

Original Article

Suppression of the CXCL4-JNK pathways contributes to the attenuation of DSS-induced acute colitis in mice

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Abstract: Inflammatory bowel diseases (IBD) are chronic disorders of the gastrointestinal tract with a complex aetiology. Recently, the incidence of the disease has been on the rise and has received additional attention throughout the world. The chemokine CXCL4 was reported to induce directed cellular migration and was involved in the development and progression of inflammatory diseases. Although it has been reported that CXCL4 expression is significantly increased in patients with IBD, the precise role and mechanism of CXCL4 in IBD remain obscure. This study aims to investigate the role of CXCL4 in dextran sulfate sodium (DSS)-induced acute colitis mouse model. The results showed that the serum level of CXCL4 was markedly increased in the acute colitis mice challenged with DSS compared to the control group. Additionally, compared to the control group, the weight loss, survival time, disease activity, colorectal length, and pathological injuries were remarkably increased in the acute colitis mice; moreover, the phosphorylation Jun N-terminal kinase (p-JNK) and the proinflammatory cytokines were significantly up-regulated in the acute colitis mice compared to the control group. In contrast, the above symptoms were notably ameliorated in the DSS-challenged CXCL4 knockout mice. Therefore, the results in the present study indicate that CXCL4 may have a crucial impact on the progression of the inflammatory response during acute colitis through JNK-mediated signalling, and it may be a candidate molecule for targeted therapy of IBD.

Keywords: Acute colitis, CXCL4, JNK, inflammatory cytokines

Introduction

Inflammatory bowel disease (IBD), which primarily includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract that is characterized by alternating phases of the clinical manifestation, including as abdominal pain, vomiting, diarrhoea, rectal bleeding, and weight loss, relapse and remission [1]. Although the precise underlying etiologies and pathogenesis of IBD have not been fully elucidated, several reports have indicated that sustained intestinal infections, mucosal barrier defects, mucosal immune dysregulation, and genetic and environmental factors are involved in the disease process [2-4]. In recent years, the incidence and prevalence of the disease have persistently increased. Moreover, there is still a lack of reliable and effective methods for diagnosing and monitoring this disease. Thus, it has gradu-

ally been appreciated as an important public health problem [5, 6]. Accumulating evidence has demonstrated that cytokines, surface molecules, and signal transduction molecules could play crucial roles in the initiation and progression of IBD. In addition, numerous researchers have been searching for a key molecule to identify, diagnose, monitor or even treat IBD [7-10].

CXC Chemokine ligand 4 (CXCL4), formerly known as platelet factor 4 (PF4), is a negative regulatory haematopoietic factor this is mainly released by megakaryocytes and platelets and was first discovered in 1955 [11]. The normal CXCL4 levels in plasma are below 1 nM, but they can rapidly reach 1-2.5 μ M upon activation [12]. The platelet-derived chemokine CXCL4 is capable of binding CXC chemokine receptor 3 (CXCR3) and is involved in numerous diverse biological processes, including immune modu-

lation, the inflammatory response, vascular endothelial cell proliferation, haematopoiesis and angiogenesis [13]. The specific and cooperative signals that regulate the CXCL4-mediated activation of different cell types will also be diverse. In neutrophils, the CXCL4-induced activation of c-Jun N-terminal kinase (JNK)/c-jun promotes strong adhesion of neutrophils to vascular endothelial cells. Additionally, in monocytes, CXCL4 mediated reactive oxygen species (ROS) formation by promoting the rapid activation of phosphatidylinositol 3-kinase (PI3K), Src-tyrosine kinase (Syk), and p38 mitogen-activated protein (MAP) kinase. CXCL4 also promoted cell survival and cytokine release by increasing the activity of extracellular signal-regulated kinase (Erk) and facilitating JNK activation, respectively [12]. Recently, mounting evidence has shown that CXCL4 expression is dramatically elevated in colorectal cancer patients, and it may be a tumour suppressor gene that inhibits the development and progression of colorectal cancer by recruiting leukocytes to the inflammation site, attracting pro- or anti-inflammatory cytokines, and restricting tumour cell growth, invasion and metastasis [14]. Moreover, it has been demonstrated that the plasma concentrations of CXCL4 were markedly elevated in patients with IBD [15]. Furthermore, an analysis of the serum protein spectrum showed that there was a positive correlation between the CD activity index and CXCL4 expression. CXCL4 was identified as a biomarker for IBD [15, 16]. Nevertheless, the function of CXCL4 in IBD is still unknown.

The JNK protein kinase, a member of the MAP kinase family, may contribute to multiple physiological processes, including the regulation of cell proliferation and apoptosis [17]. Although p38 MAP kinase, another MAP kinase, was thought to regulate an important primary signalling pathway that mediates inflammatory signals in the past, recent studies have shown that the JNK pathway was also implicated in the inflammatory response, particularly in monocytes [12]. Moreover, it was revealed that the cytokines produced by activation of the JNK signalling pathway could, in turn, further trigger the JNK signalling pathway and ultimately lead to a cytokine storm that promotes the inflammatory response [12]. Thus, it was presumed that the JNK signalling pathway may act in concert with the p38 MAP kinase pathway to mod-

ulate the inflammatory response in various inflammatory diseases. As described above, activated CXCL4 could also stimulate the JNK signalling pathway [18]. However, it is not known whether the CXCL4 and JNK interaction is involved in the progression of acute colitis.

