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ORIGINAL ARTICLE

Inhibition of Pim-1 Kinase Ameliorates Dextran Sodium Sulfate-Induced Colitis in Mice

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Abstract

Background Pim-1 kinase is involved in the control of cell growth, differentiation and apoptosis. Recent evidence suggests that Pim kinases play a role in immune regulation and inflammation. However, the role of Pim-1 kinase in inflammatory bowel diseases (IBD) remains unclear.
Aims The aims of this study were to explore the role of Pim-1 kinase in the pathology of IBD and to assess whether inhibiting Pim-1 kinase may be of therapeutic benefit as a treatment regimen for IBD.
Methods Colitic mouse model was established by the induction of dextran sodium sulfate. The expression of Pim-1 in the colonic samples of control and colitic mice was examined. Furthermore, the mice were treated with Pim-1inhibitor (PIM-Inh), then the body weight and colon inflammation were evaluated, and the production of cytokines including IFN-γ, IL-4, TGF-β and IL-17 in colon tissues was determined by ELISA. The expression of T cell master transcription factors T-bet, ROR-γt, GATA-3 and Foxp3 and Nuclear factor κB (NF-κB) and inducible nitric oxide synthase in colon tissues was detected by real-time PCR and western blot. Finally, the effect of LPS on Pim-1 expression and the effects of PIM-Inh on LPS-induced upregualtion of p65 and TNF-α in RAW264.7 cells were examined by real-time PCR and western blot.
Results Pim-1 expression was correlated with the degree of mucosal inflammation in vivo, and it was significantly induced by LPS in vitro. PIM-Inh had protective effects on acute colitis in vivo. Mechanistically, PIM-Inh reduced the proinflammatory immune response through the inhibition of the overactivation of macrophages and the down-regulation of excessive Th1- and Th17-type immune responses. Furthermore, PIM-Inh could skew T cell differentiation towards a Treg phenotype.
Conclusions Pim-1 kinase is involved in mucosal injury/inflammation and Pim-1 kinase inhibitor may provide a novel therapeutic approach for IBD.

Keywords Pim-1 kinase · Inflammatory bowel diseases · Pim-1 kinase inhibitor · Macrophage · T-Lymphocyte subsets · NF-kappa B

Abbreviations

DAI	Disease activity index
DSS	Dextran sulfate sodium
ELISA	Enzyme-linked immunosorbent assay(s)
FOXP3	Forkhead box P3
GATA-3	GATA binding protein3
IBD	Inflammatory bowel diseases
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
NF-κB	Nuclear factor κB
PIM-Inh	Pim-1 kinase inhibitor
RANKL	Receptor activator of nuclear factor kappa-B ligand

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RORγt	RA orphan receptor γ
STAT	Signal transducer and activator of transcription
TAK1	Transforming growth factor β–activated kinase 1
TGF-β	Transforming growth factor β
TNF	Tumor necrosis factor
T-bet	T-box expressed in T cells
Th	T-helper cell
TOR	Target of rapamycin

Introduction

Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammatory disorders of the gastrointestinal tract. Although the etiology of IBD remains unclear, accumulating evidence has indicated that dysfunction of the mucosal immune system plays an important role in the pathogenesis of IBD [1, 2]. Among a variety of inflammatory cells in the gut, immune cells such as macrophages and mucosal CD4 + T cells are thought to play a central role in the inflammatory response, eventually leading to the intestinal tissue damage in IBD [3]. Th1-related cytokines such as TNF-α, IFN-γ and IL-12 as well as Th17-associated cytokines such as IL-17A, IL-21 and IL-23 are markedly increased in inflamed mucosa of IBD patients [4, 5]. By contrast, regulatory T cells, which are characterized by the expression of Foxp3, play an important role in immunological tolerance, protecting the host from autoimmune diseases [6, 7]. Clinical and experimental studies have shown that the down-regulation of the established inflammatory response and the induction and maintenance of Tregs functions could ameliorate IBD [8–10].

Pim-1 is a serine/threonine kinase that is crucially involved in the control of cell growth, differentiation and apoptosis [11]. Accumulating data support that Pim-1 and Pim-2 are essential components of an endogenous pathway that regulates T cell growth and survival [12, 13]. Recently, Pim-1 kinase was shown as a new regulator of human Th1 cell differentiation which is preferentially expressed in Th1 cells compared to Th2 cells [14]. The crucial role of Pim kinase in immune cells activation and proliferation makes it an attractive target for immunomodulatory therapy [15]. Indeed, the pharmacological inhibition of Pim kinases exhibits immunomodulatory benefits [16]. These data suggest the potential of Pim-1 antagonists in treating T cell-mediated autoimmune diseases. However, it remains unclear whether Pim-1 kinase is involved in the pathology of IBD and whether the inhibitor of Pim-1 kinase could modulate inflammatory responses during the development of IBD. Therefore, in the present study, we established colitic mouse model by the induction of dextran sodium sulfate (DSS), then we detected the expression of Pim-1 kinase in colonic samples and examined the effects of small molecule Pim-1 kinase inhibitor on mucosal inflammation response during the development of acute colitis.

