Interleukin-17 Induces an Atypical M2-Like Macrophage Subpopulation That Regulates Intestinal Inflammation

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Introduction

Inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn’s disease (CD) is a chronic inflammatory disease with recurring relapses and remissions in the lower gastrointestinal tract [1,2]. Genetic, environmental factors and their interrelationships which trigger an overactive adaptive immune response to intestinal bacterial flora have been considered to play key roles in the pathogenesis of IBD. More recent evidence suggests that defects in mucosal innate immune functions may also be involved in the etiology of IBD. However the exact pathomechanisms of the disease are still not fully elucidated.

IL-17, a signature cytokine of Th17 cells, is a pleiotropic cytokine that acts on both immune and non-immune cells and is generally implicated in inflammatory and autoimmune diseases. Although IL-17 as well as their source, mainly but not limited to Th17 cells, is also abundant in the inflamed intestine, the role of IL-17 in inflammatory bowel disease remains controversial. In the present study, by using IL-17 knockout (KO) mice, we investigated the role of IL-17 in colitis, with special focus on the macrophage subpopulations. Here we show that IL-17KO mice had increased susceptibility to DSS-induced colitis which was associated with decrease in expression of mRNAs implicated in M2 and/or wound healing macrophages, such as IL-10, IL-1 receptor antagonist, arginase 1, cyclooxygenase 2, and indoleamine 2,3-dioxygenase. Lamina propria leukocytes from inflamed colon of IL-17KO mice contained fewer CD11b+Ly6C-MHC Class II macrophages, which were derived, at least partly, from blood monocytes, as compared to those of WT mice. FACScanalysis revealed that CD11b+ cells from WT mice, which were more abundant in Ly6C-MHC Class II cells, expressed increased levels of genes associated with M2/wound healing macrophages and also M1/proinflammatory macrophages. Depletion of this population by topical administration of clodronate-liposome in the colon of WT mice resulted in the exacerbation of colitis. These results demonstrate that IL-17 confers protection against the development of severe colitis through the induction of an atypical M2-like macrophage subpopulation. Our findings reveal a previously unappreciated mechanism by which IL-17 exerts a protective function in colitis.
been shown to exert pro- [12] and anti-[13,14] colitogenic activities, adding an additional layer of complexity.

These contradictory results regarding pathogenic versus protective roles of IL-17 mentioned above led us to re-examine whether IL-17 exerts protective function in a DSS-induced colitis model, focusing on the phenotypic and functional differences between macrophages in inflamed colon of WT and IL-17KO mice. Our results demonstrate that CD11b+Ly6C+ MHC Class II+ macrophages were reduced in the inflamed colon of IL-17KO mice that accompanied the development of more severe colitis as compared to WT mice. Depletion of CD11b+Ly6C+ MHC Class II+ macrophages in colon of WT mice resulted in more severe colitis. In vivo transfer experiments indicate that IL-17 promotes monocyte differentiation into M2-like macrophages. These results indicate that IL-17 protects from the development of DSS-induced colonic inflammation likely by promoting induction of unique macrophage subpopulation with anti-inflammatory and/or tissue repair functions.

Materials and Methods

Mice
C57BL/6 mice, C57BL/6 (CD45.1) congenic mice and IL-17A deficient (IL-17KO) mice (C57BL/6 background) [15] were fed a standard diet, housed under specific pathogen free conditions and used at 5–8 weeks of age. All animal experiments were conducted under protocols approved by the Animal Care and Use Committee of Mie University Life Science Center.

