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# Anti-inflammation Effects of Sinomenine on Macrophages through Suppressing Activated TLR4/NF-κB Signaling Pathway

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**Summary**: Sinomenine (SN) has been used in the clinical treatment of systemic lupus erythematosus and rheumatoid arthritis for many years. Studies showed that SN held protective effects such as anti-inflammation, scavenging free radicals and suppressing immune response in many autoimmune diseases. The purpose of the present study is to explore the mechanism of anti-inflammation of SN on lipopolysaccharide (LPS)-induced macrophages activation and investigate whether the TLR4/NF-κB signaling pathway participated in. Macrophages isolated from mouse peritoneal cavity were stimulated by 1 μg/mL LPS for 24 h. And then the cells were treated with various concentrations of SN, TLR4 inhibitor respectively for additional 48 h. Drug toxicity was detected by MTT assay and Transwell experiment was used to assess chemotaxis. Furthermore, TLR4 and MyD88 mRNA levels were detected by real-time PCR. Western blotting was used to examine TLR4, MyD88 and phosphorylated IκB protein expression in macrophages. Immunofluorescence assay was applied to observe p65 NF-κB protein expression in macrophage nucleus. We extracted macrophages with high purity and activity from the abdominal cavity of mice. SN remarkably inhibited the chemotaxis and secretion function of LPS-stimulated macrophages. It also down-regulated both the protein levels of inflammatory cytokines (TNF-α, IL-1β and IL-6) and the RNA and protein levels of the key factors (TLR4, MyD88, p-IκB) in TLR4 pathway. The expression of p65 NF-κB protein in nuclei was down-regulated, which was correlated with a similar decrease in p-IκB protein level. In conclusion, SN can inhibit the LPS induced immune responses in macrophages by blocking the activated TLR4/NF-κB signaling pathway. These results may provide a therapeutic approach to regulate inflammatory responses.
**Key words**: sinomenine; macrophage; TLR4/NF-κB pathway

Macrophages have been considered as important immune cells for a long time, which enter tissues and adjust their functions to deal with a wide range of challenges related to organogenesis, malignancy, tissue injury, sterility, or pathogenic inflammatory stimuli[1]. Macrophages regulate immune responses through engulfing bacteria, activating inflammatory cells and releasing proinflammtory factors such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6[2]. While Toll-like receptor 4 (TLR4), the specific receptor for the bacterial endotoxin lipopolysaccharide (LPS), is considered as a significant transmembrane protein and innate immune receptor, mainly expressed in antigen presenting cells, such as dendritic cells (DCs) and monocyte/macrophages.

Sinomenine (SN) is an alkaloid which is originally extracted from the Chinese medicinal herb *Sinomenium acutum*[3]. SN holds pharmacological effects on anti-inflammation, inhibiting cellular/humoral immunity, scavenging free radicals and has been used for treating rheumatoid arthritis (RA), lupus erythematosus and other immune diseases for a long time in China[4–6]. SN is shown to regulate a series of inflammation-related molecules, including nitric oxide, TNF-α, prostaglandin E3, and leukotriene C4, in LPS-treated macrophages *in vitro* and *in vivo*[7, 8]. In the present study, we investigated the role of SN in LPS stimulated macrophages. Our results indicate that SN suppresses the functions of macrophage through inhibiting the TLR4/MyD88/NF-κB signaling pathway, which sheds some light on a promising therapeutic strategy for some immunological diseases.

## 1 MATERIALS AND METHODS

### 1.1 Chemicals And Drugs

SN (purity: ≥98%) powder was obtained from Google Biology (China). A stock solution (200 mg/mL) was prepared in PBS. PBS and LPS were from

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Sigma (USA). RPMI-1640 was produced by Hyclone (China), and fetal bovine serum (FBS) was purchased from Biowest (France). PE-labeled anti-mouse CD11b and FITC-labeled anti-mouse F4/80 were purchased from Miltenyi Biotec (Germany). Mouse TNF-α, IL-1β and IL-6 ELISA kits were purchased from Google Biology. In addition, TLR4, MYD88, p-IκB and p65 NF-κB antibodies were from Abcam (USA).

**1.2 Animals**

Six male Balb/C mice (7–9 weeks old) were obtained from the Laboratory Animal Center of Zhongnan Hospital of Wuhan University, maintained in SPF Laboratory Animal Center at a temperature of 25°C and relative humidity of 50%. Normal food and tap water were provided to the animals throughout the experimental periods. All the procedures and ethics guidelines were approved by the Zhongnan Hospital of Wuhan University, China. Efforts were made to minimize the number of animals used and their suffering.