Methods

Animals

Six- to eight-week-old male BALB/c mice were purchased from the Animal Laboratory of Xiangya School of Medicine, Central South University (Changsha, China). They were acclimatized for 1 week before the experiment and housed individually in a room maintained at 22°C under a 12-h day/night cycle. All mouse experiments were reviewed and approved by the Institutional Animal Care Committee of Central South University.

Induction of Colitis

Acute colitis was induced by giving 5% DSS (molecular weight 5,000; Sigma, St. Louis, MO, USA) orally in drinking water for 7 days. The colonic tissues were collected on days 0, 1, 4, and 7 following the start of DSS treatment. PIM-Inh (sc-204330; Santa Cruz Biotechnology, Santa Cruz, CA, USA), the highly selective Pim-1 kinase inhibitor, was dissolved in 0.5% DMSO [17]. For the in vivo experiments involving PIM-Inh treatment, acute colitis mice were randomly divided into groups receiving different concentrations of PIM-Inh (5, 10 mg/kg/day, or only 0.5% DMSO solution) by intraperitoneal injection on each of the 7 days control mice were given only 0.5% DMSO solution. The colonic tissues were collected on day 8 of DSS treatment regimen.

Evaluation of Colitis Severity

Daily clinical assessment of DSS-induced colitis was performed, including measurement of the disease activity index (DAI), colon length, and histology. The DAI was determined by scoring changes in animal weight, occult blood positivity, gross bleeding and stool consistency, as described previously [18, 19]. The parameters were as follows: five grades of weight loss (0, no loss or weight gain; 1, 1–5% loss; 2, 5–10% loss; 3, 10–20% loss; 4, >20% loss); three grades of stool consistency (0, normal; 2, loose; and 4, diarrhea); and three grades of occult blood (0, negative; 2, occult blood-positive; and 4, gross bleeding). Mice were sacrificed by cervical dislocation, then the entire colon was removed from the cecum to the anus, and the colon length was measured as an indirect marker of

inflammation. The distal colon was fixed in 10% buffered formalin for histological analysis, and histological disease scores were evaluated based on the presence of crypt loss and inflammatory cell infiltration as described previously [19, 20]. All evaluation was performed independently by two persons blinded to the source of samples. Briefly, histology was scored as follows. Epithelium (E): 0, normal morphology; 1, loss of goblet cells; 2, loss of goblet cells in large areas; 3, loss of crypts; and 4, loss of crypts in large areas. Infiltration (I): 0, no infiltration; 1, infiltration around crypt bases; 2, infiltration reaching the muscularis mucosa; 3, extensive infiltration reaching the muscularis mucosa and thickening of the mucosa with abundant edema; and 4, infiltration of the submucosa. The total histological score was the sum of the epithelium and infiltration scores (total score = E + I), which ranged from 0 to 8.

Cell Culture

The macrophage cell line (RAW264.7) was purchased from the American Type Culture Collection and maintained in DMEM (4.5 g of glucose/L) containing 10% FBS at 37°C under 5% CO_2. At 80% confluency, RAW264.7 cells were treated with different doses of lipopolysaccharide (LPS, from *Escherichia coli* 0111:B4; Sigma) for 12 or 24 h, then the expression of Pim-1 was assessed by real-time quantitative RT-PCR and western blot. In other experiments, the cells were treated with different doses of PIM-Inh for 2 h, then treated by 1 μg/mL LPS for 1.5 or 24 h, and the changes in TNF-a expression and the NF-kB activation were detected by ELISA and western blot.

Cytokine Assays

The amount of murine IFN-γ, IL-4, IL-17, TGF-β in colonic homogenates and TNFα in the supernatants were quantified by commercially available enzyme-linked immunosorbent assay kits (BD PharMingen, San Diego, CA) according to the manufacturer's instructions and adapted to the protein content of the colon tissue sample.

Western Blot Analysis

Colonic tissues or cultured cells were lysed in RIPA Lysis Buffer containing 50 mM Tris (pH 7.4),150 mM NaCl, 1% Triton X-100, 1% deoxycholic acid sodium, 0.1% SDS, and protease inhibitors. The lysates were separated by 10% SDS–polyacrylamide gel electrophoresis and then electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were incubated with primary antibodies against Pim (B0712), phospho-NF-κB P65 (Ser276), inducible nitric oxide synthase (iNOS) (Assay Biotechnology, CA, USA), ROR-γt (646501) (BioLegend, San Diego, CA, USA), FOXP3 (3100-1) (Epitomics, Burlingame, CA, USA), β-actin (sc-81178) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), T-bet (13700-1-AP), GATA3 (10417-1-AP) (Proteintech Group, USA), respectively. After incubating with secondary antibodies, the immunoreactive bands were visualized using an enhanced chemiluminescence method (ECL; Pierce Supersignal, USA). β-actin was used as an inner control. For quantitative analysis, the bands were analyzed by scanning densitometry using a Quantity One software (Bio-Rad Laboratories, CA, USA).