Induction of colitis
Colitis was induced by 1.5% DSS (MW: 36,000–50,000, MP Biomedicals) dissolved in drinking water for 7 days followed by water alone. The body weight change was monitored daily up to 15 days. Healthy control animals received water only. In some experiments, DSS colitis was induced in mice adoptively transferred with monocytes or mice treated with clodronate liposomes. Briefly, monocytes (7×10⁶ cells) isolated from bone marrow were transferred via tail vein into a group of mice a day before start of DSS treatment. Another group of mice were injected with 100 μl of clodronate liposomes or control liposomes containing 2.5% FCS, 1.5 mg/ml Collagenase VIII (Sigma-Aldrich), and 0.1 mg/ml DNase I (Worthington Biochemical Corporation) by shaking (200 rpm) at 37 °C for 30 min. Resultant cell suspensions were passed sequentially through cell strainers (100 μm), resuspended in 40% Percoll (GE Healthcare) and layered over an 75% Percoll prior to centrifugation at 2,500 rpm for 20 min. Cells from 40%/75% interface were collected, washed with HBSS for three times and used for experiments.

Isolation of monocytes
Monocytes were isolated from bone marrow with EasySep mouse monocyte enrichment kit (StemCell Technologies, Vancouver, CA), according to the manufacturer’s instructions. Briefly, bone marrow cells from femora and tibiae were labeled with a cocktail of biotinylated antibodies against a panel of antigens expressed on T, B, NK, DCs, progenitor cells and granulocytes, followed by anti-biotin microbeads. Unlabeled monocytes were sorted magnetically by negative selection. Monocyte population contained more than 92% CD11b+Ly6C+ cells and less than 8% Ly6G+ cells, and used for in vivo transfer experiments.

Flow cytometry
Colonic LP cells and monocytes were incubated with anti-CD16/32 (24G2; eBioscience) to block non-specific FcR followed by cell surface staining with corresponding mixture of fluorescein labeled mAbs. 7AAD (BD Pharmingen) was used to exclude nonviable cells. The following antibodies conjugated with FITC, PE, APC, V450, or APC/Cy7 were used for flow cytometry: anti-CD45.2 (104), anti-CD45.1 (A20), anti-I-Ab (AF6-120.1), anti-Ly6C (HK1.4), anti-F4/80 (BM8), anti-Ly6G (A68), anti-CD206 (C606C2), anti-CD64 (X54-5/7.1), anti-CD112 (ATR34), anti-TCRγδ/δ (GL3) [all from BioLegend], CD3δ (NO. 72-1), anti-CCR9 (GW-1.2), anti-CCR7 (4B12), anti-CD11c (N418), anti-CD4 (RM4-5) [all from eBioscience], anti-CD11b (M1/70), Sca-1 (D7) [BD Bioscience], anti-CCR2 (475301) (R&D systems). PE-Rat IgG2a (eBR2a; eBioscience) and APC/Cy7-RatIgG2a (RTK 2758; BioLegend) were used as isotype matched control Abs. Data were acquired on a FACSscan II (BD) and processed by using FlowJo software (Tree Star) with appropriate isotype controls to determine gating.

Gene expression analysis
Total RNA was extracted from distal colon segments or purified cells with Trizol reagent (Invitrogen) and reverse transcribed into cDNA as described [18]. Realtime RT-PCR was performed by using FastStart Universal SYBR Green Master (Roche Diagnostics) according to the manufacturer’s instruction. Expression of target mRNA were normalized to the expression of β-actin mRNA for generation of ΔΔCt values, and relative mRNA expression was quantitated with the ΔΔCt method [19]. Primer sequences for these reactions were designed by using Primer Express software Version 3 (Applied Biosystems) and provided in the Table S1.

Histology
Distal colon tissue was fixed in 10% paraformaldehyde and embedded in paraffin blocks. Five micrometer sections were stained with hematoxylin and eosin.

Statistics
Statistic analyses were performed with IBM SPSS Statistics Software Version 19 (IBM). Differences between two groups were compared using the two-tailed Student’s t-test and those among multiple groups were compared using the Kruskal-Wallis with
Bonferroni post hoc test. A p value of <0.05 was considered significant. All experiments were performed more than two times. Data are presented as mean ± SEM.