**1.3 Cell Culture**

Two mL of 5% starch broth was injected into the mouse peritoneal cavity for three days. Then, all of the mice were euthanized and soaked with 75% alcohol for 10 min. The mouse was fixed on styrofoam in supine position in a bechtop. The outer skin of mouse abdomen was incised carefully to fully expose the peritoneum. After that, 6 mL of ice-cold PBS was injected into the peritoneal cavity and the fluid was collected after 30 min. To make sure that all of the collected cells only come from the peritoneal cavity, any organs should not be injured. Next, the collected cell suspension was centrifuged at 1500 r/min for 15 min at 4°C. To eliminate the influence of some red blood cell (RBC) contamination, 5–10 mL erythrocyte lysate was used. With that, all the cells were washed with 1×PBS, resuspended in RPMI-1640 (containing 10% FBS) and cultivated in a cell incubator under the condition of 37°C and 5% $CO_2$. The cells were then stimulated by 1 μg/mL LPS for 24 h, identified by a flow cytometer, and then treated with various concentrations of SN, TLR4 inhibitor or medium for 48 h.

**1.4 Flow Cytometric Analysis**

After being washed with cold PBS, $2\times10^6$ cells were incubated for 30 min at 4°C with PE-labeled anti-mouse CD11b and FITC-labeled anti-mouse F4/80 (kept in dark place). The cells were removed and washed three times with cold PBS and detected on a flow cytometer (BD Bioscience, USA) to identify the proportion of the macrophages.

**1.5 Drug Toxicity Test**

The relative activities of cells were detected by MTT. Macrophages were seeded in a 96-well plate ($1\times10^4$ cells/well, 100 μL), and treated with different concentrations of SN. Twenty μL MTT (5 mg/mL) solution was added to each well and the cells were incubated for 4 h. At the end, DMSO of 100 μL was added and incubated for 10 min and the absorbance (*A*) value was measured at 490 nm on a microplate reader (BIO-RAD Model 680, USA). The appropriate drug concentrations were selected according to the results.

**1.6 Phagocytic Function Assessment**

Cells were placed in a 6-well plate ($2\times10^6$ /well) and treated with different drugs for 48 h. Thirty min after adding ink, the ink was washed with PBS. The cells were observed and the photos collected on the Leica microscope. Then, the phagocytose percentage and the phagocytic index were calculated carefully.

The phagocytose percentage = the number of macrophages phagocytizing ink per 100 macrophages/100;

Phagocytosis index = the total number of ink granules ingested by 100 macrophages/100

**1.7 Chemotaxis Assessment**

In this experiment, chemotaxis of macrophages was determined by Transwell. The cells were digested by trypsin and suspended by RPMI 1640 medium (containing 1% BSA). Then the cell suspension liquid was arranged in the transwell cells while the RPMI 1640 (containing 10% FBS) with different drugs was placed in the 24-well plate. After 48 h, the cells of the up-surface were wiped off and stained with 0.1% crystal violet. At the end, the cells were observed and the photos were collected on the Leica microscope to count the number of migrated cells.

**1.8 Enzyme-linked Immunosorbent Assay (ELISA)**

Macrophages were cultured in 96-well plates at a density of $2\times10^5$ cells/mL and pretreated with different concentrations of SN, TLR4 inhibitor or medium alone for 48 h. Inflammatory cytokines TNF-α, IL-1β and IL-6 levels in cell culture supernatant were determined by ELISA kits according to the manufacturer's recommendations. In the end, the *A* value was measured at 450 nm on a Multiskan spectrum microplate reader. The contents of TNF-α, IL-1β and IL-6 were calculated according to the standard curve.

**1.9 Real-time Quantitative PCR (qRT-PCR)**

The RNA levels of TLR4 and MyD88 were analyzed by qRT-PCR (Takara, Japan) using an ABI Prim 7500HT machine (Applied Biosystems, USA). Total RNA was extracted with TRIzol according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 μg total RNA with an iScript cDNA synthesis Kit (Takara, Japan). In total, 80 ng of cDNA samples was applied in 20 μL reactive mixture, and the Taq DNA Polymerase and Rox Reference DyeII were used for qRT-PCR. The primer sequences were as follows: the upstream primer of GAPDH was 5′-ACCCAGAAGACTGTGGATGG-3′ and the downstream primer was 5′-ACACATTGGGG-GTAGGAACA-3′, with an amplified fragment of 172 bp. The upstream primer of TLR4 was 5′-CAGCAAAG-TCCCTGATGACA-3′ and the downstream primer

was 5′-AGAGGTGGTGTAAGCCATGC-3′, with an amplified fragment of 179 bp. The upstream primer of MyD88 was 5′-CTGTCTCCAGGTGTCCAACA-3′ and the downstream primer was 5′-ATGTAGACAGG-ACGGCATCAG-3′, with an amplified fragment of 164 bp. The amplification was carried out as follows: 1 cycle for denaturation (95°C for 30 s) followed by 40 cycles for two-stage PCR (95°C for 10 s and 56°C for 1 min). The mRNA relative quantitation was calculated using the comparative $2^{-\Delta\Delta Ct}$ method.