Real-Time PCR Analysis

Total RNA was extracted from colonic tissues or cultured cells by Trizol reagent (Invitrogen) and transcribed into cDNA. PCR was performed using a SYBR green-based PCR using iQ SYBR mix (MiniOption; Bio-Rad Laboratories). The primer sequences were as follows: Pim-1 forward: 5′-G CG GCG AAA TCA AAC TCA T-3′; reverse, 5′-TCA TCG TGC TCA AAC GGA AT-3′; RORγ forward: 5′-CAG TAT GTG GTG GAG TTT GC-3′; reverse, 5′-GCT TCC ATT GCT CCT GCT TT-3′; foxp3 forward: 5′-GGG CTT CTG GGT ATG TCC TT-3′; reverse, 5′-TGC TTG CGG CTC CTA ATG C-3′; T-bet forward: 5′-GAG GAA GGG TTT GAA GGG TG-3′; reverse, 5′-AGA AGG AGG GCG TGT TTA CC-3′; GATA3 forward: 5′-AGG GCT ACG GTG CAG AGG TA-3′; reverse, 5′-CGG AGG GTA AAC GGA CAG AG-3′; GAPDH forward: 5′-GAGTCAACGGATTT GGTCG-3′; reverse, 5′-CGGAAGATGGTGATGGGAT T-3. GAPDH was selected as internal control for RNA input and reverse transcription efficiency with the TaqMan-based assays (Applied Biosystems). All PCR reactions were done in duplicate for both target gene and internal control. After control for equal PCR efficiency of target genes and internal controls, relative gene expression was presented with the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Differences between experimental groups were assessed by one-way analysis of variance, the Tukey–Kramer multiple comparisons test (for multiple groups), or Student's *t* test (for comparisons between two groups). $P < 0.05$ was considered to be statistically significant.

Results

Pim-1 Kinase Is Upregulated in DSS-Induced Colitis

To explore the role of Pim-1 in the initiation and development of IBD, we first examined Pim-1 expression at both

mRNA and protein levels in colonic samples from mice treated with 5% DSS for 0, 1, 4 and 7 days. Real-time PCR and western blot analysis showed that Pim-1 expression was increased even 1 day after DSS induction although the difference was not significant compared to control. However, Pim-1 protein expression was significantly higher on day 7 than on day 4 or day 1 (Fig. 1a). Similarly, Pim-1 mRNA level in colon tissue was significantly increased on day 4 after DSS induction and reached the peak on day 7 (Fig. 1b). These data suggest that Pim-1 kinase is upregulated in DSS-induced colitis.

PIM-Inh Attenuates DSS-Induced Acute Colitis

The mice treated with 5% DSS for 7 days developed symptoms of acute colitis at an incidence of 100%. They exhibited body weight loss and watery or bloody diarrhea developed on day 4 of DSS administration. PIM-Inh treatment (5, 10 mg/kg/day) reduced the severity of DSS-induced colitis in a dose-dependent manner. PIM-Inh-treated mice had significantly less body weight loss and fewer signs of severe colitis compared with the DSS group ($P < 0.05$) (Fig. 2a, b). Macroscopically, the colon length was markedly shorter in DSS group than in PIM-Inh treatment group (Fig. 2c). Histopathologically, DSS-induced colitis was characterized by histological findings such as edema, infiltration of inflammatory cells into both the mucosa and the submucosa, destruction of epithelial cells, and mucosal thickening, whereas PIM-Inh-treated mice showed mild infiltration of inflammatory cells in the mucosa, minimal loss of crypts, and reduction of goblet cells (Figs. 2d, 3).

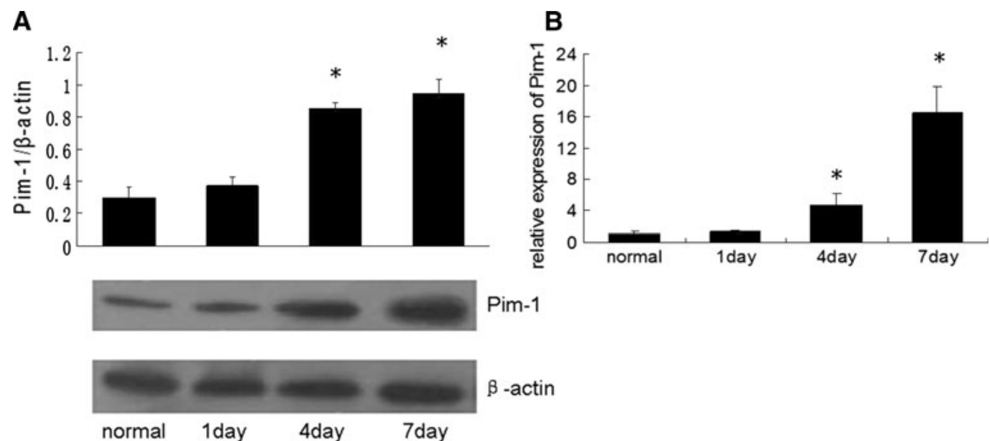

Fig. 1 Pim-1 kinase is upregulated in DSS-induced colitis. Mice were sacrificed at 0, 1, 4, 7 days after treatment with 5% DSS. The colon tissues were taken for the detection of Pim-1 expression by western blot analysis (**a**) and real-time PCR (**b**). β-actin served as loading control for western blot. Representative blots from 5 independent experiments ($n = 5$) are shown. $*P < 0.05$ versus normal controls