In this study, acute colitis was induced in mice by administering DSS in the drinking water for five consecutive days. To further verify the role of CXCL4 in the progression of acute colitis, we compared the inflammatory and immune responses in CXCL4^{-/-} and wild type mice. The results demonstrated that the CXCL4^{-/-} mice were resistant to the development of acute colitis compared to the wild type mice.

Materials and methods

Animal groups and DSS challenge

All experiments were conducted under an animal welfare protocol approved by the Institute of Medical Biology (IMB), Chinese Academy of Medicine Science (CAMS). Wild-type (WT) mice and heterozygous CXCL4 mice on the C57BL/6 background were provided by The Children's Hospital of Philadelphia and were subsequently housed and bred in specific pathogen-free (SPF) cages. The animals were fed irradiated chow and pure water. The homozygous CXCL4^{-/-} mice were obtained from the heterozygous CXCL4 mice by interbreeding and were identified by PCR and ELISA (in press). Adult mice of the same sex and genotype were co-housed in groups of no more than 5 mice per SPF cage, with 12-hour light/dark cycles, constantly monitored temperature and humidity conditions. Husbandry was performed by the IMB staff.

The WT mice and homozygous CXCL4 mice were randomly divided into the following 4 groups: WT+H₂O group (n=3), WT+DSS group (n=5), CXCL4^{-/-}+H₂O group (n=3) and CXCL4^{-/-}+DSS group (n=5). Acute colitis was induced in the WT+DSS and CXCL4^{-/-}+DSS groups by administering 3% (wt/vol) DSS (36-50 kDa, MP Biomedical, California, USA) dissolved in the drinking water for five consecutive days, followed by regular drinking water for four days, according to the method adopted by He et al [9]. The WT+H₂O group and CXCL4^{-/-}+H₂O group received regular water only. All mice were sacrificed on day 9. Serum was harvested from the eyeballs of all mice to determine the serum

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CXCL4 and cytokine levels. Subsequently, the colons were removed to measure the length (cm) from the rectum to the cecum, and then the colons were cut into two sections. One was used for the Western blotting analysis, and the other was fixed in 10% formalin for the histological examination.

Detection of the serum levels of CXCL4

The serum levels of CXCL4 were measured using the CXCL4 test kit (SINO-American Biotechnology, China) in accordance with the manufacturer's instructions. We first added 50 μ l of the Standards and Samples to the appropriate wells. The Blank well was empty. Then, we added 100 μ l of horseradish peroxidase (HRP)-conjugated reagent to the Standard and Sample wells, but not the Blank well, and incubated them for 1 h at 37°C. After 4 rinses with the Wash Solution, chromogens A and B were successively added to each well and incubated for 15 min at 37°C in the dark. Subsequently, the Stop Solution was added to each well and the optical density (O.D.) was read at 450 nm within 15 min using a microtiter plate reader.

Body weights, survival rate and clinical assessment

The animals' body weights were monitored every two days and then averaged. Additionally, the number of dead mice was recorded every two days. Nevertheless, the progression of acute colitis was clinically evaluated daily by measuring the volume of water consumed, measuring the body weights, evaluating the stool consistency, and observing the presence of blood in the faeces, as described previously. In addition, a previously validated clinical disease activity index (DAI) was also calculated using the following parameters: stool consistency (0-4), presence or absence of blood in the faeces (0-4), and body weight loss (0-4). The maximum possible score is 12 [14].

Morphological observation and histopathological scoring

HE staining was applied to observe the pathological changes in the colon. The fixed tissues were embedded in paraffin and stained with haematoxylin and eosin (H&E). The mean degree of acute colitis in the colon was calculated from observations of 20 different visual

fields of H&E-stained longitudinal sections of the colon from each animal. A histopathological score was obtained in a blinded fashion using a widely used grading tool to examine the crypts, epithelia, goblet cells, cellular infiltration, and edema. Scores of 0-1 reflect a normal morphology, and scores of 2-4, 5-7, and 8-10 represent mild, moderate, and severe colitis, respectively, as previously described [8].

