Phosphatase Wip1 Masters IL-17–producing Neutrophil-mediated Colitis in Mice

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Abstract: Wild-type p53-induced phosphatase 1 (Wip1) is currently believed to be a promising drug target for cancer therapy. Our recent studies showed that deletion of Wip1 remarkably promoted neutrophil inflammatory response. Whether Wip1 is involved in the regulation of inflammatory bowel disease is unknown. In the present study, we found that Wip1 knockout (KO) mice were more susceptible to colitis induced by dextran sulphate sodium (DSS) than wild-type mice as substantiated by the lower mouse survival ratio, rapid bodyweight loss, increased disease activity index, shorter colon length, and more severe pathology of colons in Wip1KO mice. Using full bone marrow chimera mouse models, we demonstrated that Wip1 intrinsically controls inflammatory response of immune cells. Deletion of IL-17 (Wip1/IL-17 double KO mice) significantly rescued the pathology in Wip1KO mice. Neutrophils of DSS-treated wild-type and Wip1KO mice expressed significantly higher IL-17. After adoptive transfer of sorted Wip1KO or double KO neutrophils into IL-17KO mice, mice receiving double KO neutrophils were more resistant to DSS-induced colitis than mice receiving Wip1KO neutrophils. These data collectively indicate that Wip1 modulates host sensitivity to colitis by intrinsically regulating immune cells. The enhanced IL-17 expression in neutrophils contributed to the increased sensitivity and severity of colitis in Wip1KO mice. Thus, Wip1 may be used as a drug target to treat colitis.

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Key Words: colitis, innate immunity, acute inflammation, p38 MAPK, Wip1, neutrophils

Neutrophils (also called as polymorphonuclear neutrophil granulocytes) are one of the earliest immune cells recruited to the site of infection and inflammation to protect the host from infection.[1] In addition, it is well known that neutrophils significantly contribute to various acute and chronic pathological damages in infections, autoimmune diseases, and graft rejections.[2–4] Thus, methods to properly control the inflammatory functions of neutrophils are of central importance to balance the robust host immune response and avoid severe tissue damage.[5,6] The intracellular molecular mechanisms to accomplish this balance require further investigation.

Wild-type p53-induced phosphatase 1 (Wip1, also called PP2Cδ) is a serine/threonine protein phosphatase belonging to type 2 Cδ protein phosphatases.[7] Wip1 is overexpressed in many cancers and is recognized as a novel oncogene inhibiting several p53-dependent tumor suppressor pathways, e.g., the ATM-CHK2–p53 and p38 mitogen-activated protein kinase–p53 pathways as well as the NF-κB pathway.[8–11] Currently, Wip1 is widely believed to be a promising drug target for cancer therapy. However, Wip1 deficiency significantly impacted the maturation and homeostasis of medullary thymic epithelial cells and the T-cell development in mice.[12,13] We recently demonstrated that Wip1 critically regulates bone marrow granulocyte and B-cell development and maturation.[14,15] With LPS-induced acute lung damage and ischemia/reperfusion injury mouse models, we demonstrated that Wip1-deficient mice displayed enhanced inflammatory activity such as higher TNF-α, IL-12, IL-6, and IL-1β production as well as enhanced migration ability in a cell-intrinsic manner,[3,16] indicating that phosphatase Wip1 negatively regulates neutrophil proinflammatory cytokine production and migration. In this study, Wip1 deficiency significantly increased IL-17 production by neutrophils. The role of Wip1-regulated IL-17 production by neutrophils in the development of dextran sulphate sodium (DSS)-induced colitis in mice, which is usually used as a mouse model for inflammatory bowel disease, was studied.

MATERIALS AND METHODS

Mice

C57BL/6 (B6) and CD45.1$^+$ B6 mice were purchased from Beijing University Experimental Animal Center (Beijing, China).

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Wip1KO mice were kindly provided by the Key Laboratory of Human Diseases Comparative Medicine, the Ministry of Public Health (Beijing, China). Wip1KO mice have been described[8,12,17] and were backcrossed to the C57BL/6 genetic background in our laboratory. IL-17AKO mice were kindly offered by Dr. Zhinan Yin, Nankai University.[18,19] Wip1 and IL-17A double KO mice (DKO) were produced by breeding Wip1KO and IL-17AKO mice, which have a B6 genetic background. Six- to-12-week-old sex-matched littermate mice were used for the experiments unless otherwise noted. Experimental protocols were approved by the Animal Ethics Committee of the Institute of Zoology, Beijing, China.

Monoclonal Antibodies (mAbs) and Reagents

For a flow cytometric (FCM) analysis of surface markers, cells were stained with antibodies in phosphate buffered saline (PBS) containing 0.1% (wt/vol) bovine serum albumin and 0.1% NaN_3. The following antibodies were purchased from eBioscience: anti-CD45R/B220 (RA3-6B2), anti-CD11b (M1/70), anti-Gr-1 (RB6-8C5), anti-CD45.1 (A20), and anti-CD45.2 (104). The following antibodies were purchased from BD Biosciences: anti-CD18 (M18/2), anti-CD11b (M1/70), anti-F4/80 (BM8), anti-Ly6C (AL-21), anti-Ly6G (1-A8), and anti-CD11c (HL3). The following mAbs were acquired from Biolegend: anti-CD48 (HM48–1), anti-CD150 (TCF15–12F12.2), and anti-CD45 (30-F11); anti-CXCR2 (Clone 242216) was from R&D Systems.

Bacterial lipopolysaccharide (*E. coli* 055:B5), fluorescein isothiocyanate (FITC)-dextran (average molecular weight 4000), JNK inhibitor (SP600125), and ERK inhibitor (U0126) were purchased from Sigma-Aldrich (St. Louis, MO). DSS (molecular weight is 36,000–50,000) was purchased from MP Biomedicals (Santa Ana, California). P38 mitogen-activated protein kinase inhibitor (SB203580), NF-kB inhibitor (6-Amino-4-(4-phenoxyphenylethylamino)quinazoline), and Wip1 inhibitor (2,5-bis-(2-Thienylidene)cyclopentanone) were purchased from the Merck Group (Darmstadt, Germany). The reagents were used at the indicated or the following concentrations: TLR4 agonist (LPS, 500 ng/mL), ERK inhibitor (U0126, 10 μM), JNK inhibitor (SP600125, 10 μM), p38 mitogen-activated protein kinase inhibitor (SB203580, 10 μM), STAT1 inhibitor (MTA, 10 μM), and NF-kB inhibitor (10 nM).

