A novel benzo[d]imidazole derivate prevents the development of dextran sulfate sodium-induced murine experimental colitis via inhibition of NLRP3 inflammasome

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ABSTRACT

NLRP3 inflammasome has been reported to be associated with various kinds of immunological diseases including colitis. However, there are few drug candidates targeting inflammasomes for the treatment of colitis. In the present study, we aimed at examining the effect of 1-ethyl-5-methyl-2-phenyl-1H-benzo[d]imidazole, a synthetic small molecular compound also named Fc11a-2, for the treatment of dextran sulfate sodium (DSS)-induced experimental colitis in mice via targeting NLRP3 inflammasome. Treatment with Fc11a-2 dose-dependently attenuated the loss of body weight and shortening of colon length induced by DSS. In addition, the disease activity index, histopathologic scores and myeloperoxidase activity were also significantly reduced by Fc11a-2 treatment. Moreover, protein and mRNA levels of DSS-induced proinflammatory cytokines in colon, including TNF-α, IL-1β, IL-18, IL-17A and IFN-γ, were markedly suppressed by Fc11a-2. Furthermore, a decreased CD11c+ macrophage infiltration in colons and inactivation of caspase-1 in peritoneal macrophages were detected in Fc11a-2-treated mice. The mechanism of action of Fc11a-2 was related to the inhibition of the cleavage of procaspase-1, pro-IL-1β and pro-IL-18 which in turn suppressed the activation of NLRP3 inflammasome. Taken together, our results demonstrate the ability of Fc11a-2 to inhibit NLRP3 inflammasome activation and its potential use in the treatment of inflammatory bowel diseases.

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1. Introduction

Ulcerative colitis is an idiopathic inflammatory bowel disease characterized by chronic and relapsing inflammation. The exact cause of ulcerative colitis remains undetermined but appears to be related to a combination of genetic and environmental factors [1]. Although the etiology of the disease is unknown, it has been suggested that the activation of the mucosal immune system in response to bacterial antigens with consecutive pathologic cytokine production plays a key pathogenic role [2]. To investigate this disease in mice, a chemical-induced model of acute colonic inflammation has been introduced by oral administration of dextran sulfate sodium (DSS) and characterized by a general inflammatory process associated with weight loss and histopathologic features that mimic some clinical demonstrations of inflammatory bowel diseases [3,4].

Increased levels of proinflammatory cytokines, including interleukin-1β (IL-1β), IL-6, IL-18 and tumor necrosis factor α (TNF-α), are detected in active colitis and correlate with the severity of inflammation [5–8]. IL-1β and IL-18 were likely to be essential in the early phase of the inflammatory cascade leading to inflammation in the colon [9,10]. The synthesis and secretion of proinflammatory cytokines is governed by germine-encoded receptors such as the toll-like receptor (TLR) and nucleotide-binding domain leucinerich repeat containing (NLR) protein family [11]. Many kinds of autoimmune disease, such as multiply sclerosis [12,13], colitis [14], acute lung injury [15] and atherosclerosis [16], have been reported to be associated with inflammasome. However, there are few reports about targeting inflammasomes for the treatment of these diseases. Study by Bauer et al. demonstrated a role of NLRP3 inflammasome in experimental colitis, suggesting that NLRP3 inflammasome complex may serve as a potential target for the development of novel therapeutics for patients with inflammatory bowel diseases [9].