Immunohistochemical examination

Induced nitric oxide synthase (iNOS) was used to detect the degree of inflammation in the colon. The longitudinal sections of the colon were deparaffinized with xylene and a gradient of alcohol. The deparaffinized sections were subjected to antigen retrieval by heating in a microwave for 15 min and were then treated with 3% H₂O₂ in methanol for 15 min at room temperature. Subsequently, the sections were blocked with 5% goat serum in PBS for 1 h at 37°C and incubated with a diluted anti-iNOS antibody (1:200, Boster, China) or PBS overnight at 4°C. Some sections were incubated with PBS as a negative control. After three washes in Tris-buffered saline (TBS), the sections were incubated with an HRP-conjugated secondary antibody (ZSGB-Bio, China) for 1 hour at 37°C, followed by three washes in TBS. Then, the peroxidase activity was visualized using diaminobenzidine (DAB, TIANGEN, China). After two washes with water, the nuclei in the sections were stained with haematoxylin (Solarbio, China). Finally, the sections were washed once in water and sealed with neutral resin (Solarbio, China). An Olympus microscope (CX41, Japan) attached to a digital camera (DP71, Olympus, Japan) was used to photograph the slides at $\times 10$ and $\times 40$ magnifications. The images were recorded with a computer-assisted video-imaging system (Olympus DP controller, Japan).

Western blot assay

The mouse colon tissues were frozen in liquid nitrogen. On the day of the assay, the snap-frozen tissues were homogenized in ice-cold RIPA buffer (Beyotime Biotechnology, China) containing 1 mmol/L PMSF for 30 min. The homogenates were then centrifuged at 12,000 rpm for 10 min, and the resulting supernatants were assessed with a PierceBCA Protein Assay Kit (Thermo Scientific, USA) using a microplate

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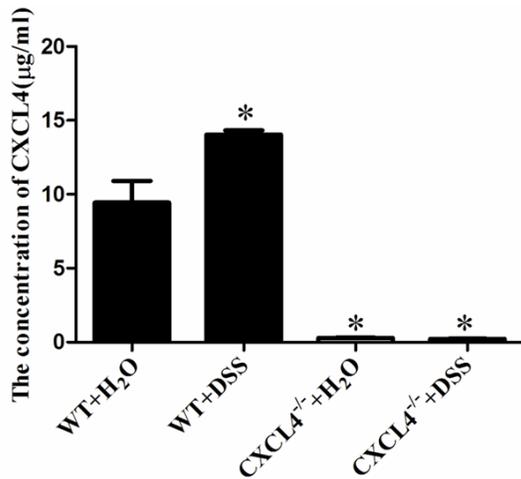


Figure 1. Detecting the concentration of CXCL4 in the mice. The concentrations of CXCL4 were detected by ELISA. The data represent the means \pm SEM (n=3). *P<0.05, compared to the WT+H₂O group.

reader. Equal amounts of protein (p-JNK: 40 μ g/20 μ l; β -actin: 20 μ g/10 μ l) were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Milipore, USA). After blocking with TBST (Tris-buffered saline, pH 7.6, 0.05% Tween 20) containing 5% non-fat dried milk for 1 h, the membranes were incubated with antibodies specific for p-JNK (1:1000 dilution, Beyotime Biotechnology, China) and β -actin (1:2000 dilution, Boster, China) overnight at 4°C. After 3 washes with TBST, the membranes were incubated with an HRP-conjugated secondary antibody (1:10000 dilution, Abmart, China) for 1 h and developed using an Enhanced Chemiluminescence Kit (Beyotime, China). The p-JNK levels were compared to those of β -actin based on the grey values calculated by Image J.

Detection of the plasma cytokine levels

The cytokine assays were performed using a Bio-Plex Mouse Cytokine 23-Plex Panel (1 \times 96-well) (Bio-Rad, USA), according to the manufacturer's instructions and as previously described [19]. The plasma samples were diluted 1:4 with mouse sample diluent. The desired number of wells was pre-wet with 100 μ l of Bio-Plex assay buffer. The multiplex bead working solution was vortexed for 15-20 s and 50 μ l were pipetted into each well. Then, the buffer was immediately removed using the Bio-Plex Pro Magnetic Wash Station. The wells were washed twice by

adding 100 μ l of wash buffer. 50 μ l of the diluted standards or prepared samples were added to each well and the plate was incubated for 30 min. Then, the plate was washed three times with 100 μ l of Bio-Plex wash buffer. 25 μ l of vortexed Bio-Plex Detection Antibody working solution were added to each well, incubated for 30 min, and then washed three times with Bio-Plex wash buffer. 50 μ l of vortexed 1 \times streptavidin-PE was added to each well, incubated for 30 min, and then washed three times with Bio-Plex wash buffer. The beads were resuspended in 125 μ l assay buffer, incubated for 30 s, and then immediately read on the Bio-Plex 200 System. The cytokine concentrations were calculated using the standard curve and the Bio-Plex manager software. The samples were run in duplicate.

Statistical analysis

All data are expressed as the means \pm S.E.M and were analysed using SPSS software (version 18.0). The serum CXCL4 levels, weight changes, disease activity scores, colorectal lengths, histopathological scores and Western blot data were analysed using one-way ANOVA, followed by the post hoc least significant difference (LSD) test. Statistical significance was set at P<0.05.

