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Macrophage immunomodulatory activity of the polysaccharides from the roots of *Bupleurum smithii* var. *parvifolium*

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ABSTRACT

Aim of the study: Radix Bupleuri, one of the most frequently prescribed crude herbs in traditional Chinese medicine, has been used for centuries to treat inflammatory diseases. However, little is known about the therapeutic mechanisms of crude polysaccharides (BPs) isolated from the roots of *Bupleurum smithii* var. *parvifolium*. Macrophages play important roles in inflammatory diseases such as systemic lupus erythematosus (SLE). The purpose of the present work was to investigate immunomodulative effects of Bupleurum polysaccharides on murine peritoneal macrophages.

Materials and methods: BALB/c mice were administered intragastrically with Bupleurum polysaccharides 20, 40, and 80 mg kg^{-1} day^{-1}, or prednisone 3 mg kg^{-1} day^{-1} or levamisole 25 mg kg^{-1} day^{-1} from day 0 to day 6. Peritoneal macrophages were isolated 5 days after intraperitoneal injection of 1 mL 5% sodium thioglycollate. Phagocytic functions of macrophages were studied; cytokines concentrations in the culture supernatants were determined by enzyme-linked immunosorbent assay and the secretion of nitric oxide (NO) was quantified by Griess reaction.

Results: Treatment with BPs enhanced phagocytic functions of macrophages (phagocytosis of apoptotic thymocytes, IgG-opsonized sheep red blood cells and chicken red blood cells) and inhibited LPS-induced productions of NO and proinflammatory cytokines (interleukin-1β, interleukin-6 and tumor necrosis factor-α).

Conclusions: Bupleurum polysaccharides up-regulated phagocytic activities but inhibited LPS-induced productions of proinflammatory mediators. These data suggested that at least part of the traditional beneficial effects of Bupleurum on inflammatory diseases could be ascribed to the immunomodulatory effects of Bupleurum polysaccharides on macrophages.

1. Introduction

Little Bupleurum Decoction is a herbal formulas originating from *Shanghan Zabing Lun*, a classical textbook of traditional Chinese medicine written by Zhang Zhongjing (220 A.D.). This Decoction is widely used for the treatment of various diseases such as SLE and chronic hepatitis C, with Radix Bupleuri as its principal ingredient (Huang et al., 2008; Ma, 2008). *Bupleurum smithii* var. *parvifolium* is affluently distributed in the northwest region of China. Our previous experiments had confirmed that a homogeneous polysaccharide (D3-S1) isolated from *Bupleurum smithii* var. *parvifolium* had inhibitory properties toward complement activation (Xu et al., 2007). In addition, the isolated Bupleurum polysaccharides improved lupus-like syndrome in mice induced by CJ-S_{131} (Wang et al., 2009a), while significantly enhanced pinocytosis of peritoneal macrophages in normal mice (Wang et al., 2009b).

Macrophages initiate the innate immune response by recognizing pathogens, phagocytosing them, and secreting inflammatory mediators. Besides, macrophages also play a crucial role in the regulation of acquired immunity (Beurel and Jope, 2009). Activation of macrophages induces increased expression of both class II MHC molecules and various co-stimulatory molecules, thereby rendering the macrophages more effective in the development of both humoral and cell-mediated immune responses (Hongmei et al., 2009).

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that affects all organs, including skin, joints,

Abbreviations: SLE, systemic lupus erythematosus; BPs, Bupleurum polysaccharides; CMC, carboxymethyl cellulose; NS, normal saline; SRBCs, sheep red blood cell; CRBCs, chicken red blood cell; IgG-SRBC, IgG-opsonized SRBC; ELISA, enzyme-linked immunosorbent assay; NO, nitric oxide; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TLRs, toll-like receptors.