**Results**

**IL-17-deficient mice exhibit more severe acute colitis following DSS administration**

To reassess the role of IL-17 in DSS-induced colitis, we administered 1.5% DSS in drinking water to age- and sex-matched IL-17KO and WT mice for 7 days followed by water consumption alone with untreated mice serving as controls. At a steady state, both genotypes displayed no gross signs of colitis such as growth retardation, weight loss or diarrhea. Although ingestion of DSS is known to cause intestinal inflammation in WT mice as a result of disruption of the gut epithelial barrier, this dose of DSS did not induce marked weight loss in WT mice (Figure 1A). By sharp contrast, IL-17KO mice experienced significant weight loss starting at day 7, reaching a maximum reduction (15%) on day 10, followed by a recovery that reached a pretreatment level at day 5 after DSS withdrawal. In addition, IL-17KO mice showed a more decrease in colon length and weight by day 10 as compared to WT mice (Figure 1B). Histological analysis also revealed aggravated colonic inflammation as evidenced by edema, high degree of ulcerations and overt inflammatory infiltrate in both mucosa and submucosa, and mucosal thickening accompanied by destruction of epithelium in IL-17KO mice (Figure 1C). Concomitant with these findings, epithelial barrier function in IL-17KO mice was

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**Figure 1. IL-17 deficient mice exhibit more severe acute colitis following DSS administration.** IL-17KO mice and WT controls were given 1.5% DSS in drinking water or water alone for 7 days followed by consumption of water alone. Colitis severity was assessed by weight loss (A), colon length (day 10) (B) and H&E histology (day 0 and 10) (Calibration bar = 200 μm) (C). Colon barrier permeability was assessed on day 10 by detection of FITC-dextran serum (D). The results are expressed as mean values ± SEM for each genotype in A (n = 5–6), B (n = 10), C (n = 2), and D (n = 5). *p<0.05; **p<0.01.

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more severely compromised by DSS administration than that in WT mice as IL-17KO mice displaying a dramatic increase in orally administered FITC-dextran translocation into the serum (Figure 1D). Although WT mice treated with DSS did not exhibit overt symptoms of progressive wasting disease, we observed an increased number of inflammatory infiltrates and IL-17+ cells in the colon of WT mice treated with DSS as compared to the colons of untreated WT mice (Figure 1C and data not shown).

Inflamed colons of IL-17KO mice expressed reduced levels of mRNA with anti-inflammatory functions

Using real-time PCR samples taken from the colons of WT and IL-17KO mice, we next assessed the expression of mRNA for a range of genes thought to be involved in inflammatory/anti-inflammatory responses. mRNA for IL-10, IL-1 receptor antagonist (IL-1Ra), arginase 1 (ARG1), cyclooxygenase 2 (COX2), and indoleamine 2,3-dioxygenase (IDO), which are produced by M2 and/or wound healing macrophages contributing to the suppres-
Figure 3. Aggravated DSS-induced colitis in IL-17KO mice is associated with reduced number of CD11b^+Ly6C^-MHC Class II^+ macrophages in the inflamed colon. IL-17KO mice and WT controls were treated with DSS as described in the legend for Figure 1. (A) LPLs were purified from pooled (n = 2–3) distal colon sections of untreated and DSS-treated mice and subjected to flow cytometry analysis. Gating strategy of IL-17/M2-Like Macrophage Axis in Colitis.
live and CD45^+ for CD11b^+ cells was shown in upper panels. Representative data from seven independent experiments are shown. Number denotes frequency of gated cells. (B) The frequency of cells for each subset is shown. Graphs represent mean ± SEM of seven independent experiments. *p<0.05. (C) Representative flow cytometry profiles of cell surface molecules implicated in M2/anti-inflammatory macrophages on each subset depicted as in A. Gray histograms represent cells stained with isotype matched control mAbs.