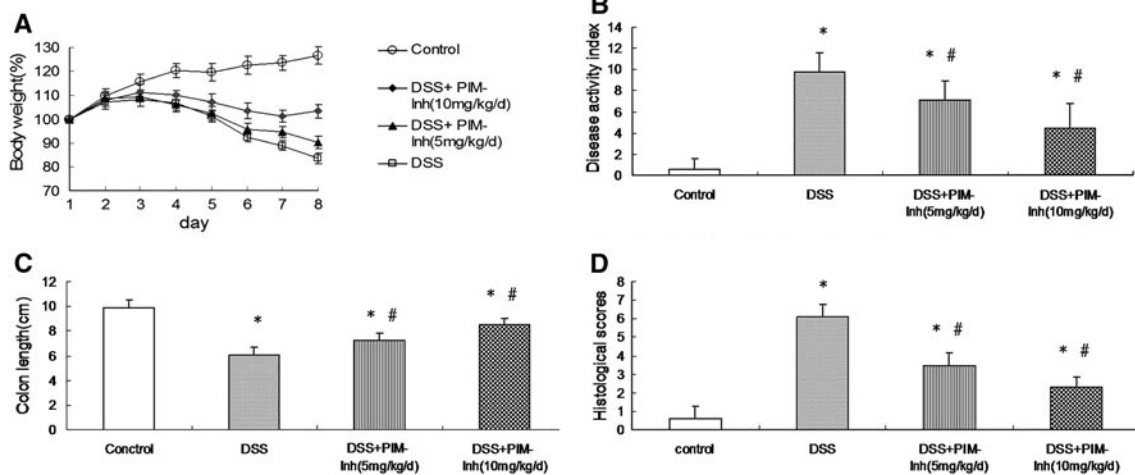

Fig. 2 PIM-Inh treatment attenuates DSS-induced acute colitis. Effect of PIM-Inh on body weight (**a**), disease activity index (**b**), colon length (**c**), and histological scores (**d**) in rats treated with DSS. Colitis was induced in rats by daily treatment with a 5% DSS solution in drinking water for 7 days. Data are presented as mean ± SD ($n = 8$–10). $*P < 0.05$ versus control group, $^{\#}P < 0.05$ versus DSS group

PIM-Inh Downregulates Th 1 and Th17 Cytokines and Upregulates Treg Cytokine Production in Acute Colitis

The activation of inflammatory cells such as Th1 and Th17 lymphocytes, and/or deficiency of regulatory T cells (Treg) contribute to DSS-induced acute colitis [21, 22]. To determine the mechanism by which PIM-Inh attenuates DSS-induced acute colitis, we examined the expression of IFN-γ, IL-4, TGF-β and IL-17 in the colon. High levels of Th1 and Th17 cytokines (IFN-γ and IL-17) were detected in the colon of DSS-treated mice. In contrast, PIM-Inh