Results

Serum CXCL4 levels

Several studies have reported that the CXCL4 levels were elevated in patients with IBD [15]. To investigate whether the trend in the CXCL4 levels in the DSS-induced acute colitis mice coincided with that in the IBD patients, we detected the serum levels of CXCL4 by ELISA. The concentration of serum CXCL4 in the WT+DSS group of mice was significantly increased compared to the WT+H₂O group of mice (14.03 \pm 0.58 μ g/ml vs. 9.44 \pm 2.95 μ g/ml, P<0.05) (**Figure 1**). There was very little serum CXCL4 in the CXCL4^{-/-}+ DSS and CXCL4^{-/-}+ H₂O groups of mice (**Figure 1**).

The effects of DSS-induced acute colitis on the animals' body weights, survival rate and clinical symptoms

IBD, a chronic inflammatory disease of the gastrointestinal tract, is characterized by abdomi-

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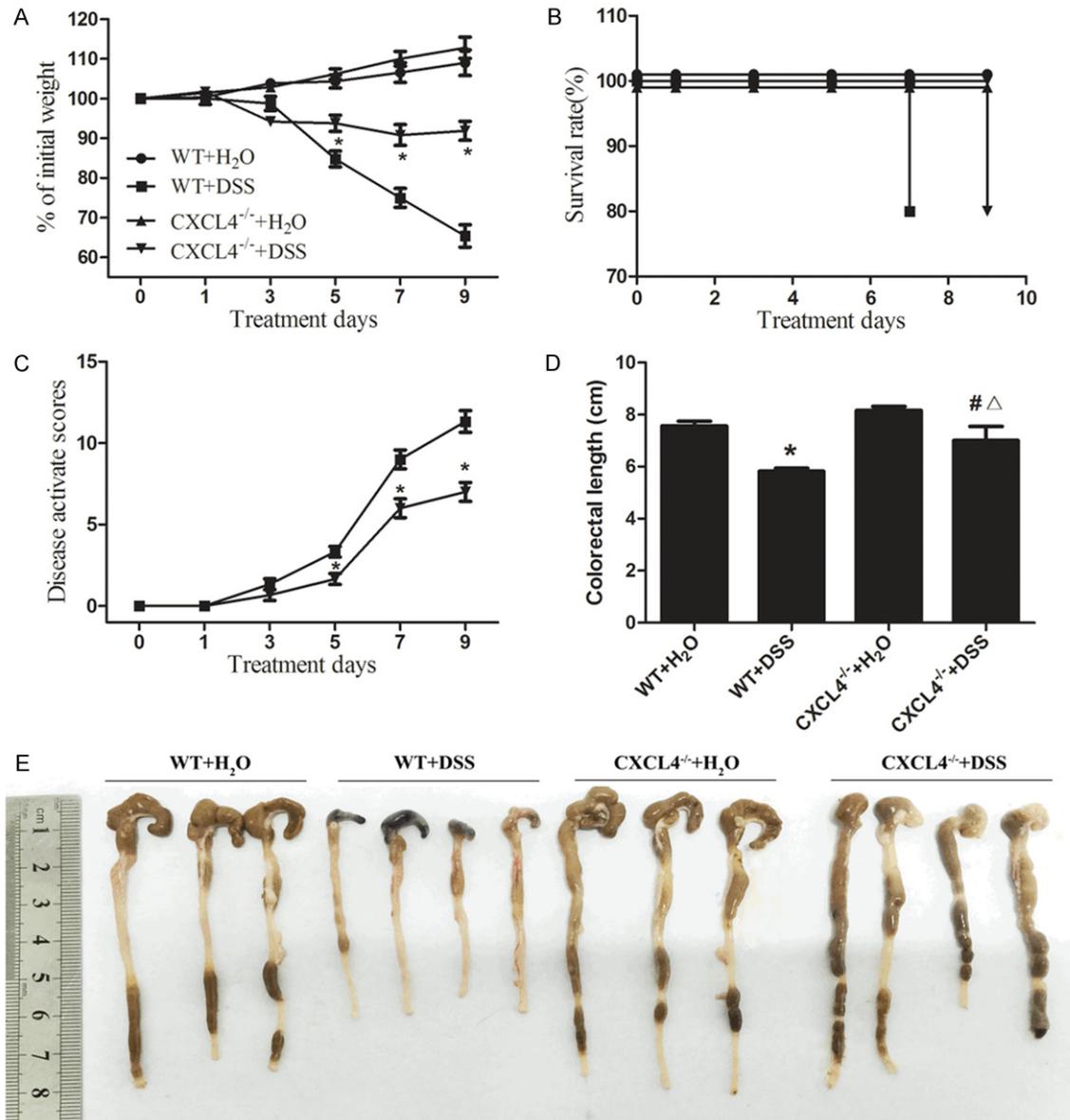