**1.10 Western-blotting Assessment**

The macrophages were lysed with RIPA lysis buffer and were centrifuged at 12 000 *g* at 4°C for 30 min to collect cellular proteins in the supernatants. Equal amounts of proteins from each sample were resolved by 6% to 15% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were treated with 3% non-fat milk on a shaker for 1 h at room temperature. Then the film was incubated with primary antibodies diluted in PBS at 4°C overnight. Mouse anti-TLR4 antibody (working concentration was 1:500), rabbit anti-MyD88 antibody (working concentration was 1:500) and mouse anti-pIκB antibody (working concentration was 1:500) were from Abcam (USA). The secondary antibodies were from Amersham Bio-sciences (USA). Equal amount of protein sample loading was verified by detecting the protein expression of mouse anti-GAPDH antibody (1:500 diluted in PBS). Immunoreactive bands were visualized via enhanced chemiluminescence, and densitometry was performed using Quantity One software (Bio-Rad Laboratories, USA).

**1.11 Immunofluorescence Assay**

In this study, p65 NF-κB expression in macrophage nucleus was observed by the immunofluorescence. Cells ($2\times10^6$/mL) were set in 6-well plates and treated with different concentrations of SN or TLR4 inhibitor for 48 h. After being fixed with 4% polyformaldehyde, penetrated with 0.5% Trixon-100 and blocked with 5% of BSA, the cells were incubated with primary anti-NF-κBp65 antibody (1:300) overnight at 4°C, followed by the secondary antibody (labeled TRICT) and DAPI. The photos were collected on the Leica microscope.

**1.12 Statistical Analysis**

SPSS (version 19.0) software was used for data analysis in this study. Comparisons among multiple groups were assessed using one-way analysis of variance (ANOVA) on the GraphPad Prim 5. All the results are expressed as mean±standard error of mean (SEM). A value of $P<0.05$ was considered to be statistically significant.

## 2 RESULTS

**2.1 Isolation of Original Generation of Cells**

We successfully extracted $(9\pm2.12)\times10^6$/mL adherent cells per mouse from peritoneal cavity. Adherent cells were visible round, elliptic, polygonal or irregular in shape with pseudopodia and protrusions by microscopy (×400). There was no cell mitosis or cell proliferation in culture for a week.

**2.2 Phenotype Identification of Macrophages**

After being stimulated with 1 μg/mL LPS, mouse macrophages presented the specific phenotype: $CD11b^{high}$ and $F4/80^{high}$ [9, 10]. The cells were stained with PE-labeled anti-mouse CD11b and FITC-labeled anti-mouse F4/80 antibodies, and identified by fluorescence. The results suggested that the proportion of macrophages was more than 95% in all cells extracted from the abdominal cavity of mice (fig. 1).

**2.3 Cytotoxicity Effect of SN on Macrophages**

Examination of cytotoxicity effect of SN on macrophages was conducted at a concentration range of 10 μg/mL to 2 mg/mL using MTT assay. Probit analysis showed cell viability of over 90% after SN treatment at the concentration of 10, 50 and 100 μg/mL (there was no significant difference). While the cell viability was reduced by 50% at the concentration of 1500 μg/mL. The result indicated that SN had no cytotoxicity on macrophages at lower concentration (≤100 μg/mL) but

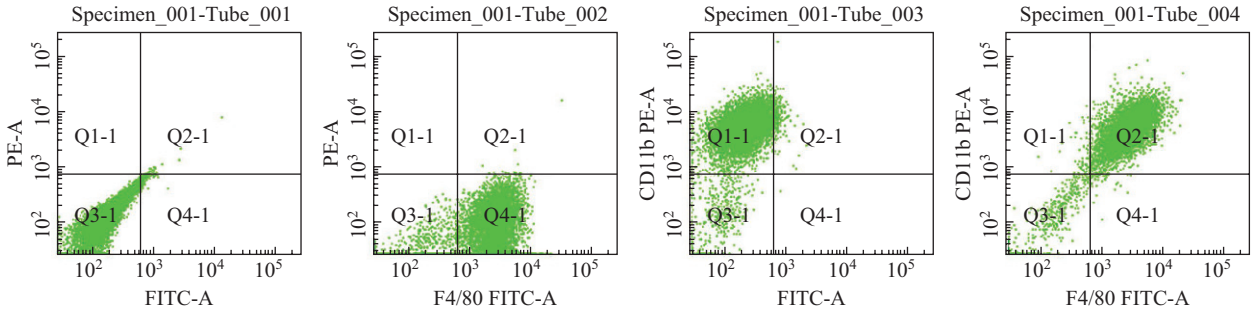


**Fig. 1** After being stimulated with 1 μg/mL LPS for 48 h, the cells were stained with PE-labeled anti-CD11b and FITC-labeled anti F4/80 antibodies