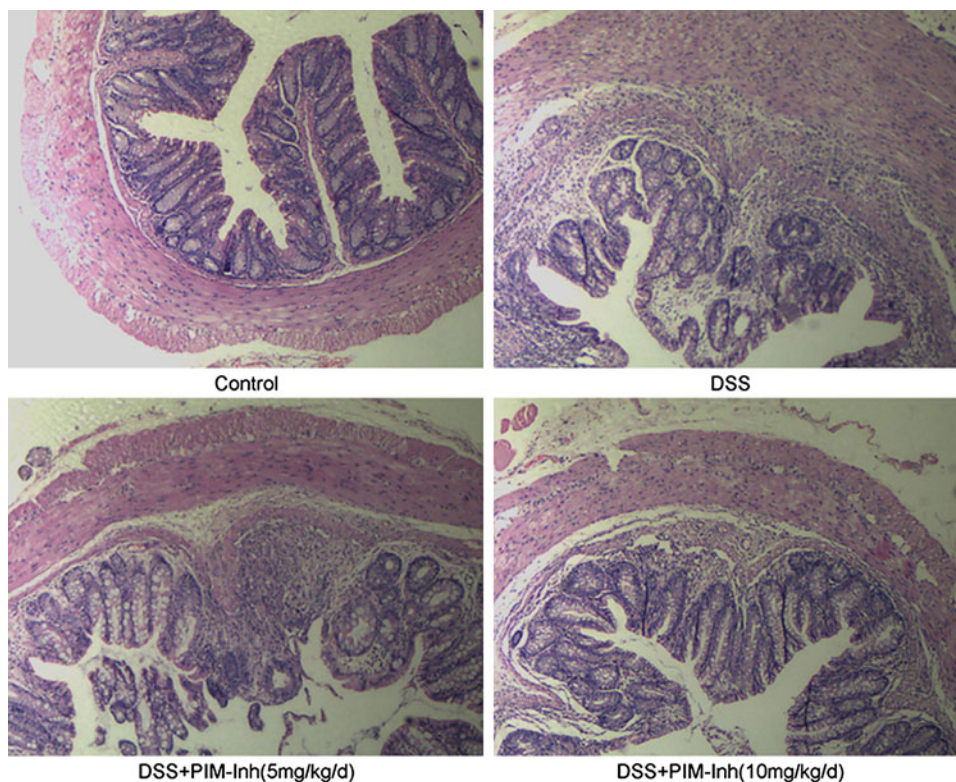

Fig. 3 Histological observation of colonic lesions induced by DSS in rats (H&E staining). Colitis was induced in rats by daily treatment with a 5% DSS solution in drinking water for 7 days. PIM-Inh (5 or 10 mg/kg) were administered daily for 7 days. Magnification ×40

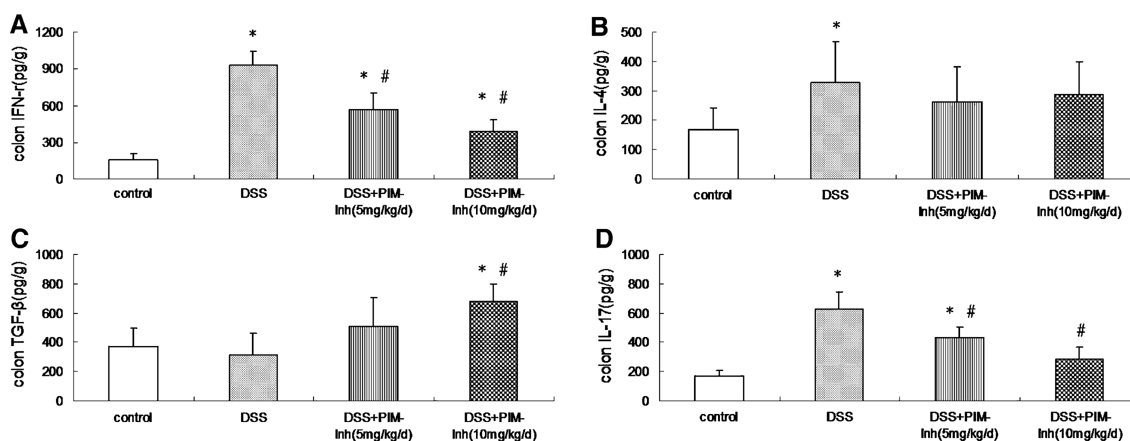

Fig. 4 Effect of PIM-Inh on the expression of IFN-γ(A), IL-4(B), TGF-β(C), and IL-17(D) in the colon. Colitis was induced by drinking 5% DSS water for 7 days. PIM-Inh (5 or 10 mg/kg) was administered daily for 7 days. Mice were sacrificed on day 8. The levels of cytokines in colonic homogenates were determined by ELISA ($n = 5$). $^{*}P < 0.05$ versus control group, $^{\#}P < 0.05$ versus DSS group

inhibited the production of IFN-γ and IL-17 in the colon in a dose-dependent manner (Fig. 4a, d). In addition, the production of Treg cytokines (TGF-β) was higher in PIM-Inh (10 mg/kg/day) treated mice compared to either DSS- or PIM-Inh (5 mg/kg/day)-treated mice ($P < 0.05$) (Fig. 4c). PIM-Inh treatment also inhibited DSS induced production of Th2 cytokines (IL-4) in the colon (Fig. 4b).

PIM-Inh Modulates T-Helper Differentiation

Next, we postulated that PIM-Inh could modulate the lymphocytes cytokine response to DSS via inducing T cell differentiation towards a Th2 or Treg phenotype. Therefore, we performed quantitative RT-PCR and western blot to analyze the expression of T cell master transcription factors T-bet (Th1), ROR-γt (Th17), GATA-3 (Th2) and Foxp3 (Treg) in the colon. The results showed that T-bet and ROR-γt were significantly up-regulated while the expression of Foxp3 was inhibited in DSS group. Administration of PIM-Inh resulted in the inhibition of T-bet and ROR-γt expression and the induction of FOXP3 expression. However, PIM-Inh did not cause significant change in GATA-3 expression (Fig. 5).