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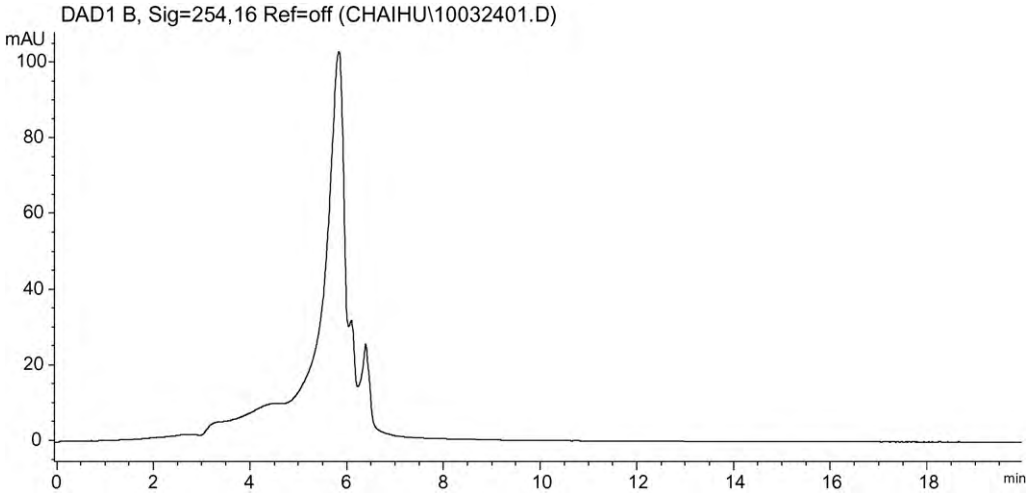

Fig. 1. HPGPC chromatogram of the *Bupleurum* polysaccharides obtained with a TSK GMPWXL gel filtration column (7.6 mm × 300 mm, TOSOH), eluted with ultrapure water at 1.0 mL/min, and detected at 254 nm.

blood and kidneys. The main effectors of disease pathology are the diverse autoantibodies, immune complexes, complement activation and autoreactive cells. Altered biology of immune cells and possibly other cell types invariably contributes in the expression of the diseases (Christina and George, 2006).

Macrophages have been considered to play important roles in the pathogenesis of SLE in numerous studies (Katsiari et al., 2009). Owing to the intrinsic defects of macrophages, SLE is one of the well-established examples in which apoptotic cell clearance is disturbed both in mice and patient (Xu et al., 2006). Recent studies also confirmed that aberrant function of lupus macrophages appeared to play a dynamic role in the initiation and perpetuation of the systemic autoimmune response and organ damage (Katsiari et al., 2009). Moreover, numerous abnormalities of the cytokine network have been described in patients suffering from SLE (Alaa et al., 2006a). Therefore secretion function of macrophages may contribute to the pathophysiology of SLE as well.

It is reported one of the primary mechanisms of immunomodulator isolated from plant polysaccharides appears to involve their ability to influence complement activation and macrophage function (Schepetkin et al., 2005). Based on our previous studies and the role of macrophage in inflammatory diseases mentioned above, we investigated the effects of BPs on some macrophage functions, including phagocytic activities and LPS-induced inflammatory factors production.

2. Materials and methods

2.1. Animals

Male BALB/c mice aged 6–8 weeks were purchased from Slaccas-Shanghai Lab Animal Ltd. (SPF II Certificate; No. SCXK2008-0016) and bred and housed under a standard laboratory condition with free access to food and water. All experimental protocols described in the study were approved by the Animal Ethical Committee of School of Pharmacy, Fudan University.

2.2. Drugs and reagents

The crude polysaccharides from *Bupleurum smithii* var. *parvifolium* were isolated according to the method described previously (Xu et al., 2007; Wang et al., 2009a). The total carbohydrate and uronic acid contents were determined by the phenol-sulfuric acid and *m*-hydroxybiphenyl methods respectively, using D-galactose and D-galacturonic acid as respective standards. The crude polysaccharides contained 74.8% of total carbohydrate and 41.5% of uronic acid. The monosaccharide composition of polysaccharides was examined by gas chromatographic analysis, the polysaccharides is mainly consisted of Ara, Gal, Glc and Rha in the ratio of 6.35:3.15:1.47:1, along with trace of Man and Xyl. High-performance gel permeation chromatography (HPGPC) analysis showed that the polysaccharides contained one major polysaccharide with several minor ones (Fig. 1), from which a homogeneous acidic polysaccharide D3-S1 was isolated and characterized as an anti-complementary agent (Xu et al., 2007).

Prednisone was provided by Tianjing Tianyao Pharmaceutical Co., Ltd. and levamisole was purchased from Sigma Co., Ltd.

2.3. Experimental protocol

BPs, prednisone or levamisole were ground and suspended in normal saline containing 0.5% sodium carboxymethyl cellulose (CMC) for administration, respectively. There were five groups in this experiment. One was given 0.5% CMC solution as vehicle-treated group, the other four groups were given BPs 20, 40, and 80 mg kg^{-1} day^{-1}, and prednisone 3 mg kg^{-1} day^{-1} or levamisole 25 mg kg^{-1} day^{-1} intragastrically from day 0 to day 6, respectively. Prednisone or levamisole was used as a positive control. Mice were sacrificed on day 7 and peritoneal macrophages were harvested for the following experiments.