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When activated by diverse stimuli, NLRP3 proteins polymerize and bind to the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) adaptor, which in turn promotes the recruitment of pro-caspase-1 [17,18]. Then pro-caspase-1 clusters and autocleaves to the activated form, caspase-1 p10/p20 tetramer, which triggers caspase-1-dependent processing of pro-IL-1β and pro-IL-18 [19] and allows for the secretion of the mature forms of these cytokines [20]. In the present study, we used phorbol myristate acetate-differentiated THP-1 monocytes to mimic the activation of NLRP3 inflammasome in vitro [21]. As a result, Fc11a-2 restrained the formation of NLRP3 inflammasome by inhibiting activation of caspase-1 and thus the activation of IL-1β/IL-18. During NLRP3 inflammasome activation, activated caspase-1 and mature IL-1β/IL-18 are secreted together by an unconventional protein secretion pathway [22]. We detected a forestalled release of caspase-1 from ASC/NLRP3 complex following Fc11a-2 treatment, which might suggest the mechanism of Fc11a-2’s action on NLRP3 inflammasome activation. Taken together, all these data suggested that treatment of DSS-induced experimental colitis by Fc11a-2 might be due to its ability to inhibit the activation of NLRP3 inflammasome.

2. Materials and methods

2.1. Mice

6- to 8-week-old female C57BL/6 mice were purchased from Model Animal Genetics Research Center of Nanjing University (Nanjing, China). Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, the United States) and the related ethical regulations of our university. All efforts were made to minimize animals’ suffering and to reduce the number of animals used.

2.2. Reagents

Fc11a-2 (1-ethyl-5-methyl-2-phenyl-1H-benz[d]imidazole, purity > 99%, chemical structure shown in Fig. 1A, a synthetic compound by our laboratory) was dissolved at a concentration of 30 mM in 100% DMSO as a stock solution, stored at −20 °C, and diluted with medium before each experiment. The final DMSO concentration did not exceed 0.1% throughout the study (all the control groups are composed of 0.1% DMSO). Cyclosporin A (CsA), phorbol myristate acetate (PMA), lipopolysaccharide (LPS) and adenosine triphosphate (ATP) were purchased from Sigma–Aldrich (St. Louis, MO). Dextran sulfate sodium (DSS, 36–50 kDa) was bought from MP Biomeicals (Aurora, OH). Myeloperoxidase (MPO) activity assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). RPMI-1640, fetal bovine serum (FBS), Alexa Fluor 546 donkey anti-rabbit IgG and Alexa Fluor® 488 donkey anti-mouse IgG (H+L) were purchased from Life Technology (Carlsbad, CA). FITC-anti-CD4, PE-anti-IFN-γ, APC-anti-IL-17A were purchased from ebioscience (San Diego, CA). Anti-phospho-STAT1, anti-phospho-ERK1/2 (Thr202/Tyr204), anti-phospho-p38 (Thr180/Tyr182) and anti-phospho-JNK (Thr183/Tyr185) were purchased from Cell Signaling Technology (Beverly, MA). Anti-NLRP3, anti-phospho-p65 and anti-caspase-1 were purchased from Epitomics (Burlingame, CA). Anti-ASC was purchased from Santa Cruz (Santa Cruz, CA). ELISA kits for murine TNF-α, IL-1β, IL-17A, IFN-γ and human IL-1β were purchased from Dakewe Biotech Co. Ltd. (Beijing, China). ELISA kits for murine IL-18 and human IL-18 were purchased from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Cell culture

Human THP-1 cells were purchased from Shanghai Institute of Cell Biology (Shanghai, China) and maintained in RPMI 1640 medium, supplemented with 100 U/ml of penicillin, 100 μg/ml of penicillin, 100 μg/ml of streptomycin and 2 mM L-glutamine at 37 °C in a humidified atmosphere of 5% CO2.

