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C-X-C Motif Chemokine Ligand 1 Promotes Colitis by Modulating the Gut Microbiota

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Keywords
CXCL1 · Gut microbiota · Colitis · Neutrophil · Inflammation

Abstract

Introduction: C-X-C motif chemokine ligand 1 (CXCL1) is a potent neutrophil chemoattractant that plays a pivotal role in recruiting neutrophils during inflammatory conditions. This study explored the role of CXCL1 in modulating the gut microbiota, influencing neutrophil infiltration, and contributing to the development of colitis. ***Methods:*** We employed quantitative PCR to assess CXCL1 expression in colon samples. A mouse model of dextran sulfate sodium (DSS)-induced colitis was utilized to explore the progression of colitis in wild-type (WT) and CXCL1-deficient (CXCL1−/−) mice. ***Results:*** Colitis attenuation was evident in CXCL1−/− mice. Significant alterations were observed in the gut microbiome, as revealed by 16S rRNA gene sequencing. Furthermore, CXCL1−/− mice exhibited reduced gut permeability and diminished endotoxin levels in peripheral blood following DSS treatment compared to WT mice. In response to DSS treatment, WT mice showed a clear increase in neutrophil infiltration, while CXCL1−/− mice exhibited lower levels of infiltration. Fecal microbiota transplantation (FMT) using stools from CXCL1−/− mice alleviated DSS-induced colitis. Interestingly, FMT from patients with colitis increased CXCL1 and Ly6G expression in the colons of gut-sterilized mice. Clinical data analysis revealed elevated CXCL1 and CD15 expression in patients with colitis, with a positive correlation between the severity of colitis and the expression of CXCL1 and CD15. ***Conclusion:*** These findings shed light on the pivotal role of CXCL1 in promoting colitis by modulating the gut microbiota.

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Introduction

Inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn's disease, constitute recurrent inflammatory disorders with a growing global incidence [1]. In the treatment of IBDs, anti-cytokines have been employed as adjuncts to conventional therapies. For instance, infliximab, an anti-TNF-α agent, acts to suppress adaptive immune system activity, effectively mitigating IBD symptoms [2]. Nevertheless, a subset of patients with IBD exhibit resistance to anti-cytokine treatments, or these therapies fall short in maintaining long-term clinical remission in more than half of cases [3, 4]. Other biological agents explored for IBD treatment, such as monoclonal IL-17 antibodies, IL-6, and CCR9 inhibitors, exhibit limited treatment efficiency and pose risks of side effects, including increased susceptibility to infections and autoimmunity [5]. Consequently, efforts to identify the mechanisms underpinning IBD onset and progression, coupled with the identification of novel therapeutic agents characterized by minimal toxicity and increased safety, remains or paramount clinical importance for IBD treatment.

Colitis, characterized pathologically by ulceration and mucosal damage, arises from various sources, including injury, infection, and inflammation [6]. Throughout the course of colitis development, innate immune cells, such as neutrophils, play crucial roles in both inflammation resolution and compromised host defense mechanisms. C-X-C motif chemokine ligand 1 (CXCL1), a potent neutrophil chemoattractant predominantly expressed in neutrophils, monocytes, and macrophages, exhibits a positive correlation with the inflammatory status in patients with IBD [7, 8]. Notably, the serum concentration of CXCL1 in patients with IBD significantly surpasses that in healthy donors [8]. Furthermore, the serum levels of CXCL1 exhibit a strong association with the severity of IBD, with observed decrease following treatment [9]. Prior investigations have indicated that CXCL1-deficient (CXCL1−/−) mice display increased susceptibility to DSS-induced colitis [10]. Collectively, these studies underscore the critical roles played by neutrophils and CXCL1 in the context of IBD and colitis development.

The gut microbiota is a pivotal regulator of immune system function, tumor development, dietary and pharmaceutical compounds metabolism, intestinal epithelium maintenance, and even behavioral via the gut-brain axis [11–13]. Colitis is widely believed to originate from an imbalance between mucosal immunity and the gut microbiota [14], a balance that is notably disrupted in patients with colitis compared to their healthy donors [15]. While previous research has shown that CXCL1−/− mice exhibit increased susceptibility to DSS-induced colitis [10], the precise role of the gut microbiota in influencing susceptibility to DSS-induced colitis in the context of CXCL1 deficiency remains elusive. This knowledge gap prompted our investigation into whether and how alterations in the gut microbiota of CXCL1−/− mice influence the development of DSS-induced colitis. This study aimed to elucidate the mechanisms through which the CXCL1/neutrophil-gut microbiota axis regulates colitis.

Materials and Methods

Clinical Samples

Clinical samples (blood, stool, and colon tissues) were collected from a total of 20 patients with colitis and 20 healthy donors at Shanghai General Hospital (Shanghai, China). Written informed consent was obtained from all patients and healthy donors. The use of human samples in this study was approved by the Ethics Committee of Shanghai General Hospital.

Histological Score

The histological score, comprising ulceration, inflammatory infiltration, and crypt damage, was assessed as previously described [16]. Ulceration was scored as follows: 0 for absence, 1 for focal and small ulcers, 2 for frequent small ulcers, and 3 for large areas devoid of surface epithelium. Inflammatory infiltration was graded as 0 for no infiltration; 1 for occasional infiltration into the submucosa; 2 for obvious infiltration only in the submucosa; 3 for infiltration in both the lamina propria (LP) and submucosa; 4 for substantial infiltration in the LP, submucosa, and surrounding blood vessels; and 5 for transmural inflammation. Crypt damage was scored as 0 for no damage; 1 for slight crypt damage; 2 for the loss of goblet cells, larger spaces between crypts, and some shortening of crypts; 3 for large areas lacking crypts; and 4 for the absence of crypts.

Mouse Models

All in vivo procedures received approval from the Animal Research Committee at Shanghai General Hospital. Wild-type (WT) and CXCL1−/− mice on a C57BL/6 background were obtained from GemPharmatech Co., China. Briefly, the CXCL1 vector was introduced into embryonic cells via electroporation, and heterozygous embryonic cells were subsequently injected into C57BL/6J blastocysts. The resultant males were bred with C57BL/6J females, yielding fertile CXCL1−/− mice without any discernible abnormalities. Both WT and CXCL1−/− mice were housed under identical specific pathogen-free conditions, including temperature, diet, and light cycles. Colitis was induced in both WT and CXCL1−/− mice using 2.5% dextran sulfate sodium (DSS, MP Biomedicals, colitis grade, 36,000–50,000) in their drinking water for a duration of 7 days, with fresh DSS water replenished every 2 days. The mice were euthanized on day 9.