PIM-Inh Inhibits Macrophage Activation in Acute Colitis

NF-κB is a crucial transcription factor which mediates transcriptional activation of many inflammatory genes. In IBD, NF-κB is activated in monocytes/macrophages of inflamed intestinal mucosa where it induces iNOS expression and the production of proinflammatory cytokines [23]. Therefore, we examined the levels of activated pNF-κB P65 and iNOS in the colon of acute colitis mice. As expected, pNF-κB P65 and iNOS levels were significantly increased in colon tissues of DSS group compared to control group. PIM-Inh treatment significantly inhibited NF-κB activation and downregulated iNOS expression in colon tissues of acute colitis mice in a dose-dependent manner. These results suggest that PIM-Inh inhibits macrophage activation in acute colitis (Fig. 6).

PIM-Inh Inhibits LPS Induced Macrophage Activation In Vitro

Based on our in vivo findings, we employed RAW264.7 macrophages as in vitro model to investigate the role of

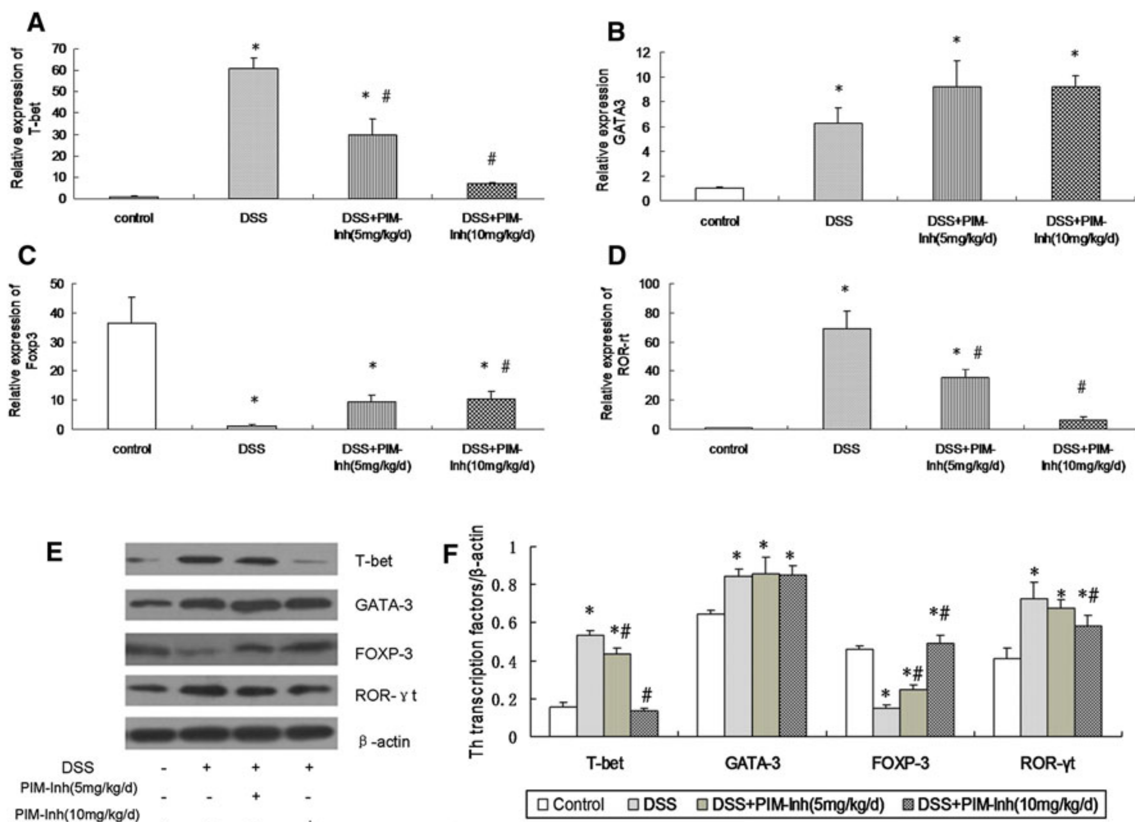

Fig. 5 Effect of PIM-Inh on Th cell expression of the master transcription factors in the colon. T-bet, GATA-3, Foxp3 and ROR-γt, mRNA expression (**a**–**d**) and protein expression (**e**, **f**) were detected by real-time PCR and western blotting, respectively. Data are shown as mean ± SD ($n = 6$). *$P < 0.05$ versus control group, $^{\#}P < 0.05$ versus DSS group

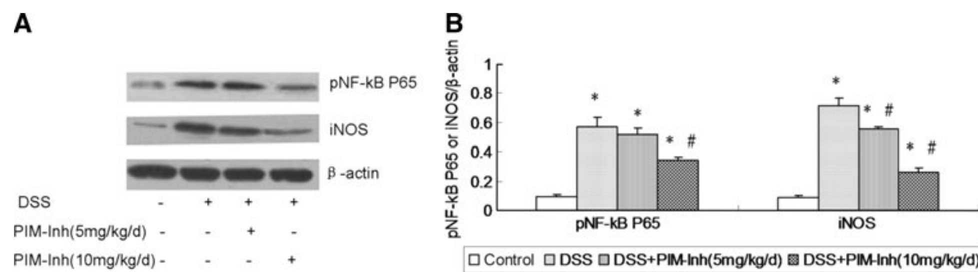

Fig. 6 PIM-Inh treatment inhibits macrophage activation in DSS induced acute colitis. The protein levels of p-NF-κB P65 and iNOS were determined by western blot. β-actin served as loading control. Representative blots from 5 independent experiments ($n = 5$) are shown. $*P < 0.05$ versus normal controls, $^{\#}P < 0.05$ versus DSS group

