C). Blimp-1^{cko} mice that were administered doxycycline showed a low degree of inflammation in the colon with normal stool, demonstrating that enhanced expression of MMPs in macrophages is a mechanism of DSS colitis.

DISCUSSION

DSS-induced colitis is usually a self-limited condition in mice, characterized by acute tissue inflammation in the

colon, and initiated by the innate immune system. Our present study highlights a novel role for Blimp-1 in human Mo-DCs and mouse $CD103^+$ DCs in the regulation of the inflammatory response in the gut. Mo-DCs carrying the *PRDM1* IBD risk allele exhibited a low level of Blimp-1 and increased production of proinflammatory cytokines on simulation with LPS. Blimp-1 deficiency in $CD103^+$ DCs rendered mice more susceptible to DSS-induced colitis with an increased mortality. Intestinal Blimp-1^{ko} DCs secrete increased levels of proinflammatory cytokines, IL-1β and IL-6, presumably in response to commensal bacteria, resulting in the enhanced activation of macrophages in the colon. Macrophages of Blimp-1^{cko} mice are induced during DSS colitis to express higher levels of MMPs with faster kinetics of expression. Inhibition of IL-1β and IL-6 or MMPs can relieve colitis symptoms, demonstrating that proinflammatory cytokines and MMPs from innate immune cells are a major mechanism of pathology in this mouse model of colitis.

In previous reports, Blimp-1 deficiency was shown to function in different cell types to cause inflammatory diseases. Mice with a T-cell–specific Blimp-1 deletion develop intestinal inflammation (34), probably mediated by a lack of functional T regulatory cells. More recently, Blimp-1 in T cells has been shown to limit Th17 differentiation, a T-cell subset that contributes to intestinal inflammation (35). Mice with an epidermal-specific deletion of Blimp-1 develop chronic skin inflammation with a higher level of granulocyte colony-stimulating factor (G-CSF) and enhanced myelopoiesis (36). This study identifies a protective function of Blimp-1 in innate immune cells and reveals how this protects against intestinal inflammation.

It is well accepted that in the steady state, intestinal myeloid cells are tolerogenic and refractory to stimulation with TLR agonists in contrast to myeloid cells in the blood or in peripheral lymphoid organs (37); therefore, uncontrolled cytokine production by myeloid cells contributes to the pathogenesis of colitis (38,39). Proinflammatory cytokines, mostly secreted from DCs not macrophages, were highly expressed in colonic myeloid cells from Blimp-1^{cko} mice compared with WT mice after DSS exposure, directly regulating the expression of MMPS from macrophages and subsequently increasing tissue damage during the development of colitis. This regulation appears to be tissue specific because the increase in MMPs in macrophages is induced by activated colonic DCs but not BM-DCs.

MMPs are implicated in tissue destruction during inflammation (30). There is a report of increased expression of MMP-1 and MMP-2 in biopsies of patients with ulcerative colitis (40). Moreover, MMP expression correlates with regions of mucosal loss in IBD patients (41). We did not observe significant changes in MMP-1 or MMP-2 (data not shown). Instead, there was an increased expression of MMP-8, -9 and -12 in macrophages isolated from Blimp-1^{cko} mice compared with macrophages from WT mice subjected to DSS colitis. Each MMP has a specific set of target molecules. MMP-8 and MMP-12 can bind to TNFα and chemokines, respectively, and convert the inactive forms into active forms. Therefore, increased expression of MMPs can increase tissue damage directly by matrix degradation and indirectly by activation of proinflammatory cytokines and chemokines, initiating an inflammatory cascade in the mucosa. In fact, the increased infiltration of neutrophils observed in the colon in Blimp-1^{cko} mice may reflect an increased secretion of neutrophil-attracting chemokines from Blimp-1^{ko} DCs and MMPs. Although we observed that an increased secretion of chemokine (C-X-C motif) ligand 1 (CXCL1) in LPS stimulated Blimp-1–deficient DCs in spleen, we did not find the difference in colonic DCs before and after DSS exposure (data not shown). It is possible that the increased MMP-12 cleaves the inactive form of CXCL1 to convert it into an active form of CXCL1 (42) or MMP-8/9 cleaves collagen generating the proline–glycine–proline peptide, which has chemotactic effects on neutrophil (43). Although neutrophil migration is beneficial for killing bacteria, it is presumed that persistent or excessive neutrophil infiltration causes tissue damage, and blocking of neutrophil infiltration can be beneficial in DSS-mediated colitis (44,45).Therefore, MMP-activated chemokines may be one component of the pathogenic inflammatory cascade in Blimp-1^{cko} mice.

While a number of studies have demonstrated the direct activation of adaptive immune cells by DCs, whether DCs can directly regulate innate immune cells has not been studied. The observation we made in this study (that $CD103^+$ colonic DCs upregulate MMP expression in macrophages) is therefore novel. This regulatory activity is tissue specific and agonist dependent. Compared to WT DCs, Blimp-1–deficient DCs secrete increased levels of IL-1β and IL-6 after stimulation with MDP, presumably through activation of the MDP receptor NOD2. We have shown that these cytokines are major contributors to MMP expression in macrophages and to susceptibility to DSS, since administration of neutralizing antibodies to IL-1β and IL-6 improved clinical symptoms of colitis and diminished MMP induction. Currently, we do not know how Blimp-1 deficiency positively regulates MDP-mediated NOD2 activation in colonic DCs. NOD2 is a member of the NLR (NOD-like receptor) family of proteins, which regulate nuclear factor (NF)-κB activation and a subsequent inflammatory cascade. A recent study showed that Blimp-1 can negatively regulate NLRP12/Monarch-1, another member of the NLR family (46). We did not find any report of Blimp-1 regulating NOD2 or NOD2 downstream molecules, but perhaps NOD2 levels are increased in Blimp-1^{ko} DCs. We are currently investigating this possibility. It is also possible that expression of IL-32 is increased in the absence of Blimp-1, since IL-32 synergizes with NOD1/2 ligands to induce IL-1β and IL-6 production (47).

CONCLUSION

The results of the present study provide several important insights into the function of DCs during intestinal inflammation. First, DC-restricted alterations can increase susceptibility to intestinal inflammation. Blimp-1 deficiency in DCs induces enhanced production of IL-1β and IL-6 on MDP stimulation; this phenotype is present only in colonic DCs but not in BM-DCs. Second, this enhanced cytokine production directly regulates MMP expression in macrophages. Direct cross-talk between DCs and macrophages has not been previously reported or shown to contribute to IBD pathogenesis. The hyperactivated phenotype of intestinal DCs observed in our study is one of the consistently observed phenotypes of DCs from IBD patients (48), suggesting that the Blimp-1 colitis risk allele may exhibit decreased expression in intestinal DCs, a new molecular pathway by lack of Blimp-1 in colitis pathogenesis. We believe that the current study suggests a novel mechanism for PRDM1-determined risk in initiation or disease progression in human IBD and demonstrates that DCs not only modify the function of cells within the adaptive immune response but also modify function of innate immune cells.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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