Single-Cell Isolation from Colon Tissues

The isolation of single cells from colon tissue was performed in accordance with a previously published protocol [17]. In brief, colon tissue segments were incubated in 30 mL of extraction media (30 mL RPMI + 93 μL 5% [w/v] dithiothreitol + 60 μL of 0.5 M EDTA + 500 μL of fetal bovine serum [FBS]) at 37°C for 15 min. Subsequently, the minced tissues were combined with 25 mL of digestion media (25 mL of RPMI + 12.5 mg of dispase + 37.5 mg of collagenase II + 300 μL of FBS), and stirred at 500 rpm at 37°C for 30 min. The resulting digest was filtered through a 100-μm cell strainer into a 50 mL tube. The strainer was rinsed with 20 mL of RPMI containing 10% FBS. The filtered solution was centrifuged at 500 × *g* for 10 min at 4°C, and the pellet was resuspend in 1 mL of RPMI containing 10% FBS, rendering the cells ready for flow cytometry analysis.

Fecal Microbiota Transplantation

We performed fecal microbiota transplantation (FMT) experiment as follows. For Figure 5a, the cecum stool from 5 WT or CXCL1−/−mice were collected into 10 mL anaerobic sterile glycerol and were stored in −80 until use. C57BL/6 mice were treated with antibiotic cocktail (ABX, 0.5 g/L neomycin, 0.5 g/L vancomycin, and 0.5 g/L primaxin) for 3 weeks. Then, the mice were orally gavaged using 200 μL cecum stool solution in anaerobic sterile glycerol for 3 times. For Figure 5d, stool samples from healthy donor (HD) and colitis patients were used for FMT in ABX-treated C57BL/6 mice. C57BL/6 mice were treated with antibiotic cocktail (ABX, 0.5 g/L neomycin, 0.5 g/L vancomycin, and 0.5 g/L primaxin) for 3 weeks. Then, the mice were garaged with 100 μL stool sample (1 g stool dissolved in 30 mL Luria-Bertani [LB] medium containing 15% glycerol under anaerobic conditions) at weeks 3–7 (twice per week). After FMT, the mice were sacrificed for further experiments.

RT-qPCR

RNA extraction from mouse colon tissues was performed using TRIzol reagent (Invitrogen) following the manufacturer's instructions. Subsequently, cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa). The primer sequences utilized in this study are provided in online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000535637). Relative mRNA expression levels were detected using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the reference gene.

Flow Cytometry

Single cells isolated from colon tissues underwent staining with antibodies at 4°C for 15 min. The antibodies employed in this study included anti-CD145-FITC (103108, BioLegend), anti-CD11b-PE (101208, BioLegend), and anti-Gr1-APC (108412, BioLegend). Neutrophils were identified based on the marker $CD45^{+}CD11B^{+}Gr1^{high}$.

Endotoxin Assay

Peripheral blood was collected in endotoxin-free tubes, followed by centrifugation at 10,000 × *g* for 5 min to obtain plasma. The endotoxin concentration in the plasma was determined using the GenScript endotoxin assay kit (L00250) as per the manufacturer's instructions.

ELISA

The concentrations of CXCL1 in serum were quantified using an ELISA kit (RAB0116, Sigma). Additionally, the concentrations of Lipocalin-2 in both serum and fecal samples were measured using a mouse Lipocalin-2/NGAL ELISA kit (MLCN20, R&D System) in accordance with the manufacturer's protocol.

Gut Permeability Assay

Mice were subjected to a 4 h fast, followed by oral administration of 440 mg/kg body FITC-dextran (4 KDa, Sigma). After 4 h, blood was collected for FITC concentration detection in serum using a fluorescence plate reader at wavelengths 485 nm/528 nm.

16S rRNA Sequencing

Stool DNA was extracted using the QIAGEN stool DNA extraction kit. Subsequently, all samples underwent 16S rDNA V4 region sequencing on an Illumina MiSeq platform. The primers were designed with adapters for single-index barcodes and MiSeq sequencing for pooling and sequencing the PCR products. A minimum of 10,000 reads were obtained for each sample. Read-pair demultiplexing was performed based on unique molecular barcodes introduced during library generation. Diversity analyses were carried out using the diversity plugin (https://github.com/qiime2/q2-diversity). The difference of microbiome at family level was determined using two-way ANOVA.

Bioinformatic Analysis

The 16S rRNA sequencing data were aligned using Clustal W in MEGA5.0 Software to generate a cladogram analysis using the Neighbor-Joining method [18]. The data we used for principal coordinate analysis (PCoA) are the sequence data, which are produced by 16S rRNA sequencing of the stools from different groups of mice. Beta diversity analysis was performed using UniFrac to compare the results of the principal coordinate analysis (PCoA) [19]. All statistical analyses were performed using R stats package. One-way ANOVA was performed to assess the statistical significance of the differences in the diversity indices. Differences were considered statistically significant at $p < 0.05$.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism version 8. Significance between groups was assessed using Student's unpaired *t* test or two-way ANOVA, with a significance level set at $p < 0.05$.

Results

Involvement of CXCL1 in Colitis Development

To investigate the role of CXCL1 in colitis, we initiated a colitis mouse model using DSS in both WT and CXCL1−/− mice. Following DSS treatment, the body weights of both WT and CXCL1−/− mice significantly decreased compared to those of mice treated with H_2O. Notably, CXCL1−/− mice exhibited reduced sensitivity to DSS treatment, as evidenced by less pronounced body weight loss when compared to WT mice (CXCL1−/− 9%