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Aggravated colitis seen in IL-17KO mice correlates with the lack of CD11b^+Ly6c^-MHC Class II^+ macrophages

Intestinal macrophages are highly versatile in function and can suppress inflammation and/or promote repair of damaged mucosal tissues [23]. Together with our results that genes involved in anti-inflammatory/tissue repair, which expressed in M2/wound healing macrophages were reduced in the inflamed colon of IL-17KO mice mentioned above, led us to examine for differences between macrophage subpopulations in the inflamed colon of WT and IL-17KO mice. As an initial step, we performed multi-color flow cytometric analysis for mononuclear cells of colonic lamina propria taken from IL-17KO and WT mice before and after induction of colitis. At a steady state, there is only a small difference in the proportion of CD11b^+ cells, and subsets within CD11b^+ cell population of colonic lamina propria leukocytes (LPLs) from IL-17KO and WT mice (Figure 2A and B). Inflamed colonic LPLs contained an increasing trend in the proportion of CD11b^+ cell infiltrates but this increase was less prominent in IL-17KO mice (Figure 3A and B). Inversely, the expression level of mRNA for IL-17, but not its related cytokine IL-17F, was increased in colon of WT mice after DSS treatment.

Extrinsic IL-17 induces differentiation of blood monocytes into CD11b^+Ly6c^-MHC Class II^+ macrophages in the inflamed colon

Based on the finding that the expression level of CCL2 mRNA was increased in the inflamed colons and CCR2 was expressed on Ly6c^-MHC Class II^+ cells within CD11b^+ cells (Figure 2 and 3C), we next sought to determine whether CCR2^+Ly6c^- blood monocytes are recruited predominantly to the inflamed colon and differentiate in situ under the influence of IL-17 into CD11b^+Ly6c^-MHC Class II^+ macrophages. To this end, monocytes were adoptively transferred into IL-17 sufficient or deficient mice, which received DSS treatment thereafter. On day 10, LPLs were harvested from these mice, among which donor cells were identified based on congenic markers and evaluated for their cell surface phenotype. As a representative result shown in Figure 5, most of donor monocytes, regardless of whether they were from WT or IL-17KO mice, gained MHC Class II expression and maintained Ly6c expression in WT host, whereas only a fraction of them gained MHC Class II in IL-17KO host. These results suggest that IL-17 is involved in the phenotypic and possibly functional maturation of monocytes by extrinsic mechanism.
Figure 4. CD11b⁺ cells from inflamed colonic LPLs of KO mice express significantly lower levels of genes implicated in M2/wound healing macrophages. IL-17KO mice and WT controls (n = 3) were treated with DSS as described in the legend for Figure 1. Then, LPLs isolated from these mice were further purified into CD11b⁺ cell on FACS Aria, from which cDNA were prepared and subjected to real-time PCR analysis.
Depletion of CD11b<sup>+</sup>Ly6C<sup>+</sup>MHC Class II<sup>+</sup> macrophages exacerbates colon inflammation induced by DSS