Fig. 1. Fc11a-2 treatment ameliorated DSS-induced experimental colitis in mice. (A) The effects of a series of small compounds on IL-1β productions in THP-1 cells activated by LPS plus ATP. LPS-primed THP-1 cells were treated with a series of compounds at a dose of 10 μM for 1 h respectively, following by 5 mM ATP treatment for 1 h. Released IL-1β in the supernatant was analyzed by ELISA. Right region: Chemical structure of 1-ethyl-5-methyl-2-phenyl-1H-benz[d]imidazole (Fc11a-2). (B-E) Mice were given 2.5% DSS in drinking water for 7 days, then mice were provided with water for another 3 days before sacrificed. (B) Loss of basal body weight of each group (n = 6 per group) during the disease process. (C) Disease activity index (DAI) was calculated (n = 6 per group). (D) Macroscopic appearances and (E) the length of colons from each group of mice were measured. Data are presented as mean ± SEM. In (B) and (C) *P < 0.05, **P < 0.01 vs. DSS-treated alone group at the same day. In (E), ***P < 0.01 vs. DSS-treated alone group.
streptomycin and 10% fetal calf serum under a humidified 5% (v/v) CO₂ atmosphere at 37 °C. Bone marrow derived macrophages (BMDM) cells were isolated according to the following procedures. Bone marrow cells were isolated from C57BL6 mice and cultured with DMEM supplemented with 10% fetal bovine serum and 20 ng/ml GM-CSF (Peprotech, Rock Hill, NJ). Culture fluid was exchanged to fresh culture medium every 3 days. Under these conditions, adherent macrophages were obtained within 7–8 days. Cells were harvested seeded on 24-well plates. After culture for 6 h without GM-CSF, the cells were used for the experiments as bone marrow derived macrophages. Peritoneal macrophages were obtained from the peritoneal cavity by injection of PBS. Cells were washed twice in PBS and suspended in RPMI-1640 medium containing 10% FBS, 10,000 U/ml penicillin and 10 mg/ml streptomycin. The macrophages suspensions in culture medium were cultured in 24-well microplates for 40 min at 37 °C in a moist atmosphere of 5% CO₂. Non adherent cells were removed by washing the plate twice with PBS. The adherent macrophages were used for experiments.

2.4. Induction of colitis and treatment

Colitis was induced in C57BL/6 mice with 2.5% DSS (molecular weight 36–50 kDa) dissolved in drinking water (days 1–7). Normal mice were given water. Vehicle control (water), Fc11a-2 (3, 10, 30 mg/kg) and CsA (20 mg/kg) were given orally from day 1 to day 10, respectively.

2.5. Clinical scoring and histological analysis

Body weight, stool consistency and the presence of gross blood in feces and at the anus were observed everyday. The disease activity index (DAI) was calculated by assigning well-established and validated scores [23]. Briefly, the following parameters were used for calculation: a) diarrhea (0 points = normal, 2 points = loose stools, 4 points = watery diarrhea); b) hematochezia (0 points = no bleeding, 2, slight bleeding, 4 points, gross bleeding). At day 10 following induction of colitis, animals were sacrificed, the colon was removed and pieces of colonic tissue were used for ex vivo analysis. For histological analysis, part of the colon was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with H&E according to standard protocols. Histological scoring was performed in a blinded way by a pathologist.

Histological evaluation of H&E-stained colonic sections was graded as follows: 0, no signs of inflammation; 1, low leukocyte infiltration; 2, moderate leukocyte infiltration; 3, high leukocyte infiltration, moderate fibrosis, high vascular density, thickening of the colon wall, moderate goblet cell loss, and focal loss of crypts; and 4, transmural infiltrations, massive loss of goblet cell, extensive fibrosis, and diffuse loss of crypts.

2.6. Assessment of myeloperoxidase (MPO) activity

Neutrophil infiltration into inflamed colonic mucosa was quantified by MPO activity assessment using the O-dianisidine method. Protein extracted from colonic tissue was used to assess the MPO level according to manufacturer’s instructions. The results were showed as activity units per mg tissue.

2.7. Cytokine analysis by ELISA

Colons from mice in each group were homogenated with lysis buffer to extract total protein. The homogenate was centrifuged at 12,000 x g at 4 °C for 15 min. The amount of total extracted protein was determined by BCA™ protein assay kit (Pierce, Rockford, IL). The amount of IFN-γ, IL-1β, IL-18, IL-17A and TNF-α in the colon homogenate was quantified by ELISA kit (Dakewe, Beijing, China).