Figure 2. DSS-induced colitis in the WT and CXCL4^{-/-} mice. **A.** Changes in the body weights of the H₂O- and DSS-challenged WT and CXCL4^{-/-} mice over nine days of treatment. The data are expressed as the percentage of the original weight prior to treatment. The data represent the means \pm SEM (n=3). **P*<0.05, compared to the WT+H₂O group. **B.** Survival rate of the H₂O- and DSS-challenged WT and CXCL4^{-/-} mice over the nine days of treatment. The data are expressed as the percentage of total mice in each group. **C.** Disease activity index of the WT and CXCL4^{-/-} mice over the nine days of treatment. The disease activity index was generated by evaluating the various presentations typically observed in the DSS-induced colitis model, including reduced mobility, vocalization, and group interactions. **P*<0.05, compared to the WT+H₂O group. **D.** The colon lengths were measured from the anus to the caecum. **P*<0.05, compared to the WT+H₂O group; #<0.05, compared to the WT+DSS group; Δ<0.05, compared to the CXCL4^{-/-}+H₂O group. **E.** Colons of the H₂O- and DSS-challenged WT and CXCL4^{-/-} mice.

nal pain, diarrhoea, weight loss and rectal bleeding [1]. Although the body weights of the WT+DSS and CXCL4^{-/-}+ DSS mice progressively decreased, the decrease in the body weights of the WT+DSS mice was significantly higher than

the CXCL4^{-/-}+ DSS mice on days 5, 7 and 9 (**Figure 2A**). Moreover, the survival time of the WT+DSS mice is 2 days less than the CXCL4^{-/-}+ DSS mice (**Figure 2B**). In addition, the clinical symptom assessment in the WT+DSS mice

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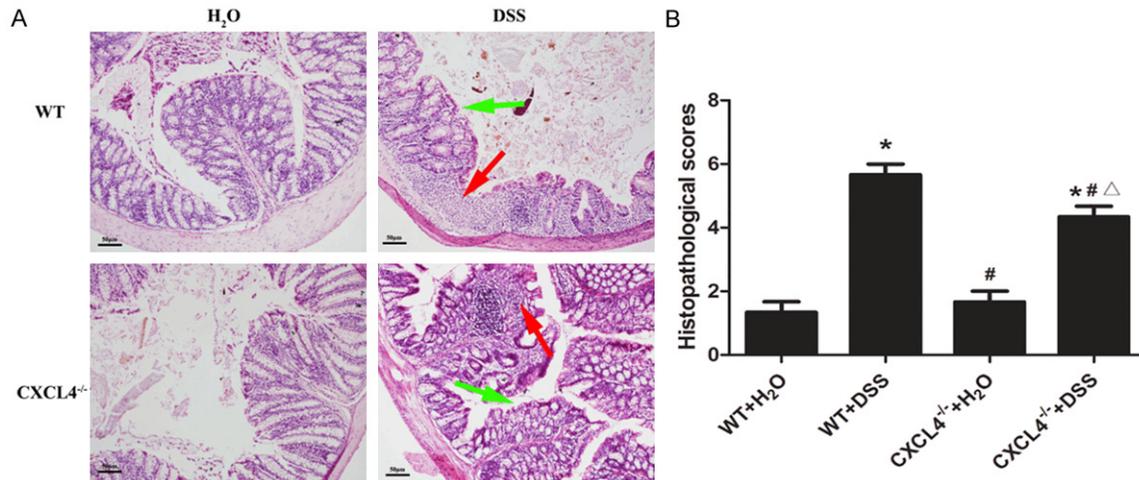


Figure 3. Histopathological changes in the colons of the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. A: Histopathological changes in the body weights of the H₂O- and DSS-challenged WT and CXCL4^{-/-} mice over the nine days of treatment. B: Average histopathological scores of the colons from the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. Histopathology was scored as 0 (no pathology), 5 (moderate pathology), or 10 (extreme pathology).

using the DAI was significantly higher than the CXCL4^{-/-}+ DSS mice on days 5, 7 and 9 (**Figure 2C**). Furthermore, the lengths of the colons from the WT+DSS mice were significantly shorter than those of the CXCL4^{-/-}+ DSS mice (**Figure 2D** and **2E**). Thus, these results suggested that the DSS-induced acute colitis in the WT+DSS mice was more intense compared to the CXCL4^{-/-}+ DSS mice.

Morphological changes

H&E staining revealed no obvious histopathological abnormalities in the WT and CXCL4^{-/-} mice that were not challenged with DSS. However, histological damage was observed in the DSS-treated mice. H&E staining demonstrated that the epithelial destruction and inflammatory cell infiltration in the CXCL4^{-/-}+ DSS mice was noticeably reduced compared to the WT+DSS mice (**Figure 3A**). The histopathological scores demonstrated that the CXCL4^{-/-}+ DSS mice develop a less severe inflammatory injury than the WT+DSS mice (**Figure 3B**). Hence, the results suggested that CXCL4 could aggravate acute colitis in mice.

The expression of iNOS in the colon

The immunohistological observations indicated that the WT mice produced more iNOS than the CXCL4^{-/-} mice after the DSS treatment (**Figure 4**). Nevertheless, the level of iNOS expression

between the WT+H₂O and CXCL4^{-/-}+ H₂O groups was not significantly different (**Figure 4**). iNOS is a marker of inflammation. Therefore, it was concluded that CXCL4 may promote the inflammatory response during acute colitis in mice.