Having confirmed that IL-17KO mice suffering more severe colitis had impaired ability to generate Ly6C<sup>+</sup>MHC Class II<sup>+</sup> cells expressing the highest M2 marker within CD11b<sup>+</sup> cell population and CD11b<sup>+</sup> cells of the inflamed colonic LPLs from WT mice abundant in Ly6C<sup>+</sup>MHC Class II<sup>+</sup> cells expressed increased level of most genes implicated in M2/wound healing macrophages (Figure 3 and 4), we next sought to determine whether CD11b<sup>+</sup>Ly6C<sup>+</sup>MHC Class II<sup>+</sup> cells are responsible for reduced colonic inflammation seen in WT mice. Liposome uptake by macrophages represents a genuine phagocytosis event [27], which has been widely used to target macrophage in vivo. Taking advantage of the fact that M2 macrophages have a high phagocytic activity [28] and intrarectal administration of clodronate-liposome, we could preferentially decrease Ly6C<sup>+</sup>MHC Class II<sup>+</sup> over other subpopulations in LPLs, but not systemically, of DSS treated WT mice (Figure 6A and Figure S3). This treatment reduced colonic CD11b<sup>+</sup> cells by 35.6% (27.5<sub>6</sub>0.9% <sub>R</sub>17.7<sub>6</sub>0.8%, p<0.01) and Ly6C<sup>+</sup>MHC Class II<sup>+</sup> cells within CD11b<sup>+</sup> population by 35.4% (33.4<sub>6</sub>1.5% <sub>R</sub>24.0<sub>6</sub>0.5%, p<0.01), resulting in an overall decrease in CD11b<sup>+</sup>Ly6C<sup>+</sup>MHC Class II<sup>+</sup> macrophages by 53.9% (9.1<sub>6</sub>0.2% <sub>R</sub>4.2<sub>6</sub>0.1%, p<0.01) in five independent experiments. On the other hand, mononuclear phagocyte populations as defined by CD11b, Ly6C and MHC Class II were marginally, if at all, affected in PBMC, spleen and bone marrow by this treatment (Figure S3). As expected, WT mice treated with clodronate-liposome exhibited a greater degree of body weight loss as compared to the mice treated with control-liposome (Figure 6B). Taken as a whole, these results indicate that CD11b<sup>+</sup>Ly6C<sup>+</sup>MHC Class II<sup>+</sup> macrophages differentiated from blood monocytes in the presence of IL-17 play a regulatory role in colonic inflammation.

Discussion

IL-17 has a critical function in the host defense response against various pathogens, but also has become notorious for its role in the pathogenesis of many inflammatory and autoimmune disorders, which makes this cytokine categorized as a proinflammatory cytokine [3]. This prevailing view was also adopted in IBD where IL-17 and IL-17 producing cells were found abundantly in the affected tissue [4]. Indeed, numerous studies have demonstrated pro-colitogenic role of IL-17 in animal models of IBD [8–10,12]. However, challenging this view, other studies in animal models [11,13,14] and recent human clinical trials [29,30] have emerged to suggest that IL-17 plays a protective role. To reassess the role of IL-17 in IBD pathogenesis and underlying mechanisms involved, we adopted a well-know DSS-induced colitis model, namely WT or IL-17KO mice were given DSS in drinking water. Upon evaluation of ensuing colitis, we found that IL-17KO mice were much more susceptible than WT mice. In addition, expression

Figure 5. Blood monocytes are recruited into inflamed colons and differentiate into CD11b<sup>+</sup>Ly6C<sup>+</sup>MHC Class II<sup>+</sup> macrophages in the presence of IL-17. Monocytes were purified from bone marrow of WT (CD45.1), WT (CD45.2) or IL-17KO (CD45.2), and adaptively transferred into WT (45.2), IL-17KO (CD45.2) or WT (CD45.1) mice (n = 3), respectively, followed by the treatment with DSS. On day 10, colonic LPLs were isolated from pooled colon, stained with anti-CD11b, anti-Ly6C, and anti-MHC Class II together with corresponding anti-CD45 congenic antibody, and subjected to flow cytometry analysis. A representative result from three independent experiments is shown.

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levels of mRNA coding for most, but not all, of the molecules contributing to suppression/resolution of inflammation and tissue repair [20,31] were significantly reduced in the inflamed colons of IL-17KO mice. Among those, genes expressed by M2/wound healing macrophages were downregulated in IL-17KO mice with notable consistency.