Pim-1 in macrophage activation. RAW264.7 cells were treated with different doses of LPS for 12 or 24 h. The results showed that LPS induced the expression of Pim-1 at both protein and mRNA levels in a dose-dependent manner, and higher expression levels were observed at 12 h than at 24 h after LPS treatment (Fig. 7a, b).

Next, we investigated whether PIM-Inh inhibits LPS-induced macrophage activation in vitro. RAW264.7 cells were pretreated with different doses of PIM-Inh for 2 h before the treatment with 1 μg/mL LPS. The level of phosphorylated NF-kB P65, a maker of NF-kB activation, was detected by western blot 1.5 h after LPS activation. In addition, TNFα level in the supernatants collected 24 h after LPS treatment was detected by ELISA. The results showed that PIM-Inh reduced the levels of P65 and TNFα in a dose-dependent pattern. Treatment with 100 μmol/L PIM-Inh exhibited the strongest inhibitory effect on TNFα production (Fig. 8a, b).

Discussion

In this study, we demonstrated for the first time that the expression of Pim-1 was upregulated during the development of acute colitis induced in mouse model while LPS induced the expression of Pim-1 in macrophages in vitro. PIM-Inh, the inhibitor of Pim-1, was able to attenuate mucosal injury/inflammation such as loose stools, fecal blood, weight loss, and histologic signs of injury. Furthermore, we found that PIM-Inh reduced the proinflammatory response in the colon through inhibiting the overactivation of macrophages and downregulating the production of Th1- and Th17-type cytokines. In addition, PIM-Inh could skew T cell differentiation towards a Treg phenotype. Collectively, these results indicate that Pim-1 kinase is involved in mucosal injury/inflammation and may contribute to the development of IBD.

NF-κB is regarded as one of the key regulators of inflammation and the activation of NF-κB is markedly induced in IBD patients. Through its ability to promote the expression of various proinflammatory genes, NF-κB strongly influences the course of mucosal inflammation [24–26]. It has been suggested that Pim-1 kinase contributes to the recruitment of RelA/p65 to κB elements to activate NF-κB signaling upon TNF-α stimulation [27]. In addition, Zemskova et al. [28] reported that Pim-1 mediates docetaxel-induced activation of NF-κB transcriptional activity in prostate cancer cells. Moreover, Pim-1/TAK1 signaling cascade plays a role in RANKL-induced NF-κB activation during osteoclastogenesis [29]. These previous studies strongly indicate that Pim-1 plays a positive role in NF-kB activation. Consistent with these, we found that PIM-Inh inhibited NF-κB activation, leading to the downregulation of iNOS expression in macrophages and

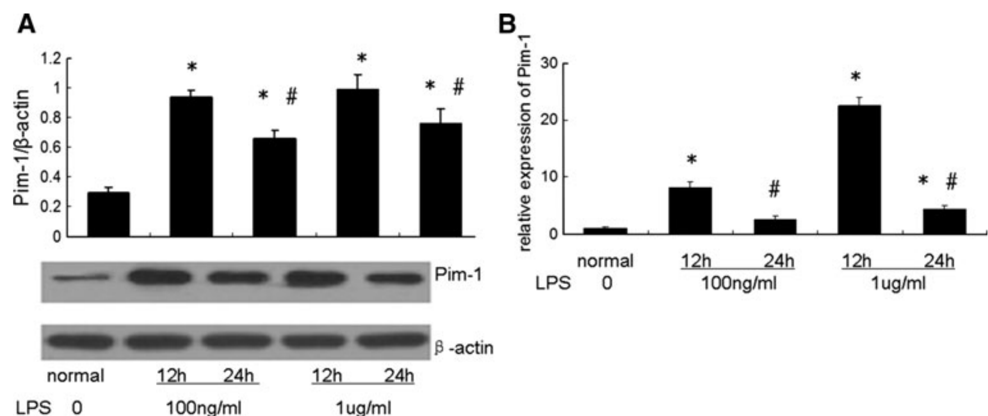

Fig. 7 LPS induces Pim-1 expression in RAW264.7 cells. RAW264.7 cells were treated with different doses of LPS and Pim-1 expression was detected by western blotting (**a**) and real-time PCR (**b**). β-actin served as loading control for western blot. Representative blots from 3 independent experiments ($n = 3$) are shown. $* P < 0.05$ versus normal controls, $^{\#}P < 0.05$ versus cells treated with LPS for 12 h