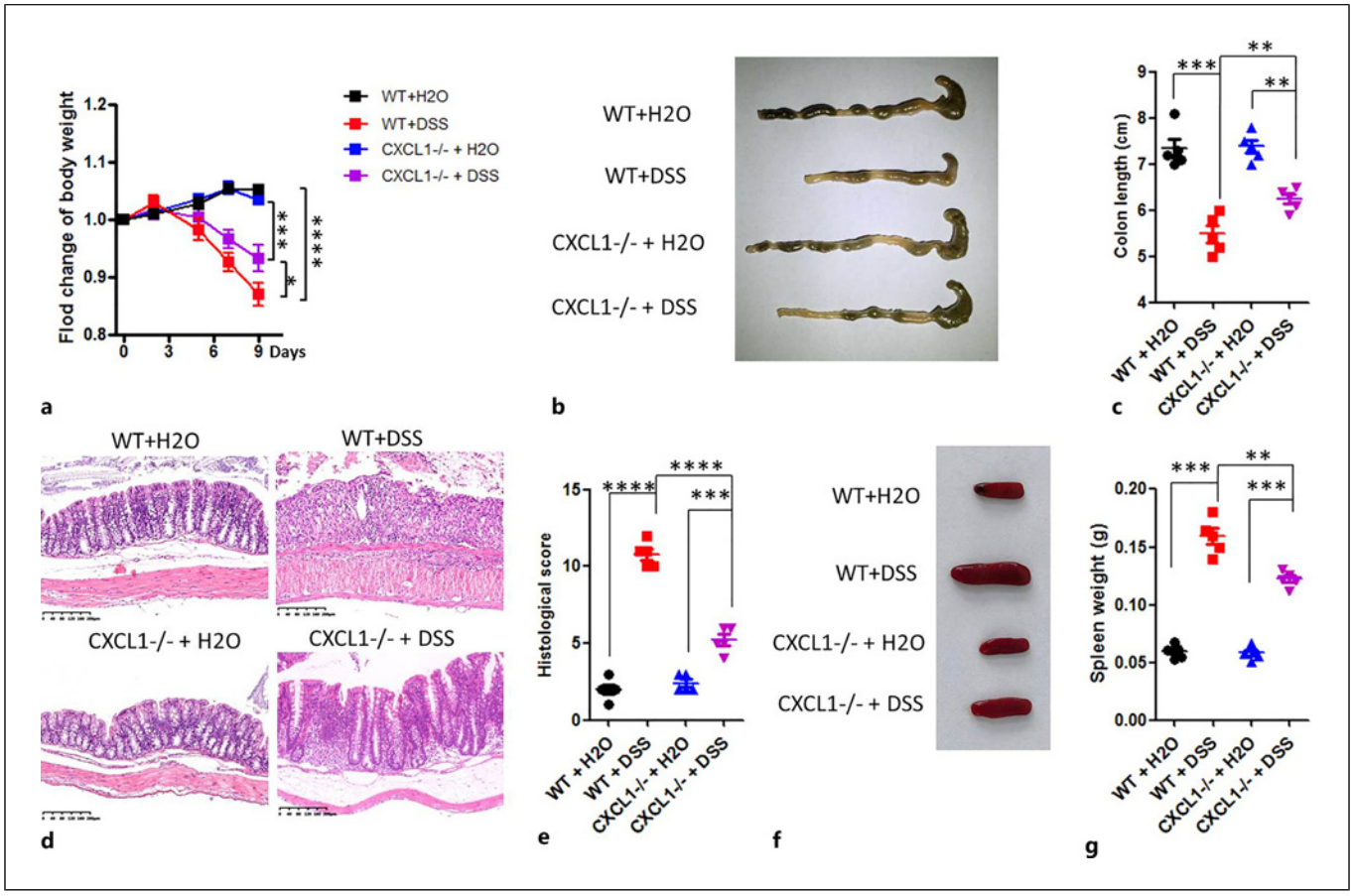

Fig. 1. CXCL1 is involved in colitis. **a** WT and CXCL1−/− mice were challenged using 2.5% DSS in drinking water (WT + DSS, CXCL1−/− + DSS) or normal water (WT + H_2O, CXCL1−/− + H_2O) for 7 days. The fold-change of body weights was measured. **b** Representative images of colons from different groups of mice. **c** The colon length of different groups of mice was detected. $n = 5$ per group. $^{**}p < 0.01$, $^{***}p < 0.001$, two-way ANOVA. **d** Representative H&E staining of colon tissues from different groups. **e** Histological score of colon tissues from different groups. $n = 5$ in each group. $^{***}p < 0.001$, $^{****}p < 0.0001$, two-way ANOVA. **f** Representative images of the spleen from different groups of mice. **g** The spleen weight of different groups. $n = 5$ in each group. $^{***}p < 0.001$, $^{****}p < 0.0001$, two-way ANOVA.

loss vs. WT 15% loss on day 9) (Fig. 1a). Furthermore, colon length significantly decreased in both WT and CXCL1−/− mice after DSS treatment (WT + H_2O vs. WT + DSS, CXCL1−/− + H_2O vs. CXCL1−/− + DSS). Importantly, the colon length of CXCL1−/− + DSS mice was greater than that of WT + DSS mice (Fig. 1b, c). Histological examination via hematoxylin and eosin (H&E) staining revealed the induction of colitis in both WT and CXCL1−/− mice following DSS treatment, with colitis severity being milder in CXCL1−/− mice compared to WT mice (Fig. 1d), as confirmed by the histological score (Fig. 1e). Additionally, spleen weights significantly increased after DSS treatment in both WT and CXCL1−/− mice (WT + H_2O vs. WT + DSS, CXCL1−/− + H_2O vs. CXCL1−/− + DSS). Notably, the spleen weight of CXCL1−/− + DSS mice was lower than that of WT + DSS mice (Fig. 1f, g).

Furthermore, we measured the concentration of lipocalin 2 in the serum and feces of mice from different groups, given its status as a marker for IBD [20, 21]. As shown in Figure 2a, b, the lipocalin 2 concentration significantly increased in both serum and feces of WT and CXCL1−/− mice after DSS treatment (WT + H_2O vs. WT + DSS, CXCL1−/− + H_2O vs. CXCL1−/− + DSS). Importantly, the lipocalin 2 concentration in the serum and feces of CXCL1−/− + DSS mice was significantly lower than that in WT + DSS mice. We also detected inflammatory cytokines (IL-1β, TNF-α, IFN-γ, and TGF-β) in colon tissues using RT-PCR. As expected, cytokine expression levels significantly increased after DSS