Level of the p-JNK protein in the colon

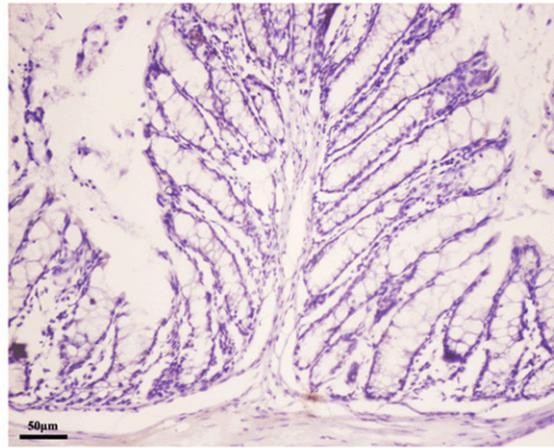
To determine the role of CXCL4 in the pathogenesis of acute colitis, we examined whether the JNK signalling pathway, which is intimately associated with inflammation, was regulated by CXCL4. Western blotting was utilized to detect the levels of the p-JNK protein, the activated form of JNK. The p-JNK protein levels were significantly up-regulated in the WT+DSS mice compared to the WT+ H₂O mice. Moreover, the DSS-treated CXCL4^{-/-} mice exhibited a dramatic increase in the p-JNK protein levels compared to the CXCL4^{-/-}+ H₂O mice (**Figure 5A**). In addition, the expression of p-JNK in the WT+DSS mice was significantly higher than that in the CXCL4^{-/-}+ DSS group ($P < 0.05$) (**Figure 5B**). Thus, these data illustrated that CXCL4 may exacerbate the development of acute colitis by activating JNK signalling.

Cytokines released in serum

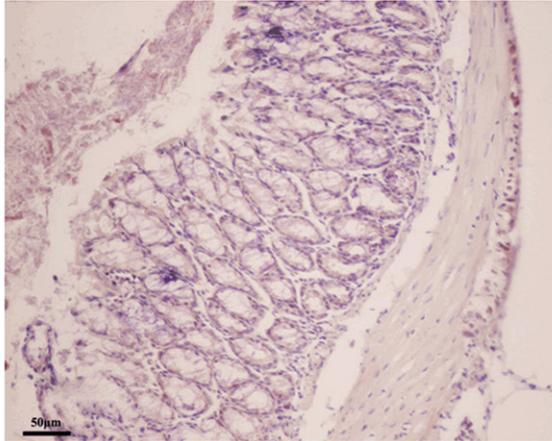
As part of a complex biological response, inflammation elicits the release of numerous inflammatory mediators, particularly cytokines [20]. Meanwhile, inflammation plays a major

A

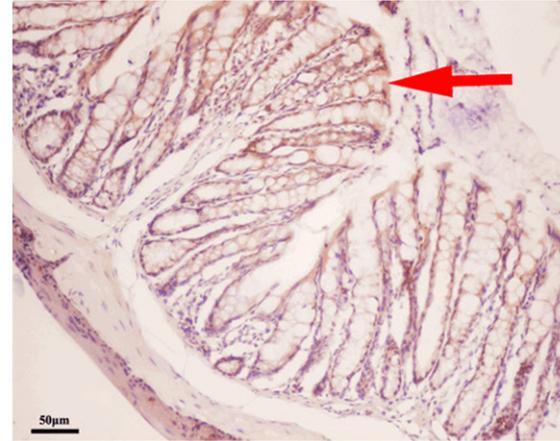
Negative control



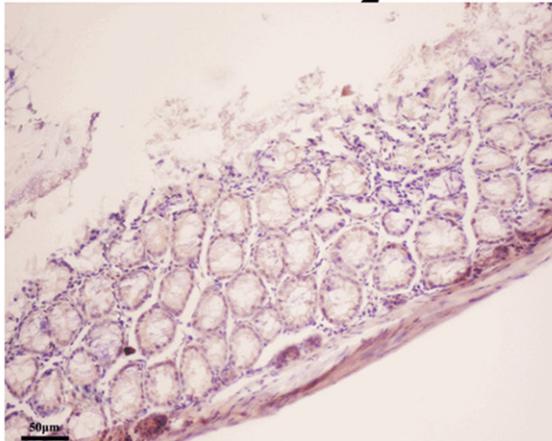
B **WT+H₂O**



C **WT+DSS**



D **CXCL4^{-/-}+H₂O**



E **CXCL4^{-/-}+DSS**

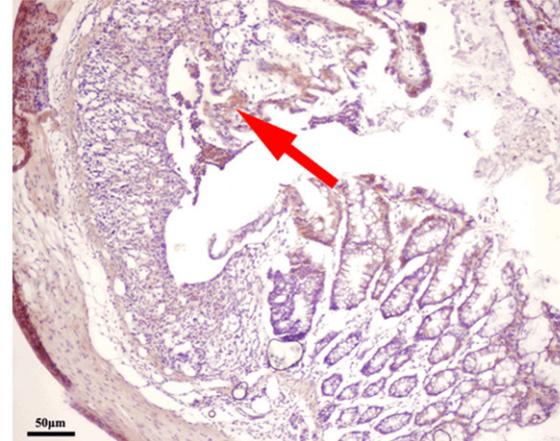


Figure 4. iNOS expression levels in the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. The levels of iNOS in the colon tissues from the H₂O- and DSS-challenged WT and CXCL4^{-/-} were measured using immunohistochemistry. A: Negative control; B: WT+H₂O; C: WT+DSS; D: CXCL4^{-/-}+H₂O; E: CXCL4^{-/-}+DSS.