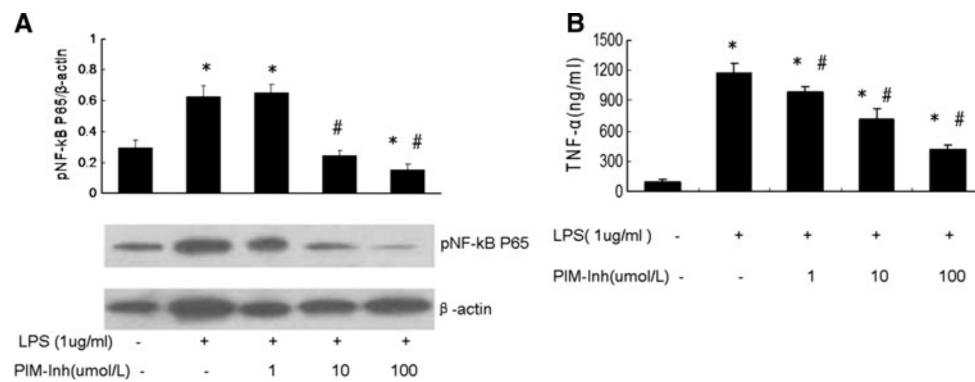

Fig. 8 PIM-Inh inhibits LPS-induced expression of pNF-kB P65 and TNF-α. RAW264.7 cells were pretreated with different doses of PIM-Inh for 2 h then treated with 1 μg/mL LPS for 1.5 or 24 h. P65 level in the cells was detected by western blot 1.5 h after LPS treatment (**a**). TNF-α level in the supernatants was measured by ELISA 24 h after LPS treatment (**b**). β-actin served as loading control for western blot. Representative blots from 3 independent experiments ($n = 3$) are shown. * $P < 0.05$ versus normal controls, $^{\#}P < 0.05$ versus cells treated with LPS alone

reduced production of TNFα, a key proinflammatory cytokine implicated in the pathogenesis of IBD. Taken together, these data suggest that PIM-Inh ameliorates experimental colitis, and that one of the mechanisms for the anti-inflammatory effect of PIM-Inh is through the inhibition of innate immune responses driven by macrophages.

A large body of evidence indicates that IBD arises from a disruption of mucosal immune homeostasis in genetically susceptible individuals, resulting in altered processing of enteric antigens, pathogenic T cell activation, and chronic inflammation [30]. IBD is characterized by excessive Th1 and Th17 cell responses [31]. The dysregulated Th1 and Th17 cell responses lead to the alterations in mucosal cytokine expression, including increased production of IFN-γ and IL-17, two key mediators in IBD. Promotion of Th1 and Th17 cell responses leads to the exacerbation of colitis. In contrast, Th2 response might be protective to IBD. Intestinal helminth infection-induced Th2 responses are associated with protection from certain types of IBD, especially Th1-associated diseases such as Crohn's disease [32]. Tregs suppress Th proliferation and effector functions to maintain immune homeostasis [33–35]. Interestingly, Pim-1 is upregulated in response to antigen and CD40 receptor ligation in B lymphocytes [36]. Pim-1 is also upregulated in response to T cell receptor (TCR) ligation in T lymphocytes [37]. Moreover, mature T-lymphocytes from Pim1/2/3-deficient mice exhibited defects in the proliferation when stimulated through TCR with anti-CD3 [38]. Inhibition of Pim-1 prevented CD4 + T cell proliferation without inducing apoptosis [39]. These data suggest that Pim-1 antagonists are effective in treating T cell-mediated autoimmune diseases. In the present study, we found that administration of PIM-Inh resulted in the inhibition of T-bet and ROR-γt expression and the induction of FOXP3 expression, but did not cause any significant change in GATA-3 expression. This is in agreement with the data that Th1-type cytokine IFN-γ and Th17-type cytokine IL-17 were significantly decreased in the colon of PIM-Inh-treated mice. These results suggest that another mechanism for the beneficial effect of PIM-Inh on colitis is the downregulation of Th1 and Th17 responses and the upregulation of Tregs responses. The exact mechanism by which PIM-Inh exhibits the inhibitory effects on Th1 and Th17 lymphocytes remains elusive, but it may be due to the regulatory role of Pim kinases in T cell proliferation and differentiation [40, 41].

Pim kinases have been proposed as potential therapeutic targets due to their crucial role in cell survival, proliferation and cytokine production. Notably, isoform-specific Pim inhibitors may not be necessary to avoid toxicity because knockout of all 3 *pim* genes led to a mild phenotype [38]. Currently, a number of Pim kinase inhibitors are at the preclinical development stage [42, 43]. In this study, our in vivo and in vitro experiments provide complementary evidence that Pim-1 kinase was involved in mucosal inflammation, and that the injury of colitis following the administration of DSS was significantly attenuated by PIM-Inh. These new findings suggest that Pim-1 kinase inhibitors may act to downregulate innate and adaptive immune responses during acute colitis and thus could be employed as a novel therapeutic approach for IBD.

Conflict of interest Authors declare that there are no conflicts of interest.

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