The figures showed the percentage of positive cells expressing various membrane markers. The percentage of macrophages with expression of F4/80 was 95% (Q4-1), while macrophages with expression of CD11b was 95.7% (Q1-1). The percentage of macrophages with co-expression of F4/80 and CD11b was 95.5% (Q2-1).

it inhibited the cell viability at a higher concentration (>1000 μg/mL) (fig. 2). Therefore, we selected drug concentration of 100 μg/mL, 500 μg/mL, 1000 μg/mL for the follow-up test.

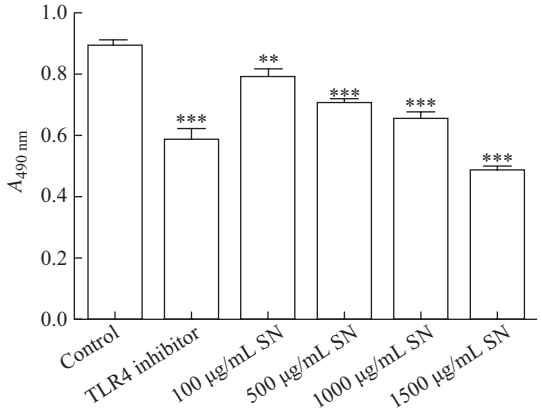


**Fig. 2** After treatment with LPS and SN (100, 500, 1000 and 1500 μg/mL) for 48 h, the cytotoxicity of SN on macrophages was determined by MTT assay. Results were expressed as means±SEM. $^{**}P<0.001$, $^{***}P<0.0001$ *vs.* the control group

### 2.4 Effect of SN on Phagocytosis of Macrophages

When organism was stimulated, the macrophages can devour and eliminate the external invasion factors. But over activation may cause the normal tissue damage in return. We calculated the number of ink granules phagocytosed by macrophages at different drug concentrations. Fig. 3 shows that there were no significant differences in the ability of devouring after treatment with different concentrates of SN.

### 2.5 Inhibition Effect of SN on Chemotaxis Function of Macrophages

In this study, the chemotactic ability of macrophages was detected by conventional Transwell method. Each drug (TLR4 inhibitor, different concentrations of SN) in the lower chamber influenced the chemotactic effect of macrophages in the upper chamber. Compared with the control group, the number of migrating macrophages decreased in the experimental groups, in which the number of the migrating cells gradually decreased with the increase of SN concentration ($P<0.05$). And compared with TLR4 inhibitor, there was no difference in migration of the SN group ($P>0.05$) (fig. 4). The results showed that SN, as TLR4 inhibitors, could decrease chemotactic migration of the macrophages to reduce inflammation in a dose-dependent manner.

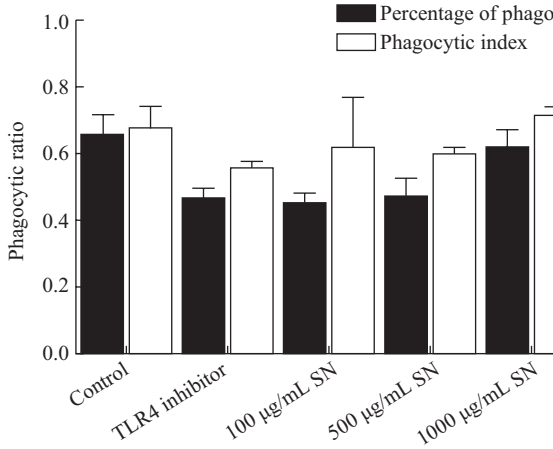


**Fig. 3** After treatment with different concentrates of SN and TLR4 inhibitor for 48 h, the ability to devour the stimulating factors of macrophages did not significantly change. Results are expressed as means±SEM. $P>0.05$ *vs.* the control group

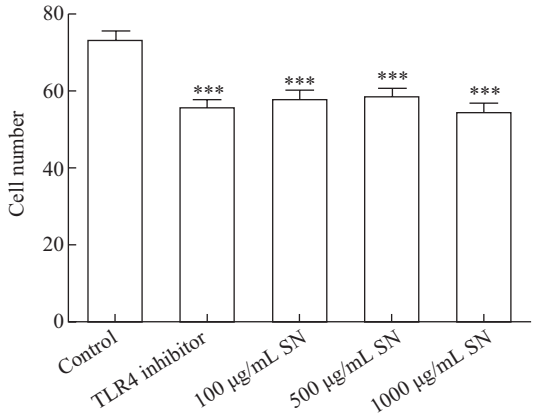


**Fig. 4** The migration of macrophage in each group decreased significantly compared with the control group ($^{***}P<0.0001$). With the increase of SN concentration, the number of migrating cells decreased gradually, but there was no significant difference between the groups ($P>0.05$).