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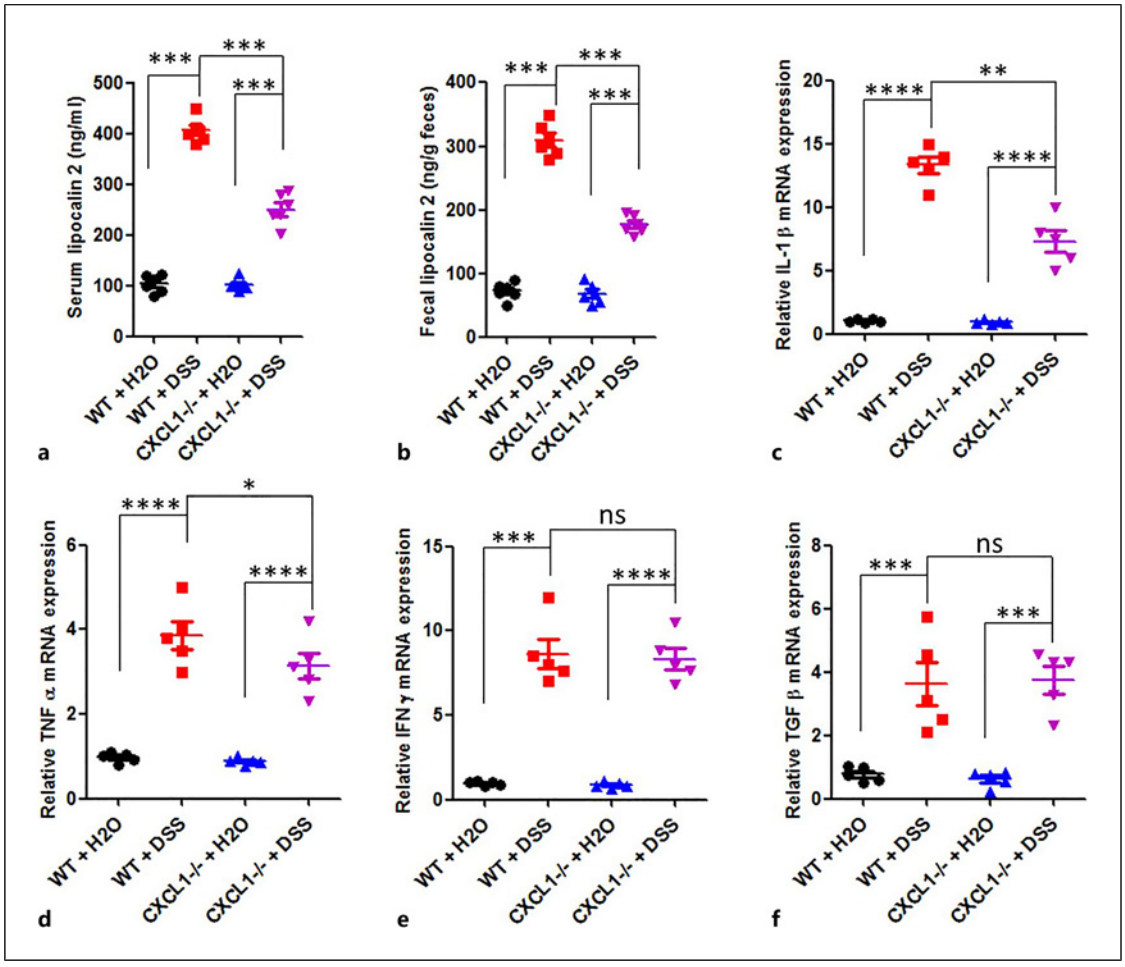

Fig. 2. Inflammatory biomarker levels increased in the colitis model. The lipocalin 2 concentration in the serum (**a**) and fecal samples (**b**) of different groups of mice was detected. $n = 5$ in each group. ***$p < 0.001$; ns, no significance; two-way ANOVA. **c–f** Relative expression levels of IL-1-β, TNF-α, IFN-γ, and TGF-β at mRNA levels in the colon tissues of different groups. $n = 5$ in each group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, no significance; two-way ANOVA.

treatment in both WT and CXCL1−/− mice. Interestingly, CXCL1−/− mice exhibited lower levels of IL-1β and TNF-α compared to WT mice after DSS treatment, while no significant difference was observed in IFN-γ and TGF-β levels between WT and CXCL1−/− mice after DSS treatment (Fig. 2c–f). These results indicate that CXCL1−/− mice displayed reduced sensitivity to colitis development following DSS treatment.

It is noteworthy that our findings contrast with a previous study that reported increased susceptibility to DSS-induced colitis CXCL1−/− mice [10]. However, the role of gut microbiota in influencing susceptibility to DSS-induced colitis remains unclear. This promoted us to investigate whether and how alterations in the gut microbiota of CXCL1−/− mice affect DSS-induced colitis.

CXCL1-Mediated Alterations in Gut Microbiota during DSS-Induced Colitis

Given the essential role of gut microbiota in colitis development [22–25], we further delved into the impact of gut microbiota on the disparity in colitis between WT and CXCL1−/− mice. Stool samples were collected from mice in different groups (WT + H_2O, WT + DSS, CXCL1−/− + H_2O, and CXCL1−/− + DSS) for 16S rRNA sequencing. As reported, 16S rRNA gut microbiome sequencing demonstrated differences in the gut microbial profile after DSS treatment in both WT and CXCL1−/− mice (WT + H_2O vs. WT + DSS, CXCL1−/− + H_2O vs. CXCL1−/− + DSS), while no significant difference was observed between CXCL1−/− + H_2O and WT + H_2O mice. Significant changes were observed at the family