2.8. Real-time quantitative PCR

RNA samples were reverse transcribed to cDNA and subjected to quantitative PCR, which was performed with the BioRad CFX96 Touch™ Real-Time PCR Detection System (BioRad, CA) using iQ™ SYBR® Green Supermix (BioRad, CA), and threshold cycle numbers were obtained using BioRad CFX Manager software. The program for amplification was 1 cycle of 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 95 °C for 10 s. The primer sequences used in this study were as follows: IFN-γ, 5'-GATATCGAGGAACCTGGCAAAA-3' (forward) and 5'-CTTCAAGACTGCTGATGATAAT-3' (reverse); TNF-α, 5'-CGAGTGACAAGCTGCTAGCC-3' (forward) and 5'-GCTTTGATATCCACGGTAATGGT-3' (reverse); IL-1β, 5'-CTTCAAGCAGGAGGTATGGTTAAT-3' (forward) and 5'-TGAGTGTGTTATGGAATGGT-3' (reverse); IL-17A, 5'-TGAGAAGATGTCTGGTGGTT-3' (forward) and 5'-CTCTGTTAGGCTGCTTTCG-3' (reverse); IL-18, 5'-GCCCTCAAACTTCCAACATA-3' (forward) and 5'-TGGATCTTTTTCTCTTAAAGG-3' (reverse); VCAM1, 5'-GGAGACACTGTATCTTCCTGCTTCG-3' (forward) and 5'-TCCCTCTGCTGATGTTGGTCTTGCC-3' (reverse); ICAM1, 5'-GTCGAAGGTGCTTCTTCTCAG-3' (forward) and 5'-GTCGCTGTAAGGTCTTCCATGACA-3' (reverse).

2.9. Immunofluorescence histochimistry

CD11c™ macrophage infiltration analysis was performed on paraffin-embedded colonic tissue sections (5 μm). Briefly, the sections were deparaffinized, rehydrated and washed in 1% PBS-Tween. Then they were treated with 2% hydrogen peroxide, blocked with 3% goat serum and incubated for 2 h at room temperature with anti-CD11c FITC (1:100). The slides were then counter-stained with DAPI for 2 min. The reaction was stopped by thorough washing in water for 20 min. Images were acquired with confocal laser-scanning microscope (Olympus, Lake Success, NY). Settings for image acquisition were identical for control and experimental tissues.

2.10. Immunofluorescence cytochemistry

Bone marrow derived macrophages (BMDM) on coverslips were fixed in 4% paraformaldehyde (PFA), permeabilized with 0.5% Triton X-100 for 20 min and blocked with 3% BSA for 30 min. Cells were immunostained with monoclonal anti-ASC together with anti-caspase-1 Ab overnight. Then Alexa Fluor 488-conjugated anti-mouse IgG and 594-conjugated anti-rabbit IgG (Life technology, CA) were immunostained for 2 h. The coverslips were counterstained with DAPI and imaged with a confocal laser scanning microscope (Olympus, Lake Success, NY).

2.11. Western blotting

The protein lysates were separated by 10% SDS-PAGE and subsequently electrotransferred onto a polycrylilide difluoride membrane (Millipore, Bedford, MA). The membrane was blocked with 5% nonfat milk for 1 h at room temperature. The blocked membrane was incubated with the indicated primary Abs, and then with a horseradish peroxidase-conjugated secondary Ab. Protein bands were visualized using Western blotting detection system according to the manufacturer’s instructions (Cell Signaling Technology, MA).
2.12. Statistical analysis

Results were expressed as mean ± SEM of three independent experiments and each experiment included triplicate sets. Data were statistically evaluated by one-way ANOVA followed by Dunnett’s test between control group and multiple dose groups. The level of significance was set at a P value of 0.05.