Isolation of Neutrophils

Mouse neutrophils from the bone marrow or peripheral blood were isolated through a Percoll gradient.[14] The enriched neutrophil fraction was recovered at the interface between 65% and 72% Percoll. The purity of the enriched neutrophils was >80% as determined by FCM. In some cases, highly purified neutrophils were sorted by a high-speed cell sorter (MoFlo, Beckman Coulter Indianapolis, IN) using anti-Ly6G/CD11b mAbs. The purity of the sorted neutrophils was usually greater than 98%.

Induction of Experimental Colitis

Colitis was induced by feeding the mouse drinking water supplemented with 3% DSS daily, causing severe erosive colitis as described previously.[20] Bodyweight and disease activity index (DAI) were assessed on a daily basis. DAI was calculated as described previously,[21] combining weight loss, stool consistency, and stool blood content/rectal bleeding. Mice were killed at the indicated timepoints and colons were removed for further analysis.

Bone Marrow Transplantation Models

A total of 1×10^7 donor CD45.2$^+$ bone marrow cells (BMCs) from Wip1KO or CD45.2 wild-type (WT) littermates were transplanted intravenously into 8-week-old CD45.1$^+$ syngeneic mice that had been lethally irradiated with 11 Gy to set up complete or mixed chimeras as described previously.[3,22]

Histological Analyses

For colitis histopathological analyses, colons were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 5 μm sections, and subsequently stained with hematoxylin and eosin, as described previously.[23] A histological colitis score was determined as previously described.[20] In brief, histological sections were scored as the following: epithelium = normal morphology (0), loss of goblet cells (1), loss of goblet cells in large areas (2), loss of crypts (3) and loss of crypts in large areas (4); and infiltration: no infiltrate (0), infiltrate around crypts (1), infiltrate reaching the lamina muscularis mucosae (2), extensive infiltration reaching the lamina muscularis mucosae and thickening of the mucosa (3), and infiltration of the submucosal layer (4). The total histological score represents the sum of both scores and ranges from 0 to 8. For each sample, 10 fields were randomly selected and the mean grade was calculated.

FCM Assays

The freshly isolated cells were stained with fluorescently labeled mAbs and washed with PBS containing 0.1% (wt/vol) bovine serum albumin and 0.1% NaN_3. The cells were then acquired on a fluorescence activated cell sorting Calibur (Becton Dickinson, CA) or Beckman Coulter Epics XL, and data were analyzed using the CellQuest software.[24] Cell numbers of various populations were calculated by multiplication of the total cell number by the percentages of each individual population from the same mouse.

Measurement of Epithelial Permeability

Mice were exposed to 3 to 4 days of DSS treatment before oral administration of 0.6 mg/g bodyweight FITC-labeled dextran (4 kDa) by standard oral gavage. Mice were killed 3.5 hours later, and blood was removed by cardiac puncture. Plasma was separated, and FITC levels in the plasma were determined by fluorometry. The distribution of FITC–dextran in sectioned colonic tissue was determined with a two-photon laser scanning microscope (LSM510, Zeiss, Jena, Germany) as described previously.[25]

Quantitative Real-Time-polymerase Chain Reaction (PCR)

Total RNA was isolated from tissues which had been stored at −80°C by using Trizol, a total RNA extraction reagent (Invitrogen, CA), according to the manufacturer's protocol. The

quality of total RNA extracted was examined by 1% agarose gel electrophoresis using 1 μL of RNA. The concentrations were detected by Nanodrop (Thermo Fisher Scientific, Barnesville, GA). cDNA was synthesized with cDNA Reverse Transcription kit (Takara, Shiga, Japan). An Applied Biosystems (ABI) 7900HT real-time PCR system was used for quantitative PCR, with primer and probe sets from Applied Biosystems.[26] PCR primer sequences are presented in Table 1. Results were analyzed using SDS 2.1 software. The cycling threshold value of the endogenous control gene (Hprt1, encoding hypoxanthine guanine phosphoribosyl transferase) was subtracted from the cycling threshold value of each target gene to generate the change in cycling threshold. The expression of each target gene is presented as the "fold change" relative to that of unstimulated WT samples (2–DDCT), as described.[27]

Quantification of Tissue Cytokine Contents

Frozen colon samples were homogenized in ice-cold RIPA lysis buffer (Thermo Fisher Scientific). TNF-α and IL-6 concentrations were measured in whole-tissue extracts by enzyme-linked immunosorbent assay following the manufacturer's instructions (R&D Systems) and expressed as pg per mg of total proteins.

Immunoblot Analysis

Collected cells were washed twice with PBS and then lysed for 30 minutes on ice in RIPA solution containing protease inhibitor cocktails (Roche) together with phosphatase inhibitors (Sigma) as described previously.[28,29] Equivalent protein concentrations were subjected to 8% to 12% SDS-PAGE (Bio-Rad, Hercules, CA). Anti–Wip1 (H-300), anti-p-S6 (Ser235/236), anti-p-Akt (Ser473), anti-p-AKT (Thr308), anti-p-STAT1 (Ser727), and anti-p-ErK (Thr202/Tyr204) were purchased from Cell Signaling Technology. Anti-Bcl-2 (sc-7382) was from Santa Cruz Biotechnology, CA and anti-β-actin was from Sigma-Aldrich. HRP-labeled goat anti-rabbit IgG (Invitrogen) and HRP-labeled goat anti-mouse IgG were used.

Adoptive Transfer of Neutrophils

In total, 2×10^6 neutrophils ($CD11b^+Ly6G^+$) sorted from bone marrow of Wip1KO or DKO mice were injected intravenously into IL-17KO mice on days 0, 3, and 5. The IL-17KO recipient mice were induced by drinking 5% DSS water on day 0. Mice were weighted and assessed for clinical signs of colitis daily.

TABLE 1. The Primer Sequences Are Used in the Present Study

Genes	Forward Primer	Reverse Primer
MCP-1	taa aaa cct gga tcg gaa cc	gca tta gct tca gat tta cgg gt
G-CSF	cat ttt cct ggg cat act tt	cca gtg ggt cgg ttt ctt gt
CXCL1	gca ccc aaa ccg aag tca tag	aga agc cag cgt tca cca ga
CXCL2	gcc cag aca gaa gtc ata gcc	ctc ctc ctt tcc agg tca gtt a
IL-6	gca atg gca att ctg att gta tg	aag gac tct ggc ttt gtc ttt ct
IL-17A	ctc aga ct acct caa ccg ttc c	atg tgg tgg tcc agc ttt cc
TNF-α	gag tga caa gcc tgt agc c	ctc ctg gta tga gat agc aaa
Hprt	cct aag atg agc gca agt tga a	cca cag gac tag aac acc tgc taa

Bacterial Culture

Samples of stool, colon, spleen, mesenteric lymph nodes, and liver were collected from DSS-treated WT or Wip1 KO mice and grinded. The obtained suspensions were plated on heated agar plates and incubated at 37°C for 48 hours, and then bacterial colony-forming units were counted.