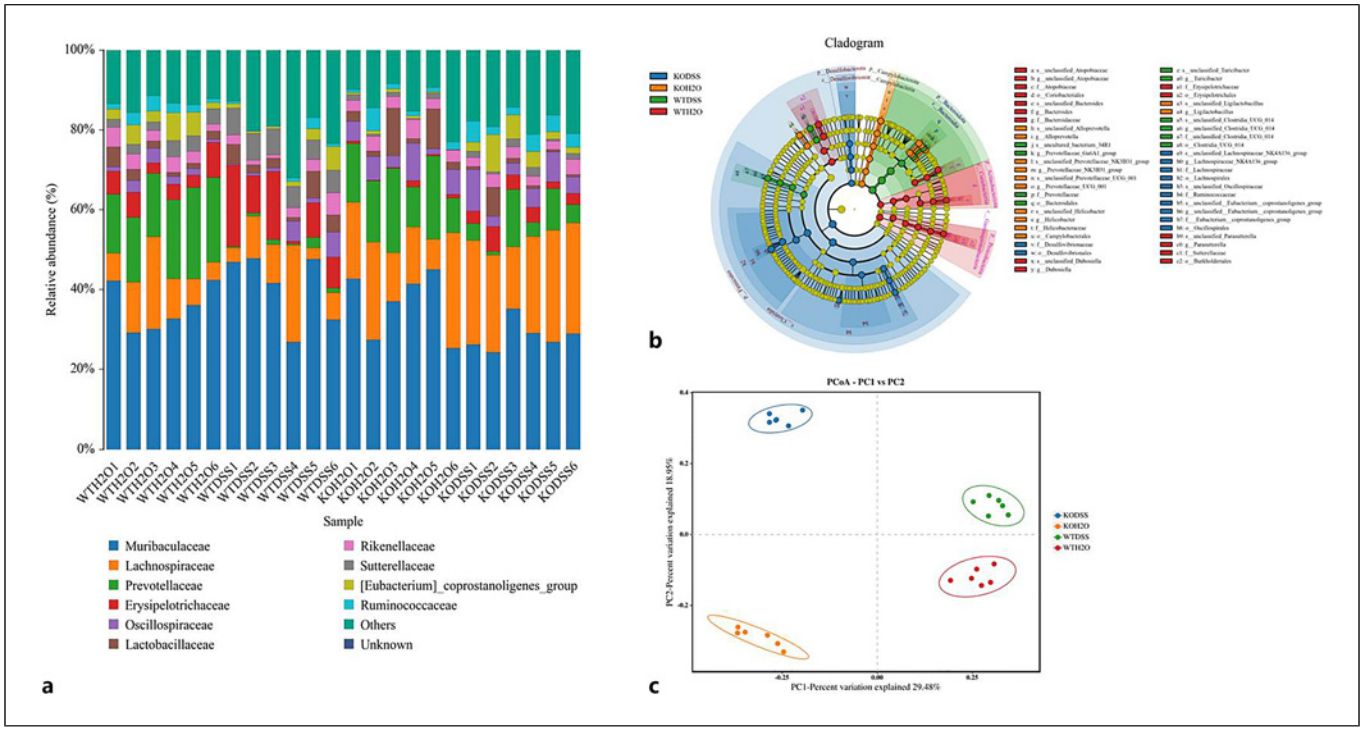

Fig. 3. CXCL1 alters the gut microbiota in DSS-induced colitis. **a** WT and CXCL1−/− mice challenged using 2.5% DSS in drinking water (WT + DSS, CXCL1−/− + DSS) or normal water (WT + H_2O, CXCL1−/− + H_2O) for 7 days. Stool samples from different groups were obtained for 16S rRNA sequencing. $n = 6$ for each group. Bar plots of the family levels in different groups are shown. Relative abundance is plotted for each sample. **b** Cladogram analysis was carried out using the data from A to identify the difference in the gut microbiota of different groups. **c** Principal coordinates analysis (PCoA) was carried out to detect the difference in the gut microbiota in different groups.

level, particularly in *Erysipelotrichaceae and Prevotellaceae* (Fig. 3a). These results indicated an increased abundance of *Erysipelotrichaceae* and a decreased abundance of *Prevotellaceae* following DSS treatment. Furthermore, CXCL1−/− mice exhibited fewer *Erysipelotrichaceae* and more *Prevotellaceae* than WT mice after DSS treatment (CXCL1−/− + DSS vs. WT + DSS). Cladogram analysis also highlighted an altered profile of commensal gut bacteria after DSS treatment in both WT and CXCL1−/− mice, with differences observed between the two groups, particularly after DSS treatment (Fig. 3b). Principal coordinate analysis demonstrated distinct separation among the four groups (Fig. 3c). Collectively, these results suggest that CXCL1−/− had minimal impact on the gut microbiome under normal conditions, but significantly influenced its composition during DSS-induced colitis.

Interestingly, alpha diversity analysis (Chao1, Shannon, and Simpson indices) indicated that DSS treatment alone did not affect gut microbiome diversity. However, CXCL1−/− mice exhibited significantly higher diversity compared to WT mice (online suppl. Fig. 1a), indicating the involvement of CXCL1 in gut microbiome diversity. Additionally, there were fewer Gram-negative bacteria and more Gram-positive bacteria in CXCL1−/− mice after DSS treatment than in the other three groups (online suppl. Fig. 1b).

Gut Permeability and Neutrophil Infiltration Changes in CXCL1−/− Mice

The observation of fewer Gram-negative bacteria in CXCL1−/− mice following DSS treatment led us to investigate the concentration of lipopolysaccharide (LPS), the main product of Gram-negative bacteria, in peripheral blood. We found that DSS treatment increased LPS concentration in both WT and CXCL1−/− mice (WT + H_2O vs. WT + DSS, CXCL1−/− + H_2O vs. CXCL1−/− + DSS), with the LPS concentration in CXCL1−/− + DSS mice being lower than that in WT + DSS mice (Fig. 4a). Additionally, we assessed gut permeability, which was enhanced by DSS treatment in both WT and CXCL1−/− mice. Importantly, gut permeability of CXCL1−/− mice was lower than that in WT mice after