3. Results

3.1. Fc11a-2 attenuated DSS-induced experimental colitis

To seek for the small compounds with anti-inflammatory activities in vitro, we screened a series of synthetic compounds by assaying their inhibitory activities of IL-1β which was produced by THP-1 cells activated by LPS and ATP. Fc11a-2, one small molecule, showed the highest inhibitory activity of IL-1β activation (Fig. 1A). The chemical name of Fc11a-2 was 1-ethyl-5-methyl-2-phenyl-1H-benzo[d]imidazole (chemical structure shown in Fig. 1A), which was confirmed by 1H NMR and 13C NMR (data not shown). It is well known that DSS induces a severe illness in mice characterized by a dramatic loss of body weight. Compared with vehicle-treated group, Fc11a-2 at 10 and 30 mg/kg and CsA at 20 mg/kg significantly attenuated the loss of body weight during the disease progression. Colitis model also showed features of significant appearance of diarrhea/loose feces and visible fecal blood, resulting in significant disease activity index (DAI) elevation. Comparatively, such changes were markedly improved by 10 and 30 mg/kg of Fc11a-2 and 20 mg/kg of CsA (Fig. 1B and C). DSS typically causes colonic shortening while such change was also improved by 10 and 30 mg/kg of Fc11a-2 and 20 mg/kg of CsA (Fig. 1D and E). Histological analysis showed distortion of crypts, loss of goblet cells, infiltration of mononuclear cells, and severe mucosal damage in the colon specimens of colitis mice (Fig. 2A). The results of standard pathological tests in mice showed much improvement in pathological changes in mice treated with 10 and 30 mg/kg of Fc11a-2 and 20 mg/kg of CsA (Fig. 2B). The myeloperoxidase (MPO) activity in colon tissues from Fc11a-2- and CsA-treated mice was also lower than that of the vehicle-treated group (Fig. 2C).

3.2. Fc11a-2 regulated the cytokine profiles in colons of mice with DSS-induced colitis

To examine the influence of Fc11a-2 on the cytokine expression in acute DSS colitis model, levels of TNF-α, IL-1β, IL-18, IL-1β, and IFN-γ in colons were measured in parallel following induction of colitis. As shown in Fig. 3, the mRNA expressions of IFN-γ, TNF-α, IL-1β, IL-18, and IL-17A in colonic homogenate were determined by ELISA. Data are presented as mean ± SEM (n = 6). *P < 0.05, **P < 0.01 vs. DSS-treated alone group.

![Fig. 2](image1.png)

**Fig. 2.** Fc11a-2 treatment prevented DSS-induced colon damage in mice. (A) Serial sections of colon tissues were stained with H&E. (B) Histopathological scores of each group were determined. (C) MPO (myeloperoxidase) activity in the colonic tissues was detected. Data are presented as means ± SEM. *P < 0.05, **P < 0.01 vs. DSS-treated alone group.

![Fig. 3](image2.png)

**Fig. 3.** Fc11a-2 suppressed proinflammatory cytokine production in colon tissues from DSS-colitis mice. (A) The mRNA expressions of inflammation-related cytokines (IFN-γ, TNF-α, IL-1β, IL-18, IL-1β, and IL-17A) and (B) adhesion molecule VCAM1 and ICAM1 in colonic tissues were determined by real-time PCR. (C) Protein levels of cytokines including IFN-γ, TNF-α, IL-1β, IL-18, and IL-17A in colonic homogenate were determined by ELISA. Data are presented as mean ± SEM (n = 6). *P < 0.05, **P < 0.01 vs. DSS-treated alone group.
IL-1β, IL-18, IL-17A, VCAM1 and ICAM1 were remarkably increased after DSS challenge. Both Fc11a-2 and CsA significantly inhibited the elevated expression of these cytokines after DSS challenge (Fig. 3A and B). Also, we analyzed the cytokine levels in colonic homogenized protein from each group. Administration of Fc11a-2 and CsA to mice significantly suppressed inflammatory cytokines at protein level (Fig. 3C).