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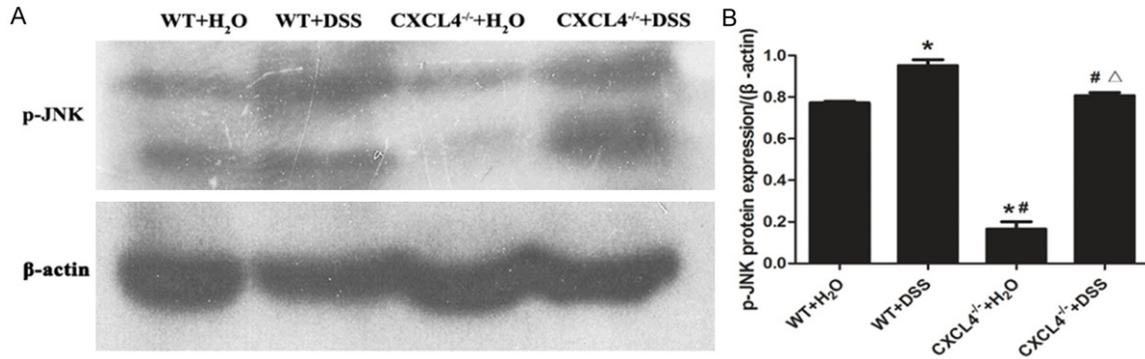


Figure 5. p-JNK expression levels in the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. A: Western blot analysis of the expression of p-JNK and β-actin in the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. B: The level of p-JNK relative to β-actin in the four groups was calculated with Image J. **P*<0.05, compared to the WT+H₂O group; #<0.05, compared to the WT+DSS group; Δ<0.05, compared to the CXCL4^{-/-}+H₂O group.

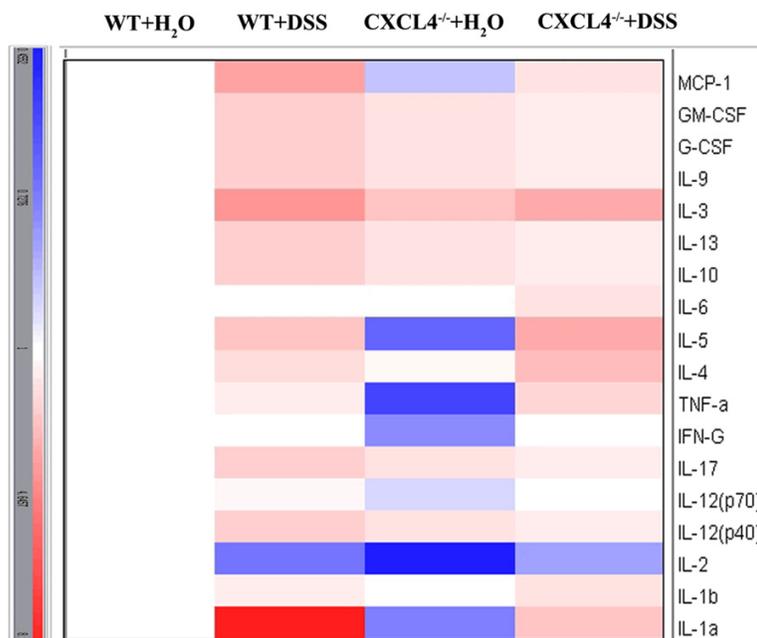


Figure 6. Cytokine expression profile in the serum from the WT and CXCL4^{-/-} mice challenged with H₂O and DSS. Cytokines, including MCP-1, GM-CSF, G-CSF, IL-9, IL-3, IL-13, IL-10, IL-6, IL-5, IL-4, TNF-α, IFN-γ, IL-17, IL-12 (p70), IL-12 (p40), IL-2, IL-1^β, and IL-1^α, were detected using a Bio-Plex Mouse Cytokine Panel. The coloured bar represents the fold change in the cytokine levels relative to the WT+H₂O group.

role in the pathogenesis of colitis [21]. We demonstrated that the levels of proinflammatory cytokines were increased in the WT mice following DSS challenge, but this response was more modest in the CXCL4^{-/-}+DSS group compared to the WT group (Figure 6). Thus, these results further demonstrated that the inflammatory process is reduced in the DSS-challenged CXCL4-deficient mice, and this trend

was similar to the p-JNK levels.