Statistical Analysis

All data are presented as the mean ± SD. One-way or two-way analysis of variance was used for comparison among multiple groups with SPSS 16.0 software according to the type of data. Student's unpaired *t* test for comparison of means was used to compare between the 2 groups. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

Wip1-deficient Mice are Susceptible to DSS-induced Colitis

DSS-induced colitis, characterized by innate immune inflammation of the gastrointestinal tract during early stages, is mediated by an inappropriate response to resident microbes, leading to a disturbed balance of local cytokine production with excessive release of proinflammatory cytokines such as IL-1β, TNF-α, IL-23, and IL-6.[30,31] Thus, we employed an acute DSS-induced colitis model to observe the different innate immune response of Wip1KO and WT mice. After administration of 3% DSS, Wip1KO mice displayed more severe symptoms of acute colitis than WT mice, as evidenced by significantly lower survival ratio (Fig. 1A), rapid bodyweight loss (Fig. 1B), increased DAI (Fig. 1C), and shorter colon length in Wip1KO mice (Fig. 1D). Macroscopic and histological analysis after 4 days of DSS treatment revealed a typical but severe colitis in Wip1KO mice, including massive inflammatory infiltrates and thickened wells (Fig. 1E). Furthermore, the increased epithelial permeability of the colons in WT and Wip1KO mice after exposure to DSS was evidenced by the distribution of FITC dextran in sectioned colonic tissue was determined with a two-photon laser scanning microscope and the FITC levels in the plasma (Fig. 1F). Consistently, bacterial courts in the tissues including stool, colons, spleens, and livers in DSS-treated Wip1KO mice were significantly higher than those in DSS-treated WT mice (Fig. 1G). Therefore, Wip1KO mice suffered from more severe colitis than WT control mice.

To characterize cell populations involved in mediating acute colitis, we analyzed the infiltrating inflammatory cells in colons by FCM. Before colitis induction, there was a moderately higher percentage and absolute number of neutrophils in the colons of Wip1KO mice than in WT mice, though the levels in