Peritoneal macrophages were isolated from BALB/c mice 4 days after intraperitoneal injection of 1 mL 5% sodium thioglycollate. Peritoneal exudate cells were obtained by lavage with 1 mL cold RPMI-1640. The cells were placed in RPMI-1640 which were supplemented with 10% (v/v) heat-inactivated, endotoxin free fetal calf serum, 100 μg/mL streptomycin, and 100 U/mL penicillin.

All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cell number and viability were assessed microscopically using trypan blue exclusion.

2.4. Assay for phagocytosis of apoptotic thymocytes

Macrophage phagocytosis of apoptotic cells was assessed *in vitro* using minor modifications of a quantitative measurement assay described previously (Licht et al., 1999). Murine thymocytes were incubated with dexamethasone for 3 h for inducing apoptotic cells.

The thymus of BALB/c mice was removed after taking out peritoneal exudate cells. The cells were stored in cold PRMI-1640 and a

single-cell suspension of thymocytes was prepared by thymic disruption in a nylon filter net, thymocytes were seeded in 20 mL of RPMI-1640 supplemented with 1 mM dexamethasone (WKP5140, Wako, Japan) at 5×10^6 cells/mL in a cell culture flask and incubated for 3 h. After that, cells were washed 3 times in order to remove dexamethasone. Samples of 20 μL were taken for cell counting by trypan blue, and the survival rate was about 50%. Then the cells were adjusted to a concentration of 2.5×10^6 cells/mL.

Macrophages were plated at a density of 1.5×10^5 cells/mL in 24-well plates (Costar, Corning, NY) and incubated for 1.5 h. Non-adherent cells were removed by washing softly, and adherent peritoneal macrophages were incubated again for 1.5 h in RPMI-1640.

Apoptotic thymocytes in 1 mL RPMI-1640 were added gently into the 24-well plates containing macrophages and co-incubated for 1 h. After interaction, the 24-well plates were washed thoroughly to remove non-engulfed thymocytes. Then macrophages were fixed for 30 min with 99.9% methanol and stained with Wright-Giemsa staining before the phagocytic rate and index was counted. Phagocytosis was assessed at 400× magnification by regular light microscopy.

The phagocytic index and phagocytic rate were calculated as follows: phagocytic rate = number of macrophages that engulf apoptotic thymocytes in 100 macrophages, phagocytic index = number of internalized apoptotic thymocytes in 100 macrophages. Only thymocytes clearly within the perimeter of the macrophages were counted and the procedure was completed by two independent observers.

2.5. Assay for phagocytosis of IgG-opsonized sheep red blood cells (SRBCs)

Phagocytic assay was performed as previously described with some modification (Song et al., 2008). Peritoneal macrophages were brought to a concentration of 5×10^5 cells/mL in 24-well culture plates and incubated for 30 min. Then the wells were washed 3 times to remove non-adherent cells and the adherent ones were harvested for the following experiment.

IgG-opsonized SRBCs (IgG-SRBCs) were prepared by incubating sheep red blood cells with 1:10 dilution of the maximal subagglutinating titer of rabbit anti-sheep red blood cells IgG (200903, Weibian, China). Prepared IgG-SRBCs in 1 mL were added gently into the 24-well plates containing macrophages at a target-to-effector ratio equal to 200:1 and co-incubated for another 30 min. The 24-well plates were washed thoroughly to remove non-engulfed SRBCs. Then macrophages were fixed for 30 min with 99.9% methanol and stained with Wright-Giemsa staining before the phagocytic rate and index was counted.

Phagocytosis was assessed at 400× magnification by regular light microscopy. The phagocytic index and phagocytic rate calculation were similar: phagocytic rate = number of macrophages that engulfed SRBCs in 100 macrophages, phagocytic index = number of internalized SRBCs in 100 macrophages.

2.6. Assay for phagocytosis of chicken red blood cells (CRBCs)

Peritoneal macrophages were plated at a density of 2×10^6 cells/mL in 35×10 mm culture plates and incubated for 2 h. The adherent ones were harvested for the following experiment.

CRBCs were prepared as follow: CRBCs were washed 3 times in barbitol buffer solution (3 mM barbitone, 150 mM NaCl, 1 mM Barbital Sodium) and suspended in this buffer at a density of 5×10^8 cells/mL; then the suspended fluid was diluted at 1:10 by RPMI-1640.