### 2.6 Inhibition Effect of SN on Secretion of Inflammatory Factors TNF-α, IL-1β, and IL-6 in Macrophages

TNF-α is an important cytokine involved in inflammatory reaction, which can be secreted by macrophages or lymphocytes, promoting T cell proliferation, promoting the production of other growth factors and cytokines (IL-1β, IL-6)[11], and mediating intestinal mucosal damage in intestinal tract. Inflammatory cytokines IL-1β and IL-6 can activate neutrophils and macrophages and up-regulate their immune responses. In this study, the concentration of inflammatory factors in the supernatant of macrophages cultured *in vitro* was investigated, and the effect of SN on the secretion of macrophages was observed. In fig. 5, compared with the control group, the concentrations of TNF-α, IL-1β and IL-6 in the supernatant of macrophages were reduced in all groups, and with the increase of SN concentration, the expression decreased gradually, and the differences were statistically significant (all $P<0.05$). While there were no significant differences between the 1000 μg/mL SN groups and the TLR4 inhibitor group ($P>0.05$). It can be seen that SN, as TLR4 inhibitor, can inhibit

the secretion of inflammatory cytokines in different degrees, and the inhibitory effect is concentration-dependent.

**2.7 Inhibition Effect of SN on Expression of TLR4 and MyD88 mRNA in Macrophages**

Relative quantity of TLR4 and My88 was assessed by the relative real-time method at cellular and molecular levels. The influence of expression caused by SN is shown by $2^{-\Delta\Delta Ct}$ (fig. 6). Compared with the control group, the mRNA expression levels of TLR4 and MyD88 in SN groups were obviously reduced, and with the increase of SN concentration, expression levels decreased gradually. Compared with TLR4 inhibitor group, the expression level of TLR4 mRNA in the SN groups and expression level of MyD88 mRNA in the 1000 μg/mL SN group had no significant difference. Preliminary results showed that SN could inhibit the RNA expression levels of key factors in TLR4 pathway, then suppress the development of further inflammation.

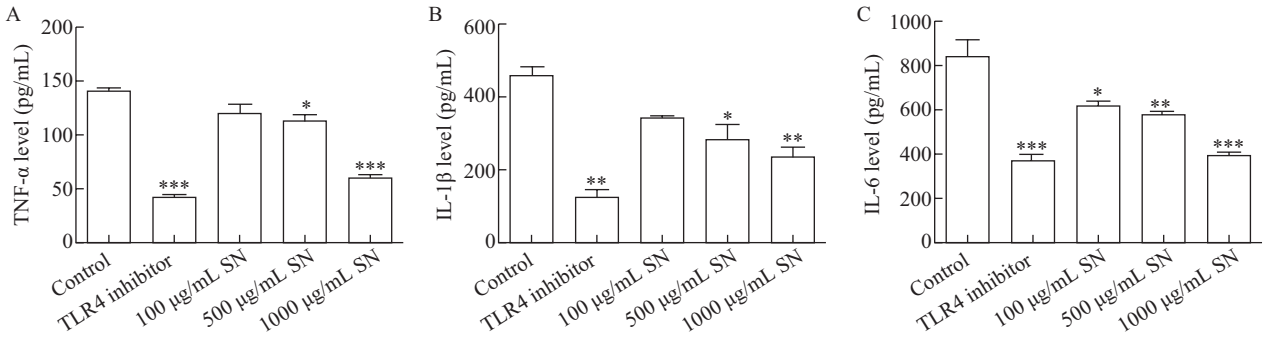


**Fig. 5** Comparison of protein levels of inflammatory cytokine TNF-α, IL-1β, and IL-6 secreted by macrophages in each group
A: the concentration of the inflammatory factor TNF-α in the supernatant. $^{*}P<0.01$, $^{***}P<0.0001$ *vs.* control group; 1000 μg/mL SN group *vs.* TLR4 inhibitor group ($P>0.05$); B: the concentration of the inflammatory factor IL-1β in the supernatant. $^{*}P<0.01$, $^{**}P<0.001$ *vs.* control group; 1000 μg/mL SN group *vs.* TLR4 inhibitor group ($P>0.05$); C: the concentration of the inflammatory factor IL-6 in the supernatant. $^{*}P<0.01$, $^{**}P<0.001$, $^{***}P<0.0001$ *vs.* control group; 1000 μg/mL SN group *vs.* TLR4 inhibitor group ($P>0.05$)