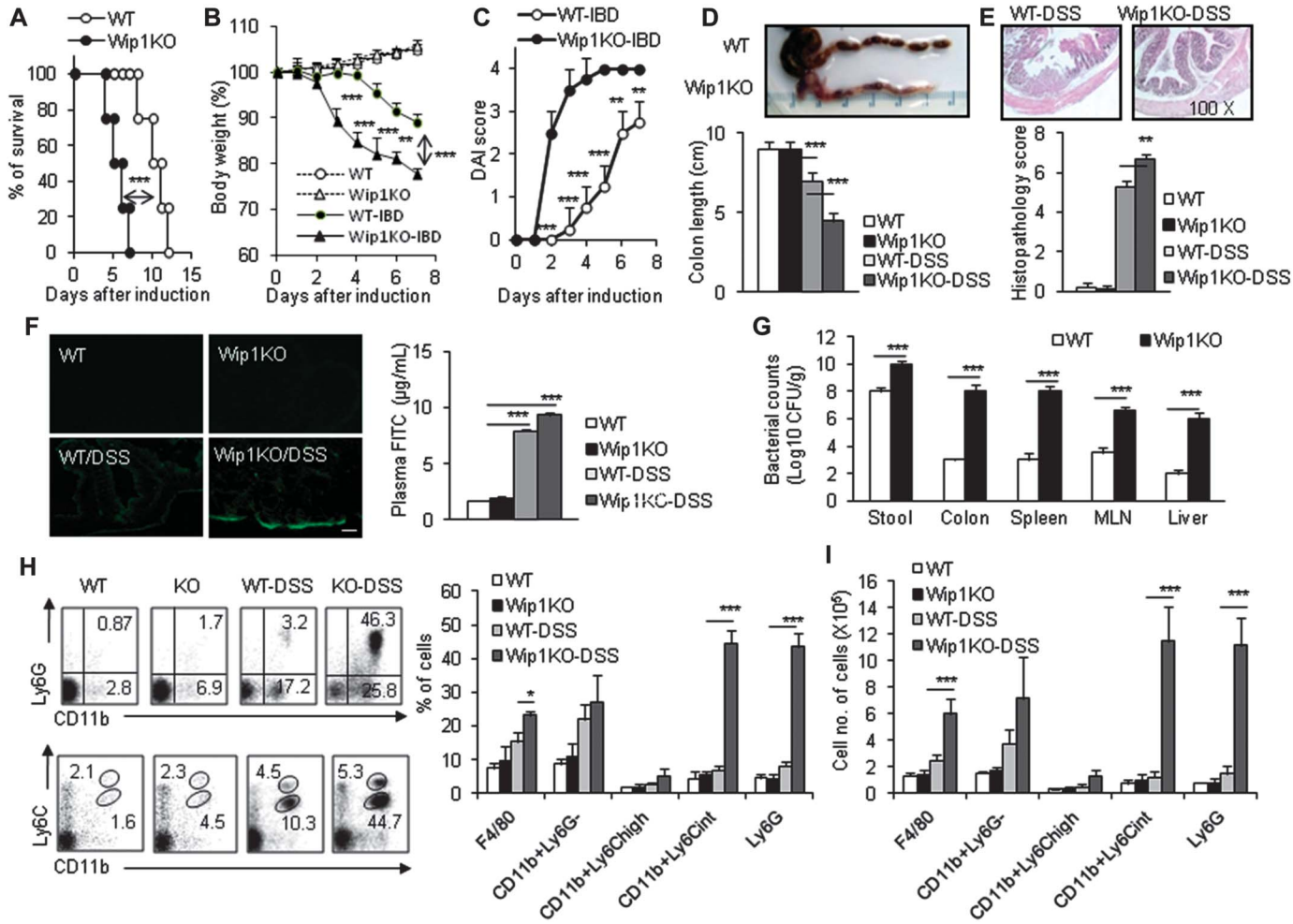

FIGURE 1. Wip1-deficient mice were susceptible to DSS-induced colitis. The percentage of mouse survival (A), bodyweight loss (B) and DAI score (C) were significantly increased in the Wip1KO mice. D, Representative colon photographs and the average colon lengths of WT and Wip1KO mice after 4 days of DSS treatment are shown. E, Histological hematoxylin and eosin(HE) staining of colon-paraffin sections of WT and Wip1KO mice after 4 days of DSS treatment are shown. Wip1KO mice showed increased mucosal leukocyte infiltration after DSS treatment. F, Fluorescence microscopy (×400) of the intestinal mucosa from DSS-treated WT and Wip1KO mice exposed to orally administrate FITC-labeled dextran shows FITC permeation into the tissue. Intestinal permeability was equal by the appearance of orally administered FITC-labeled dextran in the plasma from WT and Wip1KO mice exposed to DSS (mean ± SD, $^{***}P < 0.001$, n = 5), data are representative of 2 experiments. G, The bacterial counts in tissues were increased in Wip1KO mice compared with those of WT mice after DSS treatment. H, Representative fluorescence activated cell sorting analysis and the percentages of infiltrating neutrophils ($CD11b^+Ly6G^+$and $CD11b^+Ly6C^{low}$) and macrophages/monocytes ($CD11b^+Ly6C^{hi}$) in the gated $CD45^+$ mucosal cells are shown. I, The total number of infiltrated immune cells in the colons of WT and Wip1KO mice treated with and without DSS are shown. Data represent the mean of at least 2 independent experiments with n = 3 or 4 each time. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ (WT versus Wip1KO).

both untreated Wip1KO and WT mice were low (Fig. 1H). However, neutrophils represented the predominant mucosal cell populations in acute DSS-induced colitis in both WT and Wip1KO mice (Fig. 1H, I), as reported previously.[31] Impressively, Wip1KO mice exhibited markedly higher percentage and absolute numbers of $CD11b^+Ly6G^+$ or $CD11b^+Ly6C^{low}$ infiltrating neutrophil-like cells, moderate increases of $CD11b^+F4/80^+$ monocytes/macrophages or $CD11b^+Ly6C^{hi}$ infiltrating monocyte-like cells compared with WT mice ($P < 0.001$, Fig. 1H, I). Furthermore, we compared cytokine and chemokine expressions in colon samples from Wip1KO and WT mice before and after DSS treatment by real-time PCR and enzyme-linked immunosorbent assay. Before DSS treatment, only basal levels of these molecules could be detected. However, after DSS treatment, the expressions of CXCL1 (KC), CXCL2 (MIP-2), IL-6, IL-17A, and IL-12 were greater in the colon tissues of Wip1KO mice than in those of WT mice ($P < 0.001$, Fig. 2A). The induction of TNF-α and IL-6 protein expression in colon tissues was markedly higher in Wip1-KO mice than in WT control ($P < 0.001$, Fig. 2B). Consistent with the mRNA alterations in colon tissues, the protein levels of