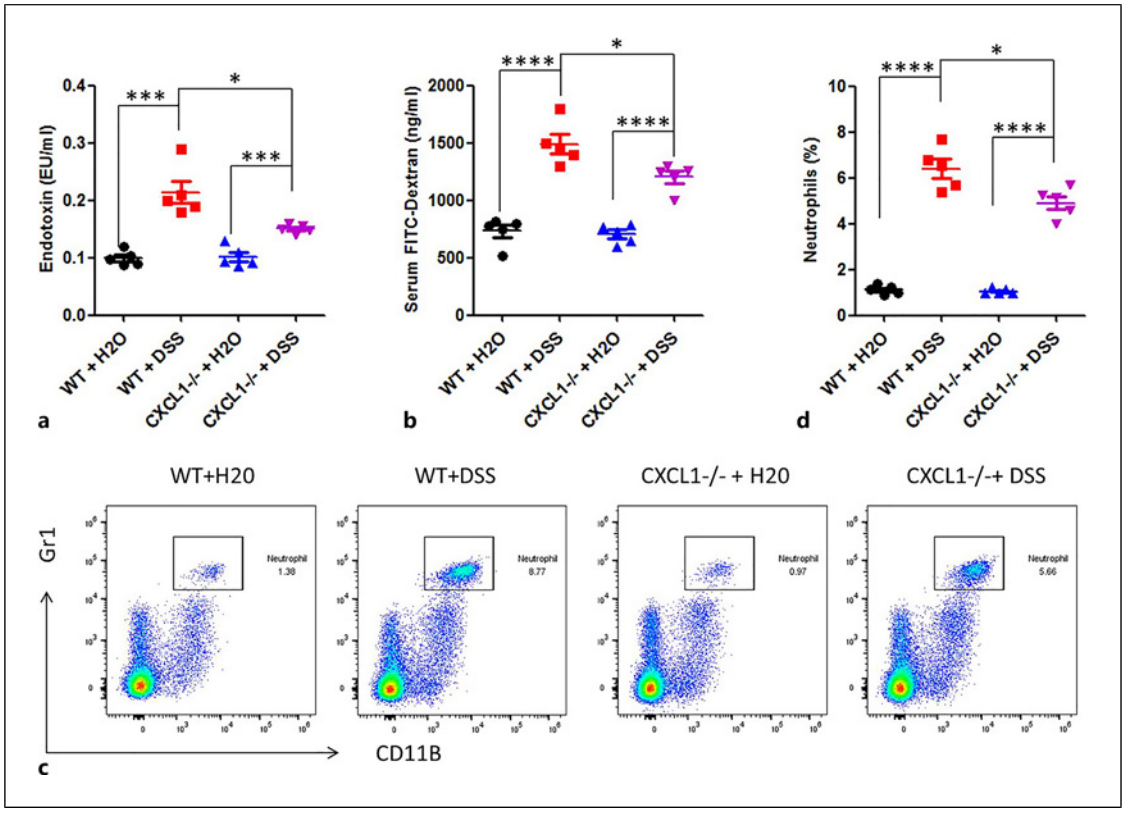

Fig. 4. CXCL1 changes the gut permeability and neutrophil infiltration. **a** WT and CXCL1−/− mice were challenged using 2.5% DSS in drinking water (WT + DSS, CXCL1−/− + DSS) or normal water (WT + H_2O, CXCL1−/− + H_2O) for 7 days. Peripheral blood from different groups was collected for endotoxin detection. $n = 5$ for each group. *$p < 0.05$, ***$p < 0.001$; two-way ANOVA. **b** WT and CXCL1−/− mice were challenged using 2.5% DSS in drinking water (WT + DSS, CXCL1−/− + DSS) or normal water (WT + H_2O, CXCL1−/− + H_2O) for 7 days. The mice were orally gavaged using 440 mg/kg FITC-dextran. Blood was collected after 4 h, followed by measuring the FITC-dextran concentration. $n = 5$ for each group. *$p < 0.05$, ****$p < 0.0001$, two-way ANOVA. **c** Representative flow cytometry picture to stain $CD11B^{+}Gr1^{high}$ neutrophils in colon tissues of different groups of mice. **d** Frequency of neutrophils from different groups of mice. $n = 5$ for each group. *$p < 0.05$, ***$p < 0.001$; two-way ANOVA.

DSS treatment, suggesting a correlation between CXCL1 and gut permeability (Fig. 4b). We also examined the presence of neutrophils in colon tissues (online suppl. Fig. 2a). As expected, the number of neutrophils in colon tissues increased significantly after DSS treatment in both WT and CXCL1−/− mice. Moreover, the number of neutrophils in the colon tissues of CXCL1−/− mice was lower than that in WT mice after DSS treatment (Fig. 4c). Furthermore, the proportion of neutrophils in the peripheral blood of mice from different groups was determined using flow cytometry (online suppl. Fig. 2b). After DSS treatment, the proportion of neutrophils in peripheral blood significantly increased in both WT and CXCL1−/− mice, but the increase was less pronounced in CXCL1−/− mice compared to WT mice. These findings suggest that CXCL1 depletion reduced gut permeability and neutrophil infiltration in DSS-induced colitis.

FMT Using CXCL1−/− Mice Stools Mitigated Colitis

To gain further insights into the role of the gut microbiome in colitis, we performed FMT in gut-sterilized mice. These mice had been treated with an antibiotic cocktail for 3 weeks [26]. Subsequently, FMT was then carried out using stools from either WT or CXCL1−/− mice, followed by colitis induction using 2.5% DSS in drinking water (Fig. 5a). Mice transplanted with CXCL1−/− mouse stools displayed less severe colitis compared to those transplanted with WT mouse stools (Fig. 5b, c), suggesting that the gut microbiome of CXCL1−/− mice conferred resistance to DSS-induced colitis.

We further investigated whether stools from patients with colitis could induce colitis and neutrophil infiltration in gut-sterilized mice. Stool samples were collected from both patients with colitis and healthy donors