A characteristic of an inflammatory landscape in the colon of patients with IBD is an increased number of macrophages derived from blood monocytes [1,2]. As compared to a healthy colon, these macrophages produce an increased amount of inflammatory cytokines and express cell surface molecules involved in the activation of their own and T cells [1,2]. In a mouse model of colitis, these macrophages are CD11b+ F4/80+ MHC Class II+ CX3CR1+ driving inflammation through various effector mechanisms [25,32]. Hence, aberrant activation and functions of intestinal macrophages have been proposed to contribute to the IBD pathogenesis. However, recent studies also indicate that macrophages are functionally highly promiscuous, some of which also produce factors that dampen inflammatory responses while facilitating tissue repair [20,23,33]. Indeed, anti-TNF therapy for patients with IBD induces macrophages with regulatory functions, which promote wound healing [34]. In an animal model of colitis, macrophages have also been shown to exert disease ameliorating effects. These macrophages include CD11b+ F4/80+ MHC Class II+ cells coexpressing CD11c and/or also CX3CR1 [35,36]. It has also been shown that they are recent emigrant from blood monocytes [37]. Recent study also points that CD64 as a specific macrophage marker that can discriminate dendritic cells from macrophages in the murine intestine under both steady-state and inflammatory conditions [24]. In our present study, we found a significant decrease in the frequency of CD11b+Ly6C+MHC Class II+CD64+ macrophages, which were derived, at least in part, from blood monocytes, in the inflamed colon of IL-17KO mice as compared to WT mice. Although statistically non-significant due to the variation in number of cells recovered from LPLs in each experiment, the absolute numbers of CD11b+Ly6C+MHC Class II+ macrophages were almost consistently reduced in IL-17KO mice. Furthermore, depletion of this population by topical administration of clodronate-liposome resulted in the exacerbation of DSS-induced colitis in WT mice, clearly indicating that these macrophages ameliorate, rather than exacerbate, colitis. In support of this notion, recent studies have shown that CD11b+Ly6C+MHC Class II+ macrophages in inflamed intestine produce both inflammatory and anti-inflammatory molecules to control invading pathogen while limiting collateral tissue damage [30,39]. Commercially available Abs do not allow to detect CX3CR1 by flow cytometry or immunohistochemistry, we were unable to determine whether CD11b+Ly6C+MHC Class II+ macrophages express CX3CR1 and belong to the same or overlapping population mentioned above, an important issue which needs to be further investigated.

CD11b+ cells isolated from the inflamed colons of WT mice enriched in CD11b+Ly6C+MHC Class II+ macrophages expressed higher levels of mRNA encoding anti-inflammatory and tissue repair functions as compared to CD11b+ cells from IL-17KO mice. A discrepancy remains, however, in that CD11b+ cells from the inflamed colons of IL-17KO mice expressed higher levels of Ym1 mRNA, which is a marker for M2 macrophages [40]. Recent study indicates that GM-CSF is critical in the expression of Ym1 [41]. Therefore, the higher levels of GM-CSF in the inflamed colons of IL-17KO mice as compared to WT mice (data not shown) may explain this seemingly paradoxical observation. Taken as a whole, it is possible that through the production of factors with “anti-inflammatory/tissue repair” functions, CD11b+Ly6C+MHC Class II+ macrophages suppress inflammation while quickly repairing colon tissue before serious damage to the tissue occurs, resulting in less severe colitis seen in WT mice. Recent studies also suggest that a subset of macrophages in the colon play a crucial role in the maintenance and/or proliferation of differentiation of functional Foxp3+ regulatory T cells. Together with our present results that inflamed colon of IL-17KO mice contained reduced levels of Foxp3+ T cells, it is also possible that CD11b+Ly6C+MHC Class II+ macrophages ameliorate colitis through enhancement of Foxp3+ regulatory T cell function [23]. In support of this notion, we observed that the depletion of CD11b+Ly6C+MHC Class II+ macrophages in colon of WT mice by clodronate-liposome was associated with the reduction in Foxp3+CD4+ T cells by 36% (30.3±0.63% → 19.2±0.62%, p<0.01, n = 4).