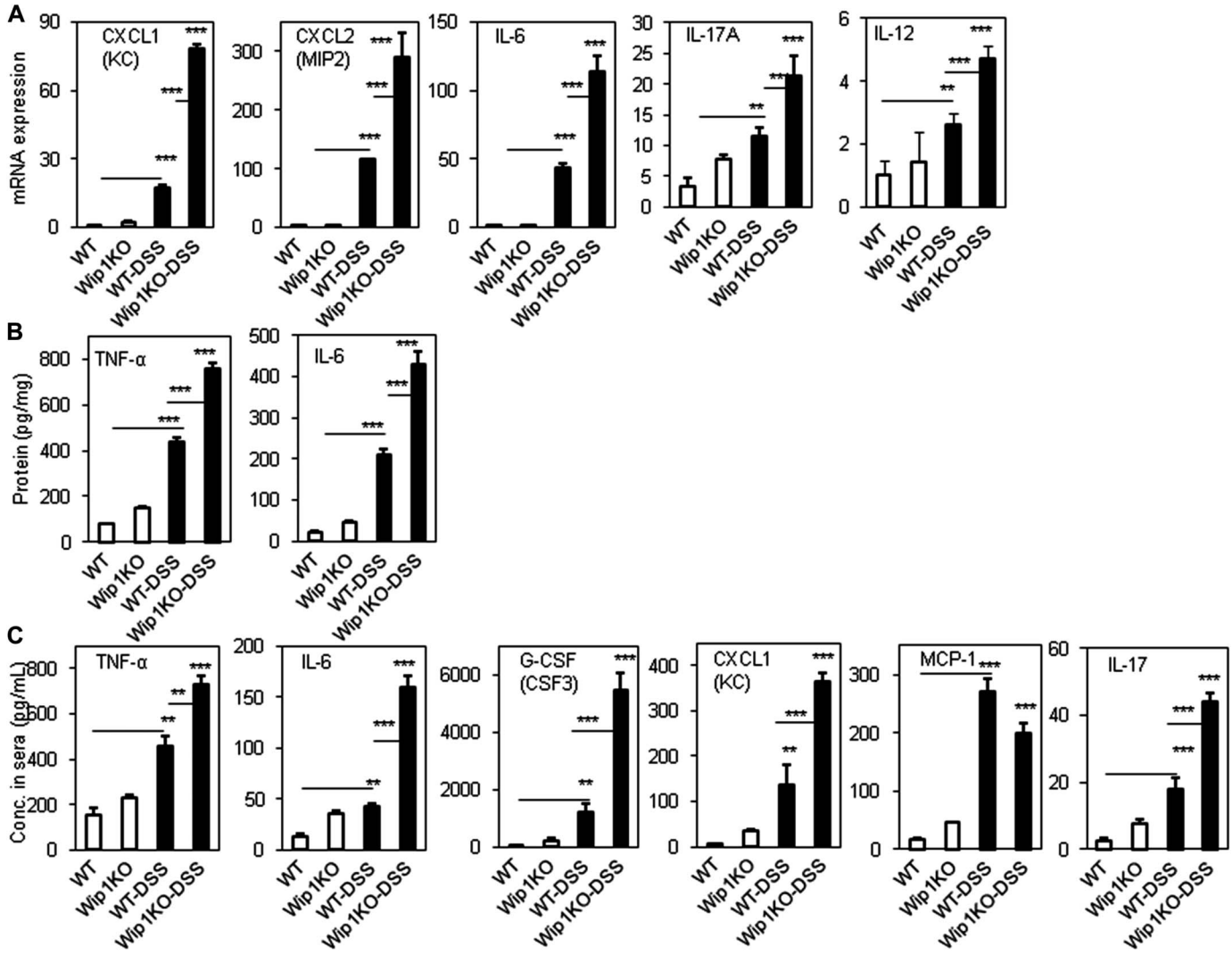

FIGURE 2. Chemokines and inflammatory cytokines were significantly increased in the colons and sera of DSS-treated Wip1KO mice. A, Mucosal chemokine (CXCL1 and CXCL2) and cytokine (IL-6, IL-17A, and IL-12) mRNA expressions were measured using real-time PCR. B, Mucosal IL-6 and TNF-α concentrations were measured by enzyme-linked immunosorbent assay (ELISA) and expressed as pg/mg total proteins in WT and Wip1KO mice treated with or without DSS. C, The concentrations of TNF-α, IL-6, G-CSF, CXCL1, MCP1, and IL-17 in the sera were detected by ELISA. Data are represented as mean ± SD. and are representative of 3 experiments (n = 4–5 mice per group). $^{**}P < 0.01$ and $^{***}P < 0.001$ (WT versus Wip1KO or between the indicated groups).

TNF-α, IL-6, G-CSF, CXCL1, CXCL2, and IL-17A were significantly higher in the sera of Wip1KO mice than those in WT mice after colitis induction ($P < 0.001$, Fig. 2C). In addition, the early pathological and cytokine changes in the intestine of WT and Wip1KO mice were detected by 3 days after DSS treatment. The pathological changes, neutrophil infiltration, and the mRNA expressions of cytokines and chemokines including CXCL1, CXCL2, IL-6, IL-17A, and TNF-α were significantly higher in DSS-treated Wip1KO mice than those in DSS-treated WT mice (see Fig. 1, Supplemental Digital Content, http://links.lww.com/IBD/B239). From these results, we concluded that Wip1 deficiency significantly increased the host sensitivity to the pathogenesis of DSS-induced colitis in mice.

Wip1 in Immune Cells Intrinsically Control the Sensitivity to DSS-induced Colitis

To define whether the deficiency of Wip1 in neutrophils is critical for the acute inflammation in these models, we performed reciprocal bone marrow transfer experiments between WT and Wip1KO mice as described in the materials and methods. Full chimera mice were confirmed by FCM assays (data not shown) and then treated with DSS to induce colitis in 12 weeks after bone marrow transplantation. WT and Wip1KO mice receiving Wip1KO BMCs showed lower survival ratios, substantial body-weight loss, increased DAI, shortened colon length, severe colon damage, and significant histological inflammatory cell infiltration in colons compared with WT and Wip1KO recipients of WT

BMCs, respectively ($P < 0.001$, Fig. 3). In contrast, Wip1KO mice receiving WT BMCs showed identical symptoms and colon damage to that of WT mice receiving WT BMCs ($P > 0.05$, Fig. 3). Consistently, FCM analysis showed that neutrophils are the predominant cells in the pathological colons (data not shown). Thus, Wip1 deficiency in hematopoietic cells exacerbates innate-cell–mediated acute colitis in the DSS-induced colitis mouse model.

IL-17 is Involved in the Increased Sensitivity of Wip1-deficient Mice to Colitis

It is well known that TNF-α and IL-6 are closely involved in the pathogenesis of DSS-induced colitis. Our recent studies showed that Wip1-deficient neutrophils produce more TNF-α and IL-6 in LPS-induced acute lung damage.[3] It is not surprising that the increased expression of local inflammatory cytokines like TNF-α and IL-6 likely contributes to the more