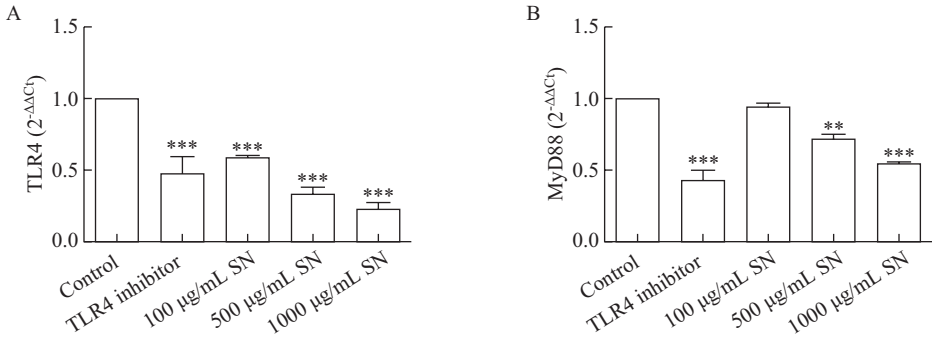


**Fig. 6** TLR4 and MyD88 mRNA levels of macrophages in each group
A: relative TLR4 mRNA expression. $^{***}P<0.0001$ *vs.* control group; SN groups *vs.* TLR4 inhibitor group ($P>0.05$); B: relative MyD88 mRNA expression. $^{**}P<0.001$, $^{***}P<0.0001$ *vs.* control group; 1000 μg/mL SN group *vs.* TLR4 inhibitor group ($P>0.05$)

**2.8 SN Inhibited Transfer of p65 NF-κB Proteins into Nucleus**

In this study, nuclear translocation and transcription of NF-κB were qualitatively studied by immunofluorescence method. We observed the fluorescent expression in the nucleus to reflect the transcription of NF-κB from one aspect (fig. 7). NF-κB was significantly translocated into the nucleus after being induced by aseptic inflammation in combination with LPS. While the expression of NF-κB in the nucleus of macrophages decreased because of drug stimulation in each group. These results indicated that SN could reduce the nuclear translocation and transcription of NF-κB to a certain extent, thereby inhibiting the immune inflammatory reaction.

**2.9 SN Inhibited Activation of TLR4, MyD88 and p-IκB Proteins**

In this study, we detected the protein expression of TLR4, MyD88 and p-IκB by Western blotting. Meanwhile the expression of NF-κB was further reflected by the expression of p-IκB protein. Results in fig. 8 showed that the protein expression levels of TLR4, MyD88 and p-IκB in all the SN groups were decreased as compared with the control group. And