Discussion

Numerous data on epidemiology have reported that the incidence and prevalence of IBD, a condition that seriously affects the patient's quality of life, have increased over the past 50 years [6]. Although progress has been made in understanding IBD, its aetiology and pathogenesis are not completely understood [3]. There is a clinical need to exploit a new therapeutic drug to prevent or treat IBD, and numerous investigators have focused on cytokines, surface molecules, or even signal transduction molecules [22]. A considerable number of studies have demonstrated that CXCL4 expression was significantly elevated in IBD patients [15, 16]. Here, our study indicated

that CXCL4 could exacerbate the progression of acute colitis by activating the JNK signalling pathway, which caused the subsequent activation of downstream cytokines and led to an increased inflammatory response.

The experimental DSS-induced murine colitis model is a well-established and consistent method used to investigate the development,

progression and changes in acute colitis in the gut [23]. We adopted this colitis model and observed that the serum levels of CXCL4 were remarkably up-regulated. This result exhibited the same tendency as that found in Meuwis' laboratory [15]. Thus, we further generated a CXCL4 knockout mouse to investigate the function of CXCL4 during the progression of acute colitis. In the present study, we verified that the weight loss, survival time, disease activity and colorectal lengths of the DSS-treated CXCL4 knockout mice were notably decreased compared to the normal mice subjected to the same treatment. In the clinic, weight loss, survival time, and disease activity are closely associated with the degree of IBD, and weight loss is one of clinical symptoms of IBD [24]. Therefore, these findings supported the hypothesis that CXCL4 could accelerate the development of acute colitis. In addition, it has been reported that there is a direct correlation between the outward signs of disease and the pathological changes in the gut tissue [23]. In our study, we also observed a disruption of the mucosal structures and a massive infiltration of inflammatory cells in the colons of the WT+DSS group, but these pathological injuries were ameliorated in the CXCL4^{-/-}+ DSS group. Moreover, iNOS was expressed at higher levels in the WT+DSS group than in the CXCL4^{-/-}+ DSS group. iNOS, a marker of inflammation, is capable of synthesizing superoxides, particularly nitric oxide (NO), which has been recognized as a gaseous signalling molecule involved in the regulation of diverse physiological and pathophysiological mechanisms in the cardiovascular, nervous and immune systems [25]. NO can act as a cytotoxic agent in inflammatory disorders, and the inhibition of iNOS may be a beneficial treatment for inflammatory disease [26]. Together, our results further suggested that CXCL4 exacerbated the inflammatory response in the DSS-treated mice.

Based on the above phenomenon, we analysed the pathway downstream of CXCL4 to elaborate the underlying mechanism of CXCL4 in acute colitis. The pathways involved in the CXCL4-mediated signal transduction cascade can result in the induction of a broad spectrum of acute and delayed functions, including phagocytosis, a respiratory burst, survival, and the secretion of cytokines [12]. Moreover, it was

confirmed that the immediate CXCL4-stimulated monocyte functions (oxygen radical formation) were regulated by p38 MAP kinase, Syk, and PI3K, whereas the delayed functions (survival and cytokine expression) were controlled by Erk and JNK [27]. Additionally, the JNK-induced expression of pro-inflammatory cytokines could cause a cytokine storm, which disturbed homeostasis and provoked an inflammatory response [28]. Furthermore, accumulating literature has revealed that the cytokine responses, including proinflammatory cytokines such as tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin (IL)-1, IL-6, and IL-12 and anti-inflammatory cytokines such as IL-4, IL-10, and IL-11, are the key pathophysiological elements of IBD [20, 29]. Therefore, we examined the levels of the p-JNK protein and confirmed that the p-JNK levels were significantly increased in the WT+DSS group compared to the WT+H₂O group, but the p-JNK levels were significantly reduced in the CXCL4^{-/-}+ DSS group compared to the WT+DSS group. Furthermore, cytokine release, which is related to inflammation, was increased in the WT+DSS group compared to the CXCL4^{-/-}+ DSS group. These results implied that CXCL4 may activate JNK; subsequently, the JNK signal accelerated cytokine release. Following CXCL4 knockout, JNK activation and cytokines release were suppressed to a certain extent. Among all of the tested cytokines, the changes in IL-1 α expression were the most significant. Nevertheless, the binding of IL-1 α to its receptor can rapidly initiate proinflammatory cytokine release by activating JNK [28]. Thus, we presumed that the increased cytokine release could, in turn, trigger additional JNK signalling and exacerbate the inflammatory response.

In summary, the data in the present study revealed that an aggravated inflammatory response was generated in the DSS-induced acute colitis mouse model, whereas the aggravated inflammatory response was sharply repressed in the CXCL4 knockout mice subjected to the same treatment. Furthermore, the aggravated inflammatory response may result from the initiation of the JNK signalling pathway as a result of the up-regulation of CXCL4 expression during acute colitis. Hence, CXCL4 may be a candidate molecule for targeted therapy of acute colitis.

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Disclosure of conflict of interest

None.

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