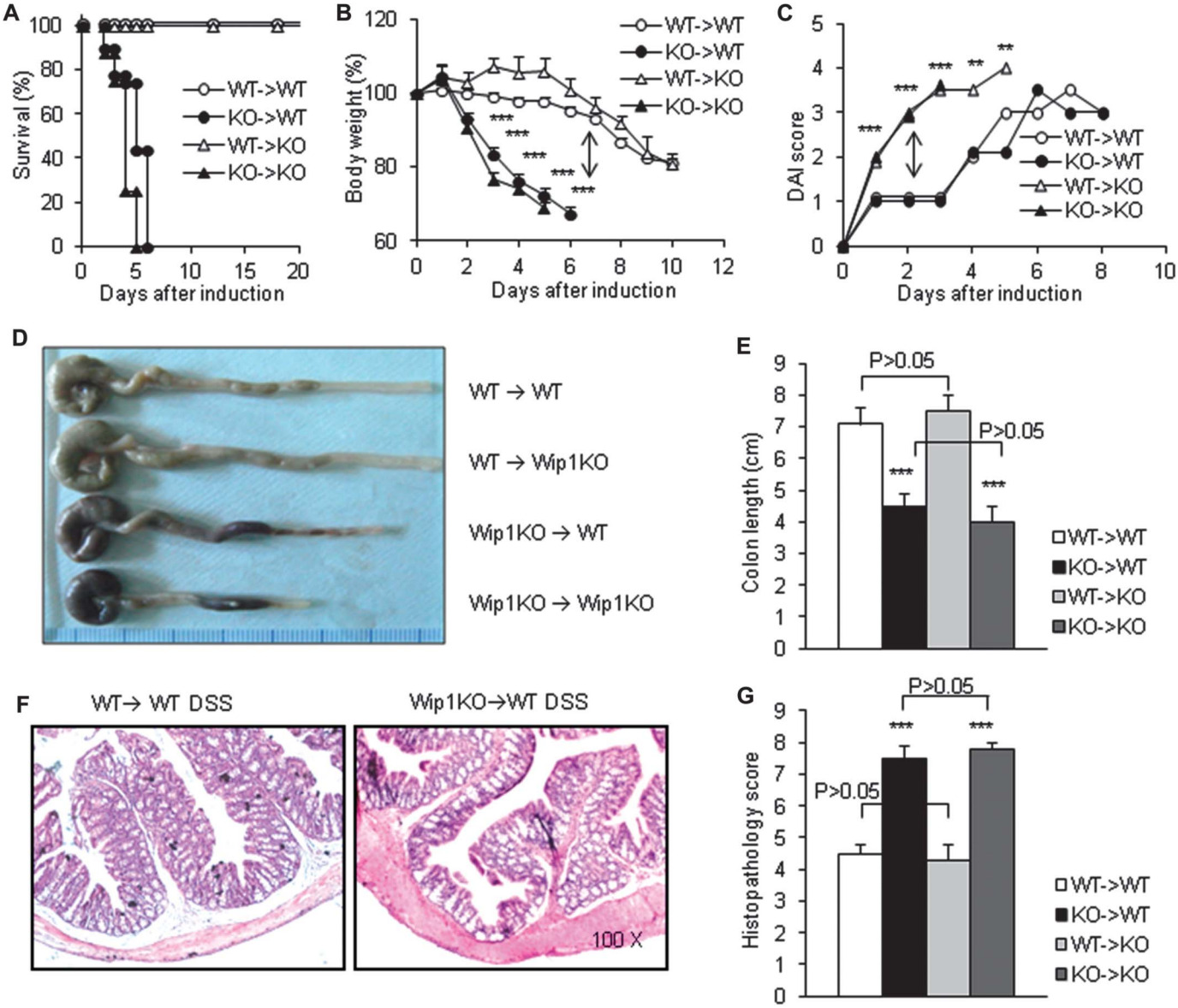

FIGURE 3. Mice with a Wip1 deficiency in immune cells were susceptible to DSS-induced colitis. The percentage of mouse survival (A), bodyweight loss (B) and DAI score (C) were significantly increased in the Wip1KO → WT and Wip1KO → Wip1KO mice. Representative colon photographs (D) and the average colon lengths (E) of WT → WT, WT → Wip1KO, Wip1KO → WT, and Wip1KO → Wip1KO mice after 5 days of DSS treatment are shown. F, Histological HE staining of colon-paraffin sections of WT → WT and Wip1KO → WT mice after 5 days of DSS treatment is shown. G, Histopathologic score of colon of WT → WT, Wip1KO → WT, WT → Wip1KO, and Wip1KO → Wip1KO mice after 5 days of DSS treatment are shown. Data are indicated as mean ± SD (n = 5) and are representative of 2 experiments. ***$P < 0.001$ (WT versus Wip1KO or the indicated groups).

severe colitis damage in Wip1KO mice. However, the significantly increased IL-17 mRNA and protein expression in Wip1KO colitis mice prompted us to investigate the role of enhanced IL-17 production by Wip1KO neutrophils in colitis pathogenesis. To address this issue, we produced Wip1 and IL-17A DKO mice by breeding Wip1KO with IL-17AKO mice. As shown in Figure 4, deletion of IL-17 alone significantly prolonged the survival of mice and decreased colon pathology compared with WT mice. Importantly, deletion of IL-17 significantly delayed the death of Wip1KO mice (Fig. 4A), rescued the bodyweight loss of Wip1KO mice ($P < 0.01$, Fig. 4B), and decreased the increased DAI score of Wip1KO mice ($P < 0.01$, Fig. 4C) in a DSS-induced colitis model. Meanwhile, the decreased colon length in DKO mice was less than that in Wip1KO mice (Fig. 4D). The colon pathological alterations in DKO mice were weaker than those in Wip1KO mice (Fig. 4E). In addition, the infiltrated neutrophils in the colon of DKO mice decreased slightly compared with Wip1KO mice after DSS-treatment as indicated by the percentages and the total number of neutrophils in the colons ($P > 0.05$, Fig. 4F, G). Thus, enhanced IL-17 production is closely involved in the increased pathogenesis of DSS-induced colitis in Wip1KO mice.