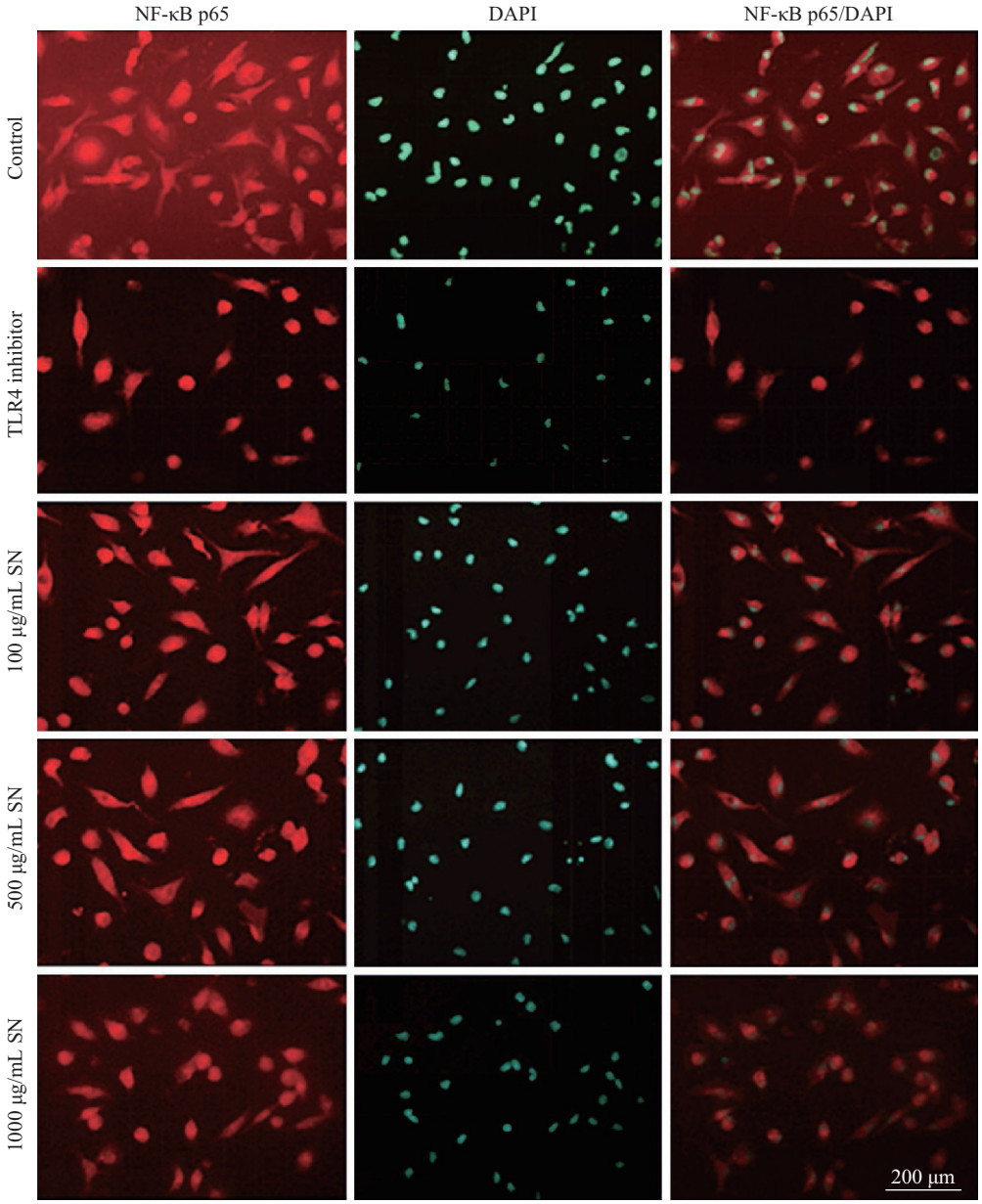


**Fig. 7** Expression of NF-κB in the nuclei of macrophages in each group
Compared with the control group, the expression level of NF-κB was decreased in the other groups. The decrease was the most obvious in the inhibitor group and 1000 μg/mL SN group.

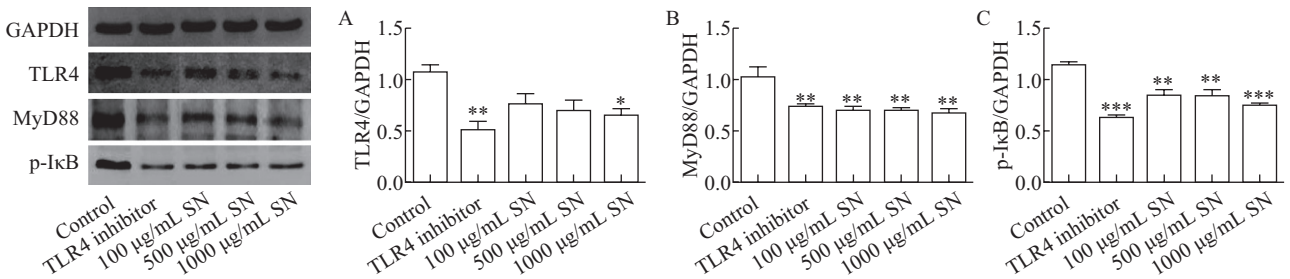


**Fig. 8** The protein levels of TLR4, MyD88 and p-IκB in macrophages of each group
A: Compared with the control group, the level of TLR4 protein decreased significantly in the TLR4 inhibitor group and 1000 μg/mL SN group ($^{*}P<0.01$, $^{**}P<0.001$). There was no significant difference in the expression of TLR4 between the SN groups and the TLR4 inhibitor group ($P>0.05$). B: Compared with the control group, the expression of MyD88 decreased in all the other groups ($^{**}P<0.001$). There was no significant difference between the SN groups and the TLR4 inhibitor group ($P>0.05$); C: Compared with the control group, the expression of p-IκB protein decreased in the other groups, and the expression of p-IκB decreased gradually with the increase of SN concentration ($^{**}P<0.001$, $^{***}P<0.0001$).

with the increase of SN concentration, the expression levels decreased gradually. Results showed that SN could inhibit the protein expression levels of TLR4, MyD88 and p-IκB, which are the key factors in the TLR4/NF-κB signaling pathway.