3.3. Fc11a-2 reduced DSS-induced activation of MAPK and STAT1 signaling

Activations of mitogen-activated protein kinases (MAPKs), STAT1 and NF-κB all play essential roles in transcriptional induction of various genes involved in inflammation, such as TNF-α, IL-1β, IFN-γ and IL-17A [24,25]. As shown in Fig. 4, DSS treatment caused different levels of phosphorylations of p38, ERK, JNK, p65 and STAT1 in the injured colons from mice. Both Fc11a-2 and CsA treatment markedly reduced the phosphorlylations of ERK, JNK and STAT1 but had little effect on activations of p65 and p38.

3.4. Fc11a-2 attenuated macrophage infiltration and reduced cleaved caspase-1 expression of peritoneal macrophages in DSS-induced colitis mice

To further investigate the mechanism of protection from colitis by Fc11a-2, we examined macrophage infiltration in the colon tissues from each group. We observed a large number of CD11c+ macrophages in colonic samples from vehicle-treated DSS mice. These infiltrated macrophages were mainly located in the mucosa of the lesion site. In contrast, few infiltrating macrophages were detected in either Fc11a-2-treated or untreated colonic samples (Fig. 5A). Macrophages incubated with DSS in vitro secreted high levels of IL-1β in a caspase-1-dependent manner [9]. We examined the regulation of caspase-1 activation in murine peritoneal macrophages in vivo from different groups. Consistently, Fc11a-2 treatment markedly inhibited caspase-1 activation in vivo in comparison with that of vehicle-treated mice (Fig. 5B). Inflammasome-derived IL-1β and IL-18 are required for the differentiation of Th helper 17 (Th17) and T helper 1 (Th1) cells [26]. Actually, our in vivo investigation showed that Fc11a-2 suppressed Th1 differentiation in mice with DSS-induced colitis (Fig. 5C).

3.5. Fc11a-2 inhibited the activation of NLRP3 inflammasome in vitro

IL-1β and IL-18 were processed as an inactive cytoplasmic precursor (pro-IL-1β and pro-IL-18) which has to be cleaved by caspase-1 to produce the mature active form. We examined the ability of Fc11a-2 to inhibit the activation of pro-IL-1β/pro-IL-18 by NLRP3 inflammasome. As shown in Fig. 6A, Fc11a-2 exhibited a significant inhibition on IL-1β/IL-18 activation at the dose of 3 μM. Recent evidence indicates that a caspase recruitment domain-containing protein called ASC binds pro-caspase-1 (p45) to induce the autocleavage of pro-caspase-1 to produce the mature active form (p10 and p20). Our results showed that Fc11a-2 inhibited the activation of caspase-1 induced by addition of ATP in vitro (Fig. 6B and C) but had little effect on NF-κB activation (Fig. 6D). It is reported that in the presence of signal I (NF-κB signaling), the NLRP3 inflammasome is activated by mitochondrial apoptotic

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**Fig. 4.** Fc11a-2 decreased activations of MAPK and STAT1 signaling pathways in colon tissues from DSS-colitis mice. (A) Colonic homogenate from each group of mice were subjected to Western blot. (B) Phosphorylations of MAPK protein, STAT1 and p65 were normalized to GAPDH. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01 vs. DSS-treated group.
Fig. 5. Fc11a-2 inhibited NLRP3 inflammasome activation in mice with DSS-induced colitis. (A) Sections of colonic tissue were immunostained with DAPI (blue) and anti-CD11c-FITC (green) and observed by confocal laser-scanning microscope, 100×. (B) Peritoneal macrophages were isolated from normal, vehicle-treated and Fc11a-2-treated colitis mice (10 mg/kg) at day 7. After 5 mM ATP stimulation for 30 min, proteins were collected for Western blot. Data shown here are representative of three different experiments. (C) Fc11a-2 inhibited Th1/Th17 differentiation in mice with DSS-induced colitis. Splenocytes was isolated from each group of mice and restimulated with PMA/ionomycin/monensin for 4 h. Th1 and Th17 cells were analyzed by intracellular staining of IFN-γ and IL-17A in the CD4 gate. Numbers in quadrants indicate percent cells in each throughout. Data are presented as means ± SEM. *P < 0.05 vs. DSS-treated group. (For interpretation of the references to color in the artwork, the reader is referred to the web version of the article.)