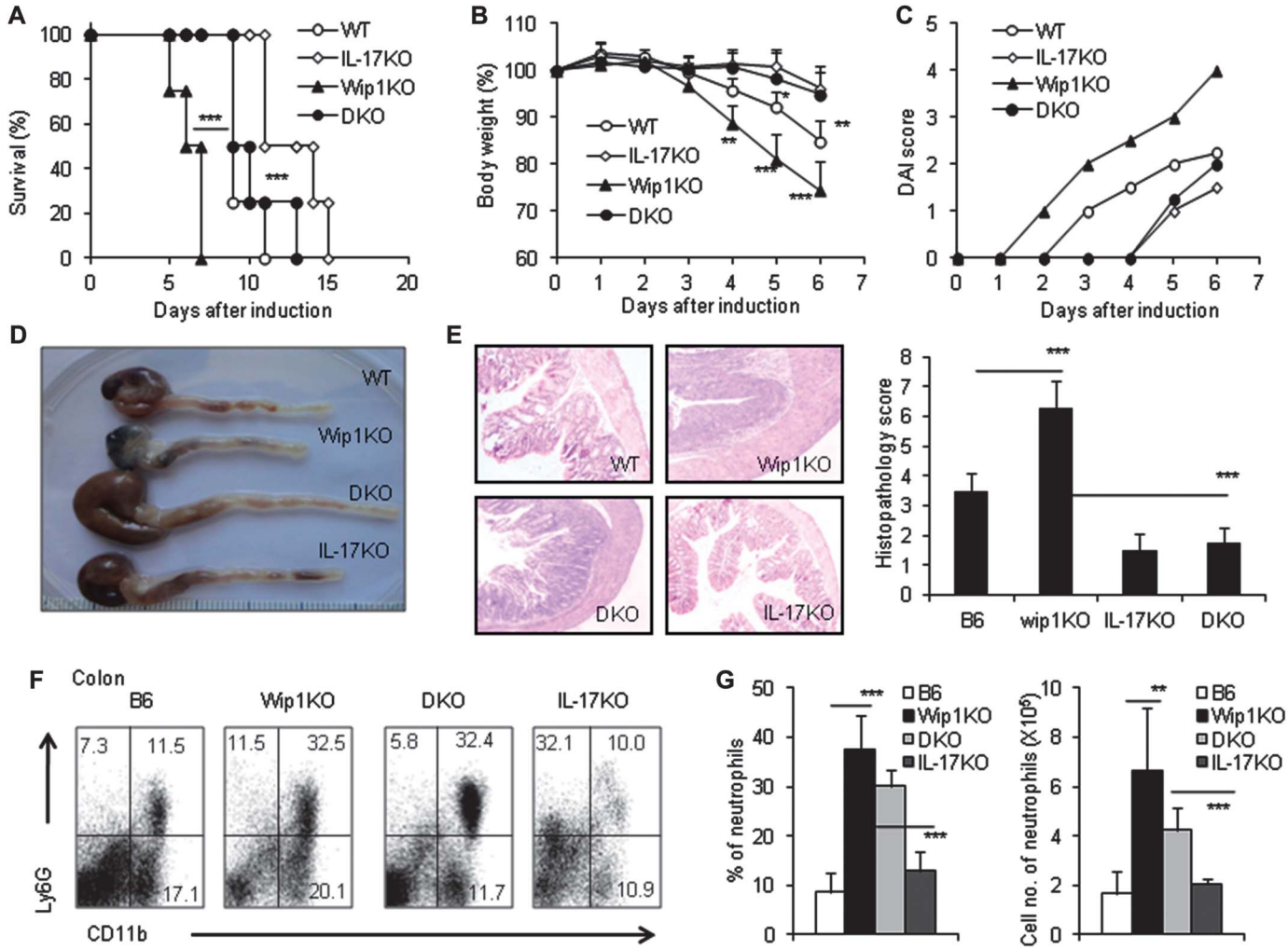

FIGURE 4. IL-17 was involved in the increased sensitivity to DSS-induced colitis in Wip1-deficient mice. DKO mice had significantly higher percentage of mouse survival (A), less bodyweight loss (B), and less DAI score (C) than those of Wip1KO mice. Representative colon photographs (D) and the average colon lengths of WT, Wip1KO, DKO, and IL-17KO mice after 5 days of DSS treatment are shown. E, Histological HE staining of colon-paraffin sections of WT, Wip1KO, DKO, and IL-17KO mice after 5 days of DSS treatment is shown. Histopathology scores of colon of WT, Wip1KO, DKO and IL-17KO mice after 5 days of DSS treatment are shown. F, Representative fluorescence activated cell sorting analysis and the percentage of infiltrating neutrophils ($CD11b^+Ly6G^+$) and macrophages/monocytes ($CD11b^+Ly6G^-$) in the gated $CD45^+$ mucosal cells are shown. G, The percentage and total number of the infiltrated neutrophils in the colons of WT, Wip1KO, DKO, and IL-17KO mice treated with DSS. Data are indicated as mean ± SD and represent 2 independent experiments with n = 3 to 5 mice per group each time. $^{**}P < 0.01$ and $^{***}P < 0.001$ (WT versus KO or the indicated groups).

IL-17 Produced by Wip1-deficient Neutrophils Promotes the Sensitivity to Colitis

Recently, it was reported that IL-17 significantly contributed to the severity of IBD in mice and humans. Our data also showed that neutrophils isolated from DSS-treated mice expressed high levels of IL-17 compared with neutrophils of untreated mice ($P < 0.001$, Fig. 5A). Meanwhile, Wip1-deficient neutrophils isolated from DSS-treated Wip1KO mice expressed significantly more IL-17 than those from DSS-treated WT mice ($P < 0.001$, Fig. 5A). To determine whether neutrophil produced IL-17 mediated the increased susceptibility to DSS-induced colitis, we adoptively transferred isolated Wip1KO or DKO neutrophils into IL-17KO mouse recipients during DSS treatment as described in the materials and methods. IL-17KO mice receiving DKO neutrophils survived significantly longer than IL-17KO mice receiving Wip1KO neutrophils ($P < 0.01$, Fig. 5B). In line with the mouse survival, IL-17KO mice receiving DKO neutrophils displayed less bodyweight loss ($P < 0.01$, Fig. 5C) and decreased DAI score ($P < 0.01$, Fig. 5D) in a DSS-induced colitis model compared with IL-17KO mice receiving