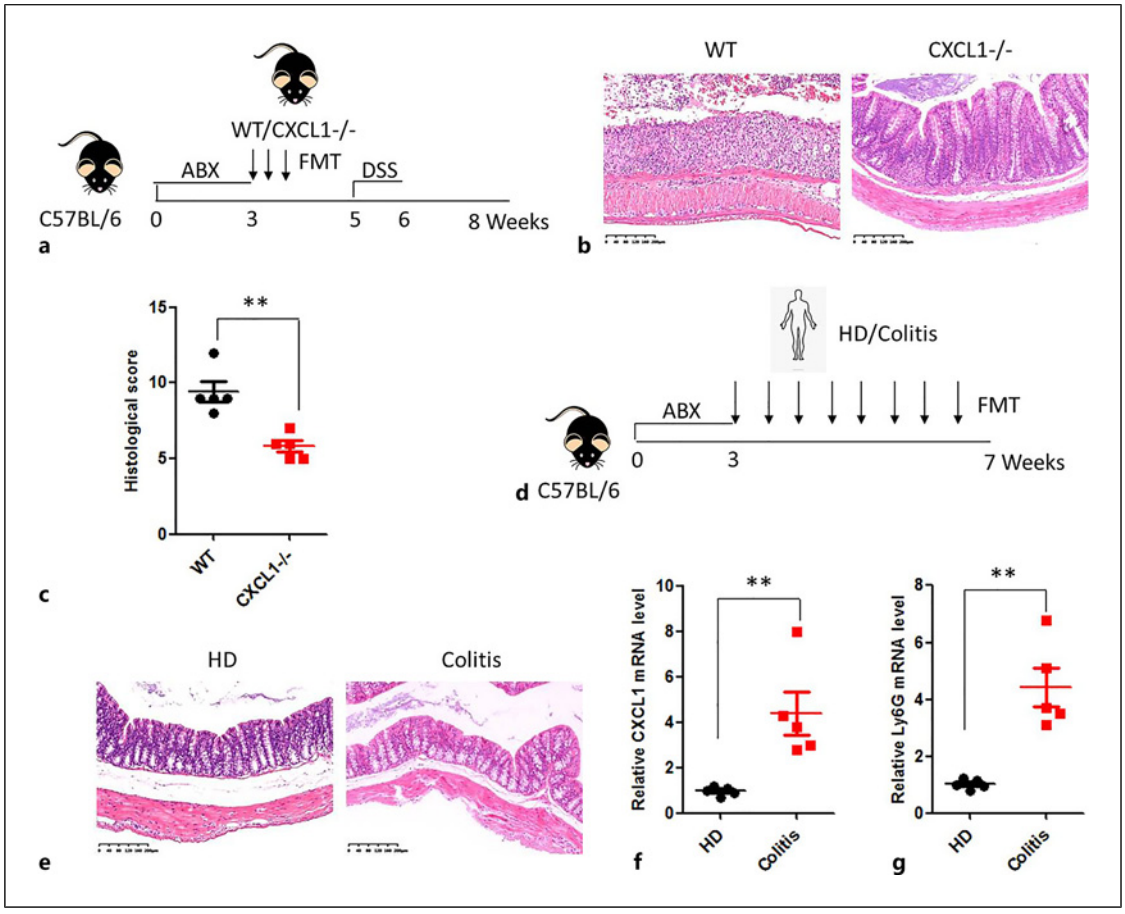

Fig. 5. FMT using CXCL1−/− mice stools suppresses colitis. **a** C57BL/6 mice were treated with an antibiotic cocktail (ABX, 0.5 g/L neomycin, 0.5 g/L vancomycin, and 0.5 g/L primaxin) for 3 weeks. Then, the mice were orally gavaged using cecum stools from WT or CXCL1−/− mice. After 2 weeks (week 5), mice were challenged using 2.5% DSS for 1 week. Mice were euthanized at week 8 for further study. **b** Representative H&E staining of colon tissues from experiment (**a**). **c** Histological score of colon tissues from different groups. $n = 5$ in each group. $^{**}p < 0.01$, Student's t test. **d** C57BL/6 mice were treated with ABX for 3 weeks. Then, the mice were orally gavaged using stool samples from healthy donors (HD) or patients with colitis. After 4 weeks (week 7), mice were euthanized. **e** Representative H&E staining of colon tissues from experiment (**d**). **f**, **g** Relative mRNA expression levels of CXCL1 (**f**) and Ly6G (**g**) in the colon tissues of mice that received FMT using stool samples from healthy donors (HD) and patients with colitis. $n = 5$ in each group. Data represent mean ± SEM. $^{**}p < 0.01$, Student's t test.

for FMT in gut-sterilized mice. After 4 weeks, colitis was assessed using H&E staining (Fig. 5d). Notably, no clear evidence of colitis was observed in mice that received FMT with stool from either patients with colitis or healthy donors (Fig. 5e). Nevertheless, Ly6G and CXCL1 mRNA expression levels in the colon tissues of mice that received FMT with stools from patients with colitis were significantly higher than those in mice that received FMT from healthy donor stools (Fig. 5f, g). These results suggest that the gut microbiome of patients with colitis elevated CXCL1 and Ly6G expression in gut-sterilized mice, implying a connection between the gut microbiome and colitis.

Positive Correlation of CXCL1 and CD15 Expression with Colitis

We collected 20 clinical samples (blood and tissue) from patients with colitis and 20 samples from healthy donors. As expected, the serum CXCL1 concentration was significantly higher in patients with colitis compared to in healthy donors (Fig. 6a). Given that CD15 is a marker for human neutrophil [27, 28], we then detected CD15 expression level in the tissues of patients with colitis and healthy donor. The mRNA expression levels of CXCL1 and CD15 in the tissues of patients with colitis were much higher than those in healthy donors (Fig. 6b, c). Notably, the mRNA levels

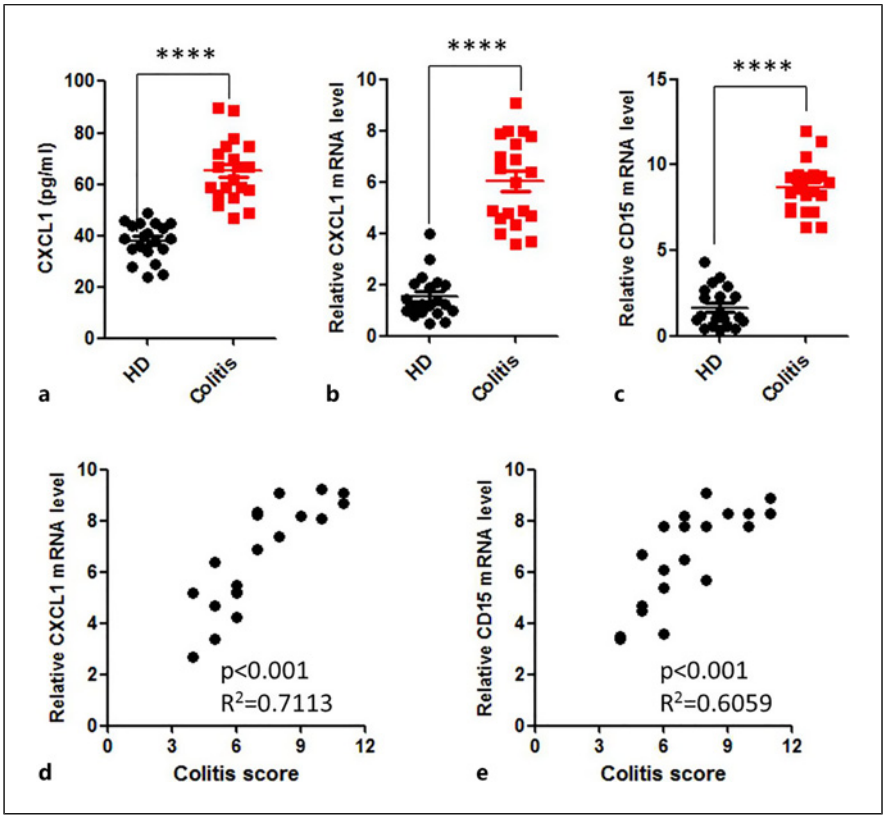

Fig. 6. CXCL1 and CD15 expression levels are positively correlated with colitis. **a** The blood samples of 20 HD and 20 patients with colitis were collected. The serum CXCL1 concentration was determined using ELISA. $n = 20$ in each group. ****$p < 0.0001$, student's t test. **b**, **c** A total of 20 healthy donors (HD) and 20 colitis patients' colon samples were collected. The expression levels of CXCL1 (**b**) and CD15 (**c**) at the mRNA level were detected. $n = 20$ in each group. ****$p < 0.0001$, Student's t test. **d**, **e** In 20 colitis patients, the correlation between colitis score and CXCL1 (**d**) and CD15 (**e**) levels was determined.

of both CXCL1 and CD15 positively correlated with colitis scores (Fig. 6d, e). These findings indicate that CXCL1 and CD15 expression levels are positively correlated with colitis and may serve as potential biomarkers.

Discussion

In recent years, the global incidence of IBD has been on the rise, making it a growing global health concern [29]. Emerging research has shed light on the multifaceted factors influencing IBD development, encompassing environmental influences, immune responses, and gut microbiota dynamics [30–32]. Among these factors, CXCL1 has been recognized for its role in initiating inflammation and contributing to colitis development [33]. However, the intricate relationship between CXCL1 and the gut microbiota, particularly under colitis conditions, remains incompletely understood. Our findings reveal that CXCL1−/− mice exhibit minimal alterations in gut microbiota under normal conditions, indicating that CXCL1 has limited influence on the gut microbiota in the absence of colitis. However, a significant shift in the gut microbiota profile occurs in CXCL−/− mice within the context of the DSS-induced colitis mouse model. Furthermore, colitis in CXCL1−/− mice is less severe compared to that in WT mice following DSS treatment. This attenuation of colitis is accompanied by reduced neutrophil infiltration and decreased expression of inflammatory cytokines in colon tissues, which is attributed to the lower gut permeability observed in CXCL1−/− mice during DSS-induced colitis. Strikingly, FMT from CXCL1−/− mice results in a substantial amelioration of colitis in the DSS-induced colitis mouse model, highlighting the significant impact of gut microbiota composition on colitis severity. Conversely, FMT from patients with colitis enhances the expression levels of CXCL1 and Ly6G in the colon tissues of gut-sterilized mice. Our clinical data further support these findings, as they reveal higher serum CXCL1 concentrations in patients with colitis compared to healthy donors and a positive correlation between the expression levels of CXCL1 and CD15 with colitis severity scores. These data indicate that CXCL1 promoted colitis by modulating the gut microbiota and neutrophil infiltration.