## 3 DISCUSSION

SN has pharmacological actions such as anti-inflammation, immunosuppression and scavenging free radicals. It can inhibit both mRNA and protein levels of TLR2 and TLR4 in DCs in the peripheral blood of patients with rheumatoid arthritis. It is also able to suppress the maturation of DCs, then prevent the abnormal activation of T lymphocytes, and finally reduce the inflammatory response[9]. Researches showed that SN may reduce the inflammatory mediators and cytokines to inhibit the invasion of the inflammatory cells, decrease the inflammatory injury, accelerate the repair of tissue and promote wound recovery[10, 12].

As primary phagocytes in immune response stage, macrophages migrate to an infected or damaged site in a free or fixed form to eliminate the pathogen, activate related lymphocytes and secrete inflammatory factors, which triggers inflammation and induces tissue injury. According to different stimulating factors, macrophages can differentiate into different phenotypes, most commonly into M1 and M2 macrophages. M1 macrophages are mainly activated by interferon-gamma (IFN-γ) and LPS which are produced by Th1 cells and gram-negative bacteria, respectively, to participate in phagocytosis of bacteria and chemotaxis of inflammatory cells, secrete various pro-inflammatory factors such as IL-1β, IL-6, TNF-α, and promote Th1 immune response. In this research, the macrophages were simulated with 1 μg/mL LPS and different drugs. The results showed that the phagocytic activity, migration ability and secretion ability of macrophages were increased after the simulation of LPS. While SN and TLR4 inhibitors can inhibit migration and secreting function of macrophages induced by LPS, but have no effect on phagocytosis. So, we believe that during the immune responses of macrophages, SN can inhibit the migration of macrophages to the site of injury in a dose-dependent manner and decrease the secretion of pro-inflammatory factors. This directly reduced the releasing of proinflammatory factors, then inhibited the hyperfunction of inflammation.

TLR4 signal transduction pathway is an important pathway for immune response in mononuclear macrophage. It can typically identify LPS in the context of CD14. LPS binds to the LPS-binding protein (LBP), and the binding of LPS to LBP enhances the sensitivity of monocyte response to LPS[13–20]. Once TLR4 is engaged by LPS, it may then activate myeloid differentiation factor (MyD) 88 dependent TLR4 signaling pathway and IL-1R related kinase (IRAK) and so on, eventually cause phosphorylation of I-κB, transfer the NF-κB into the nucleus, and promote the release of TNFs and ILs[21–25]. As one kind of important transcription factors, NF-κB can regulate the proliferation, growth and differentiation of T and B lymphocytes. NF-κB plays an important role in humoral and cellular immunity. In quiescent cells, NF-κB is retained in the cytoplasm through an interaction with inhibitory IκB proteins. In response to a variety of stimulis, inhibitory IκB is degraded by the ubiquitin-proteasome pathway, leading to nuclear translocation of NF-κB and regulating a variety of cytokines simultaneously, such as TNF-α, IL-1β and IL-6, etc[26–28]. These cytokines can promote the production of NO, and then aggregate the inflammatory chemokines to expand the inflammatory response[29]. The expression levels of mRNA and protein of key factors in TLR4/MyD88 pathways were examined by real-time PCR and Western blotting. While the expression of NF-κB in macrophage nuclei was qualitatively studied under the conjunct effect of LPS and SN. Results showed that SN not only inhibited the gradual amplification of inflammation on the level of mRNA, but also further reduced the expression levels of key proteins and down-regulated the nuclear expression of NF-κB in macrophages. Then the hyperfunction of immunologic response was reduced. This study confirmed that SN could inhibit immune responses of macrophages by restraining the expression of key factors in TLR4/My88/ NF-κB signaling pathway.

According to the research of the therapeutic target and related mechanism of SN, we found that SN could inhibit chemotaxis and secretion of inflammatory factors in macrophage in different conditions. And it suppressed the excessive hyperfunction of inflammation by inhibiting the activation of TLR4/NF-κB signaling pathway. Inflammatory bowel disease (IBD) is also one kind of autoimmune diseases, including ulcerative colitis (UC) and Crohn's disease (CD). The hyperfunction of immune response leads to the damage of the gut mucosal barrier and intestinal tissue in intestinal susceptible populations. Rahman *et al* found that the specificity response of TLR4 was enhanced in peripheral blood macrophages of UC patients when stimulated by bacteria and TLR ligands. It led to over-expression of a series of pro-inflammatory molecules, and ultimately caused inflammation[30]. Recent studies have found that TLR4, MyD88 and NF-κB in the colonic mucosa of UC rats were significantly much more hyperactive than those in the control group, suggesting that TLR4/NF-κB mediated signal transduction pathway is closely related to the occurrence and development of UC. As one kind of immunosuppressive drug, SN can inhibit

the function of macrophages and the expression of inflammatory related factors through TLR4/NF-κB signaling pathway in our study, which may provide a new idea for treatment of another autoimmune disease, such as IBD.

**Conflict of Interest Statement**

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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