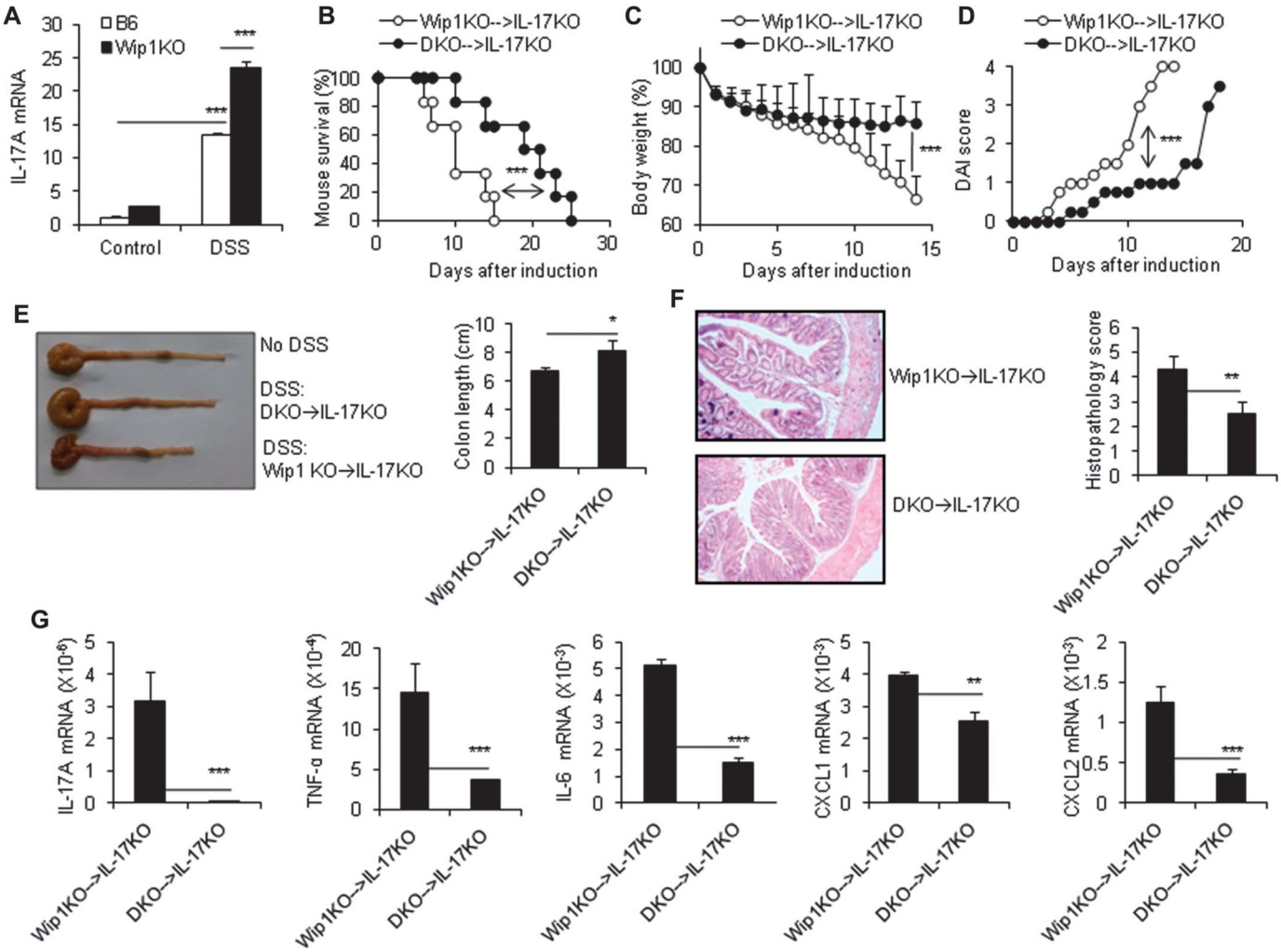

FIGURE 5. Neutrophil-produced IL-17 contributed to the increased sensitivity to DSS-induced colitis in Wip1-deficient mice. A, The IL-17 expression by isolated neutrophils from WT and Wip1KO mice treated with DSS. Wip1KO neutrophils expressed significantly more IL-17 than WT neutrophils after DSS treatment as determined by real-time PCR. IL-17KO mice were intravenously injected with either Wip1KO or DKO neutrophils and then treated with DSS to induce IBD as described in the materials and methods. IL-17KO mice receiving DKO neutrophils showed decreased mouse survival (B), less bodyweight loss (C) and lower DAI scores (D) than IL-17KO mice receiving Wip1KO neutrophils. E, Representative colon photographs and the average colon lengths of IL-17KO mice receiving Wip1KO and DKO neutrophils, respectively, after 10 days of DSS treatment are shown. F, Histological HE staining of colon-paraffin sections and the histological HE staining score of colon-paraffin sections in IL-17KO mice receiving Wip1KO or DKO neutrophils after 10 days of DSS treatment are shown. G, Mucosal chemokine (CXCL1 and CXCL2) and cytokine (TNF-α, IL-6 and IL-17A) mRNA expressions in colon tissues of IL-17KO mice receiving Wip1KO or DKO neutrophils were measured by real-time PCR after 10 days of DSS treatment. Data are indicated as mean ± SD (n = 4–5) and are representative of 2 independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (DKO versus Wip1KO or the indicated groups).

Wip1KO neutrophils. Meanwhile, the decreased colon lengths in IL-17KO mice receiving DKO neutrophils were less than that in mice receiving Wip1KO neutrophils ($P < 0.05$, Fig. 5E). Importantly, the colon pathology in IL-17KO mice receiving DKO neutrophils was weaker than that in IL-17KO mice receiving Wip1KO neutrophils ($P < 0.01$, Fig. 5F). Consistent with the severer pathological alteration, the expressions of inflammatory cytokines and chemokines such as TNF-α, IL-6, CXCL1, and CXCL2 in colon tissues of IL-17KO mice receiving DKO neutrophils were significantly higher than those in IL-17KO mice receiving Wip1KO neutrophils ($P < 0.001$, Fig. 5G) as detected by real-time PCR, whereas undetectable IL-17 expression in IL-17KO mice receiving DKO neutrophils was observed (Fig. 5G). Thus, these data indicate that neutrophil-produced IL-17, at least as one of the contributing cytokines, was closely involved in the increased pathogenesis of DSS-induced colitis in Wip1KO mice.

DISCUSSION

In this study, we provided evidence to support that IL-17 produced by neutrophils participates in the pathogenesis of DSS-induced colitis. In addition to TNF-α, IL-6, and granular proteins (elastase, gelatinase, lactoferrin, MPO, and MMP9), the enhanced IL-17 production by neutrophils was one of the reasons for the increased sensitivity of Wip1KO mice to colitis. Considering the critical role of Wip1 in neutrophil inflammatory response, supported by the present and our recent studies,[3] we speculated that Wip1 might be a potential target for therapeutic intervention to modulate neutrophil function in inflammatory conditions such as IBD.

IL-17 production by neutrophils can be induced in various models such as mucosal candidiasis, fusarium corneal infections, kidney ischemia-reperfusion injury, and psoriasis that have been recently identified.[32–36] Intra-articular injection of zymosan, a ligand for Toll-like receptor 2, causes IL-17 production by neutrophils in an arthritic mouse model.[37] The activated peritoneal neutrophils produce IL-17A and IL-23 in response to myeloperoxidase-specific anti-neutrophil cytoplasmic autoantibodies in vitro by means of their Fc region and classical complement pathway, which initiate the first steps of chronic autoimmune inflammation by allowing the local microenvironment to develop Th17-mediated autoimmunity.[38] In agreement with these reports, neutrophils isolated from DSS-treated mice expressed significantly higher IL-17. These data collectively indicate that neutrophils may be one of the major sources for IL-17 during inflammation.

Importantly, we found that the IL-17 production of neutrophils is involved in the pathogenesis of DSS-induced colitis as indicated by the studies with IL-17KO mice and mouse models with an adoptive transfer of WT or IL-17KO neutrophils. The critical role of Th17 cell subpopulation and IL-17–producing lymphoid tissue inducer cells in IBD have been previously recognized.[39,40] However, gut Th17 cells or IL-17 itself is also important for maintaining intestinal immune responses and barrier function.[41,42] The contribution of IL-17–producing neutrophils to IBD development added a more innate source for IL-17 in the pathogenesis of IBD. Furthermore, Wip1-deficient neutrophils produced more IL-17, so adoptive transfer of Wip1-deficient neutrophils promoted more severe clinical colitis symptoms and pathology including bodyweight loss, DAI score, decreased colon length, and hematoxylin and eosin staining. Deletion of IL-17 in Wip1-deficient neutrophils significantly rescued these effects in an adoptive transfer mouse model. However, the survival of these DSS-treated DKO mice is somewhat poorer than that of DSS-treated IL-17KO mice, indicating that other pathways than IL-17 may be involved in the increased pathogenesis and poor survival in Wip1KO mice. The importance of TNF-α, IL-6, and IL-12 in the pathogenesis of colitis has been highly recognized.[43–46] The increased TNF-α, IL-6, and IL-12 in Wip1KO mice likely also contributed to the poor survival after DSS treatment, which was not studied in this study. It is worthwhile to note that Wip1 is emerging as an important oncogene because of its role in regulating several key tumor suppressor pathways and is believed to be a promising drug target for cancer therapy.[47,48] This study suggests that Wip1 inhibitors might simultaneously increase the severity of IBD during their applications to treat cancers. However, increasing Wip1 expression may be used to treat IBD in the future.

In summary, Wip1-deficient mice were highly sensitive to DSS-induced colitis. Phosphatase Wip1 was an intrinsic negative regulator of IL-17 production in neutrophils. In addition to TNF-α, IL-6, and other inflammatory mediators, the enhanced IL-17 production by neutrophils significantly contributed to more severe colitis pathologies in Wip1KO mice.

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