CXCL1 is a potent chemoattractant for neutrophils, orchestrating their recruitment to sites of inflammation [34]. Elevated serum CXCL1 levels have been documented in patients with IBD, and these levels correlate positively with the inflammatory state in patients with IBD [4, 5]. Neutrophils play a multifaceted role in maintaining intestinal homeostasis by eliminating microbes that translocate across the intestinal barrier, which separates commensal microbes from the host [35]. Under normal conditions, neutrophils contribute to mucosal healing and bolster the immune response. However, in pathological states, such as IBD, the excessive infiltration of neutrophils into the intestine can exacerbate mucosal damage [36]. In active ulcerative colitis, both systemic and intestinal neutrophil activity is heightened [37]. Consequently, therapies targeting neutrophil activity have been explored in the clinical management of IBD, aiming to dampen inflammation without compromising host defense mechanisms [37, 38]. Our study confirms the increase in neutrophil infiltration within colon tissues following DSS treatment in both WT and CXCL1−/− mice, aligning with previous findings. Notably, the frequency of neutrophils in CXCL1−/− mice subjected to DSS treatment was significantly lower than that in WT mice, suggesting that CXCL1 depletion mitigates neutrophil recruitment into the colon tissues. This reduction in neutrophil infiltration was associated with a less severe colitis phenotype, as corroborated by histological assessment.

The gut microbiota has been increasingly recognized as a pivotal player. Dysbiosis of the gut microbiota, characterized by alterations in its composition, has been observed in both colitis mouse models and patients with colitis [39–42]. This close association between gut microbiota and colitis underscores the potential of modulating gut microbiota as a therapeutic approach for IBD. Probiotics, for instance, have demonstrated the capacity to enhance intestinal barrier function, stimulate the production of anti-inflammatory factors, and inhibit the growth of pathogenic bacteria, ultimately leading to the amelioration of colitis [43]. FMT using healthy gut microbiota decreases gut permeability and alleviates the severity of colitis, enhances short-chain fatty acid production, and repairs immune dysfunction [44]. Consistent with these findings, our study identified significant dysbiosis of the gut microbiota in both WT and CXCL1−/− mice during DSS-induced colitis, reinforcing the link between gut microbiota and colitis pathogenesis. Importantly, the influence of CXCL1−/− on gut microbiota under normal conditions was found to be moderate or weak, as indicated by minimal differences between WT + H_2O and CXCL1−/− + H_2O groups. However, clear alterations in gut microbiota were observed under DSS-induced colitis conditions (WT + DSS vs. CXCL1−/− + DSS). Notably, a considerable decrease in Gram-negative bacteria and an increase in Gram-positive bacteria were observed in CXCL1−/− mice following DSS treatment, signifying the significant impact of CXCL1−/− on gut microbiota composition during colitis. These shifts in the gut microbiota composition subsequently contributed to changes in gut permeability, peripheral endotoxin levels, and neutrophil infiltration, collectively affecting the severity of colitis.

Neutrophil infiltration in the colon tissues was observed in the DSS-induced colitis mouse model. In humans, CXCL8/IL-8 is a major neutrophil chemoattractant chemokine. As the *Clostridium difficile* toxin induces CXCL8/IL-8 production and intestinal epithelial barrier dysfunction in human colorectal adenocarcinoma Caco-2 cells [45], and the IL-8 antagonist coupled with probiotics exhibits variably enhanced therapeutic potential in ameliorating ulcerative colitis [46], this suggests that CXCL8/IL-8 may be a potential target for colitis treatment in humans.

It is essential to acknowledge that our results contradict those reported by She-Donohue et al. [10], who found that CXCL1−/− mice exhibited increased susceptibility to DSS-induced colitis. However, their study did not delve into the alterations in gut microbiota nor investigate how changes in gut microbiota may influence susceptibility to DSS-induced colitis. One crucial factor to consider is the distinct gut microbiota profiles maintained in different animal facilities, which can impact susceptibility to DSS treatment. Our study underscores the significance of gut microbiota composition in shaping the outcomes of colitis. The mice housing in different animal facility holds different gut microbiota profile, and this may affect the susceptibility to DSS treatment.

In summary, our investigation elucidates the multifaceted role of CXCL1 in modulating gut microbiota, neutrophil infiltration, and colitis development. Colitis was suppressed in CXCL1−/− mice. Alteration of the gut microbiota profile was indicated by 16S rRNA sequencing. Gut permeability and endotoxin concentrations in the peripheral blood of CXCL1−/− mice decreased after DSS treatment. Moreover, neutrophil infiltration in colon tissues was inhibited in CXCL1−/− mice after DSS treatment. FMT using the gut microbiota of CXCL1−/− mice suppressed DSS-induced colitis. FMT using colitis patient stools enhanced CXCL1 and Ly6G expression levels in the colon tissues of gut-sterilized mice. Importantly, clinical data analysis indicated higher expression levels of CXCL1 and CD15 in samples from

patients with colitis, and CXCL1 and CD15 expression levels were positively correlated with colitis severity. These results demonstrate that CXCL1 promotes colitis by altering the gut microbiota.

Statement of Ethics

This study using human samples was reviewed and approved by the Ethics Committee of Shanghai General Hospital (2023SQ068), and written informed consent was obtained from all patients and healthy donors. In addition, all in vivo manipulations were approved by the Animal Research Committee at Shanghai General Hospital (2021AW015).

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Author Contributions

Zhanjun Lu and Hang Zhao designed the study. Hang Zhao, Wenhua Li, Xin Zhou, Liang Pan, and Zhanjun Lu performed the in vivo experiments and analyzed the data. Yun Feng, Pingyu Gao, Jie Ji, Huanyan Zhang, Kai Zhao, and Chi Wang analyzed the data from flow cytometry studies and 16S rRNA sequencing. Hang Zhao and Zhanjun Lu wrote the manuscript. Hang Zhao, Chi Wang, and Zhanjun Lu revised the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding authors.

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