Fig. 6. Fc11a-2 inhibited IL-1β/IL-18 processing in THP-1 cells induced by LPS and ATP. (A) LPS-primed THP-1 cells were treated with Fc11a-2 (1, 3, 10, 30 µM) for 1 h, following by 1 h incubation of 5 mM ATP. Released IL-1β/IL-18 in the supernatant was analyzed by ELISA. (B) Protein levels of pro-caspase-1, cleaved caspase-1, ASC and NLRP3 were determined by Western blot. (C) Caspase-1 activity was measured. *P < 0.05, **P < 0.01 vs. LPS + ATP group. (D) THP-1 cells were treated with LPS alone or with Fc11a-2 (3, 10, 30 µM) for 4 h. Then proteins were collected and phosphorylation of NF-κB was analyzed by Western blot. Triptolide (TP) was used as a positive control. **P < 0.01 vs. LPS group. (E) LPS-primed THP-1 cells were treated with or without Fc11a-2 (30 µM) for 1 h, followed by 1 h incubation of 5 mM ATP. Mitochondria membrane potential was detected by JC-1 staining. Data are presented as mean ± SEM of three different experiments. NS means no significance.
signaling which licensed production of IL-1β/IL-18. NLRP3 secondary signal activators such as ATP induce mitochondrial dysfunction, resulting in the release of oxidized mitochondrial DNA (mtDNA) into the cytosol, where it binds to and activates the NLRP3 inflammasome [27,28]. However, our study revealed that Fc11a-2 had a very modest effect on protecting mitochondrial from dysfunction (Fig. 6D and E).

3.6. Fc11a-2 restrained pro-caspase-1 autolysis by suppressing its release from ASC/NLRP3 complex

Next, we sought to determine the mechanism how Fc11a-2 inhibited the activation of inflammasome in vitro. As shown in Fig. 7A, ATP time-dependently induced cleavage of caspase-1, while Fc11a-2 inhibited the cleavage of caspase-1 induced by ATP. Upon ATP stimulation, cytoplasmic caspase-1 first co-localizes with ASC which translocates from nucleus [29] and then auto-cleaves and releases from the complex. Immunoprecipitation (IP) and immunofluorescence analysis (Fig. 7B and C) showed that Fc11a-2 treatment interrupted this process by inhibiting the auto-cleavage of caspase-1 so that less amount of activated caspase-1 was released from the complex.

4. Discussion

Nowadays, therapeutic options and approaches for inflammatory bowel disease continue to evolve. Ulcerative colitis is a chronic inflammatory condition of the intestine which not only impairs patients’ quality of life, but also contributes to the risk of colon cancer [30]. Generally, treatment of inflammatory bowel disease depends on the severity of the disease. Most of the time immunosuppressants such as prednisone, TNF inhibition, azathioprine and methotrexate may be required to control the symptoms [31]. However, these immunosuppressants also have potential side effects including steroid dependence [32] and serious infections [33]. Therefore, novel strategies with fewer adverse effects are urgently needed.

Over the past years, with the use of the less biased approach of genome-wide screening with microsatellite DNA markers, study has suggested that Crohn’s disease and ulcerative colitis share many common genetic and mechanistic features [34]. NOD2 (also referred to as caspase activation and recruitment domain 15), which is expressed in macrophages, is responsible, at least in part, for this linkage. Its variants appear to result in reduced macrophage activation of NF-κB in response to lipopolysaccharide [1]. Inflammasomes are cytosolic multiprotein complexes...
Conflict of interest

Y. Sun and Q. Xu are currently exploring the commercial implications of these findings and declare a potential conflict of interest.

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(1) Study conception and design: QX, YS; (2) Acquisition, analysis and/or interpretation of data: WL, WG, JW, QL, FT; (3) Drafting/revision of the work for intellectual content and context: YG, Y Shen, JL, RT; (4) Final approval and overall responsibility for the published work: QX, YS.

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