Figure 6. Depletion of CD11b+Ly6C+MHC Class II+ macrophages accelerates colon inflammation in WT mice induced by DSS treatment. WT mice were given 1.5% DSS in drinking water for 7 days followed by consumption of water alone for another 3 days, during which the mice were treated with clodronate-liposome or control liposome intrarectally on days 1, 1, 3, and 5 as described in Materials and Methods. (A) Representative FACS plots showing reduced CD11b+Ly6C+MHC Class II+ macrophages in colon of clodronate-liposome treated mice. (B) Changes in body weight over time were expressed as a percentage of the original weight. Data represent as mean ± SEM. The experiments were repeated two times with at least three mice per group per experiment. doi:10.1371/journal.pone.0108494.g006
Perhaps more importantly, our study revealed that monocytes differentiate to express molecules and genes implicated in M2/ wound healing macrophages under the influence of IL-17 in the inflamed colon. In support of this notion, recent studies show that IL-17 stimulates differentiation of M2/anti-inflammatory macrophages [12,43]. However, in the present study we showed that along with elevated expression of mRNA for M2 associated transcription factors and anti-inflammatory/tissue regenerative factors, CD11b⁺ myeloid cells in the inflamed colons of WT mice also expressed higher levels of mRNA for M1 associated transcription factors. Thus, macrophages differentiated under the influence of IL-17 did not fit comfortably with the M1/M2-paradigm of differentiated macrophages. We speculate that macrophages may acquire the unique M2 dominant properties adapted to the inflamed colon microenvironment under the influence of a unique cytokine milieu involving IL-17.

In summary, our study shows, for the first time, that CD11b⁺Ly6C⁺MHC Class II⁺ macrophages differentiated in the inflamed colon under the influence of IL-17 represent M2-like/ wound healing macrophages which may have regulatory functions. Whether the induction of this population by IL-17 solely influences the protective function of IL-17 in colitis remains arguable, since we also observed that inflamed colonic tissue of IL-17KO mice expressed reduced levels of claudin-1/2, β-defensin-1/2, and mucin-2, all of which have been shown to be regulated by IL-17 signaling. However, our data demonstrate a previously unappreciated mechanism by which IL-17 exerts protective functions in colitis and targeting IL-17/M2-like macrophase axis may represent an important future therapeutic approach in the treatment of mucosal inflammatory diseases such as IBD.

Supporting Information

Figure S1 Flow cytometric analysis of LPL from WT and IL-17KO mice. IL-17KO mice and WT controls were given 1.5% DSS in drinking water for 7 days followed by consumption of water alone for another 3 days. LPLs were purified from pooled distal colon sections of untreated and DSS-treated mice and subjected to flow cytometry analysis after staining with mAbs specific for indicated cell surface and intracellular molecules. Plots were shown after electric gating for 7AAD⁻ and CD45⁺ cells. Number denotes frequency of gated cells. Representative results of at least two independent experiments are shown. (TIF)

Figure S2 Flow cytometry analysis of CD11b⁺ cells before and after cell sorting. IL-17KO mice and WT controls were given 1.5% DSS in drinking water for 7 days followed by consumption of water alone for another 3 days. LPLs were purified from pooled (n = 10) distal colon sections of untreated and DSS-treated mice followed by cell sorting by FACS. Representative results out of two independent experiments are shown. (TIF)

Figure S3 Representative flow cytometry plots on CD11b⁺Ly6C⁺MHC Class II⁺ macrophage population in organs other than colon of WT mice treated with clodronate-liposome. WT mice were given 1.5% DSS in drinking water for 7 days followed by consumption of water alone for another 3 days, during which the mice were treated with clodronate-liposome or control liposome intrarectally on days −1, 1, 5, and 5 and their body weight changes were monitored. The experiments were repeated two times with at least three mice per group per experiment. (TIF)

Table S1 Primer sequences used for realtime RT-PCR. (DOCX)

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Author Contributions

Conceived and designed the experiments: KN TK. Performed the experiments: KN NS TK NM. Analyzed the data: KN TK. Contributed reagents/materials/analysis tools: DM IT MM KT YT MT HS NK. Contributed to the writing of the manuscript: KN TK.

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