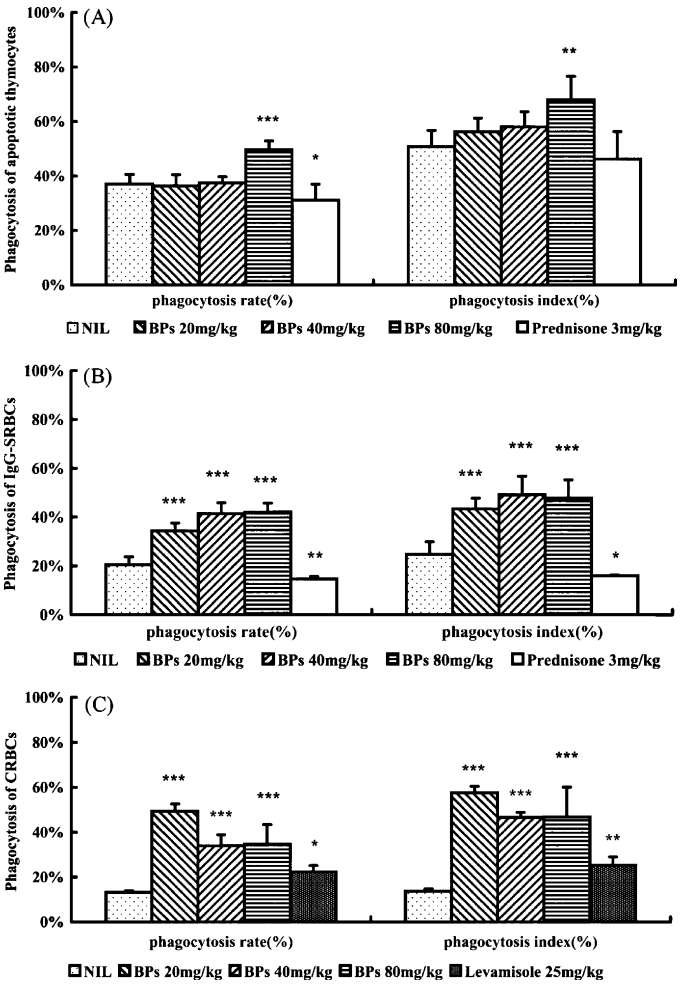

Fig. 2. Effect of BPs on phagocytosis. Mice were treated with BPs 20 mg kg^{-1} day^{-1}, 40 mg kg^{-1} day^{-1}, 80 mg kg^{-1} day^{-1} and prednisone 3 mg kg^{-1} day^{-1} or levamisole 25 mg kg^{-1} day^{-1} from day 0 to day 6 and were intraperitoneal injected with 1 mL 5% sodium thioglycollate on day 2. Mice were sacrificed on day 7, peritoneal macrophages were prepared from mice. (A) For phagocytosis of apoptotic thymocytes, peritoneal macrophages were incubated for 3 h and co-incubated with self-apoptotic cells (apoptotic thymocytes induced by dexamethasone) in fresh medium for another 1 h. (B) For phagocytosis of IgG-SRBCs, peritoneal macrophages were incubated for 30 min at 37 °C. Then they were co-incubated with IgG-SRBCs in fresh medium at a target-to-effector ratio equal to 200:1 for another 30 min. (C) For phagocytosis of CRBCs, peritoneal macrophages were incubated for 2 h, and were co-incubated with CRBCs for 1 h. Non-engulfed apoptotic cells, SRBCs and CRBCs were washed out and the cells were fixed and stained with Giemsa-Wright staining before the phagocytic rate and index were counted. Data were expressed as means ± S.D.; $n = 6$ mice for each group. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, tested by ANOVA and Fisher's PLSD.

The prepared CRBCs were added gently into 35×10 mm culture plates with adherent macrophages at the ratio equal to 25:1 and co-incubated for 1 h. The plates were washed thoroughly to remove non-engulfed CRBCs and macrophages were fixed with 99.9% methanol and stained with Wright-Giemsa staining.

Phagocytosis was assessed at 400× magnification by regular light microscopy. Calculation of phagocytic index and phagocytic rate was similar: phagocytic rate = number of macrophages engulfed CRBCs in 100 macrophages, phagocytic index = number of internalized CRBCs in 100 macrophages.

2.7. Determination of nitric oxide (NO)

Macrophages were plated at a density of 2×10^6 cells/mL in 48-well culture plates and incubated in medium alone or medium

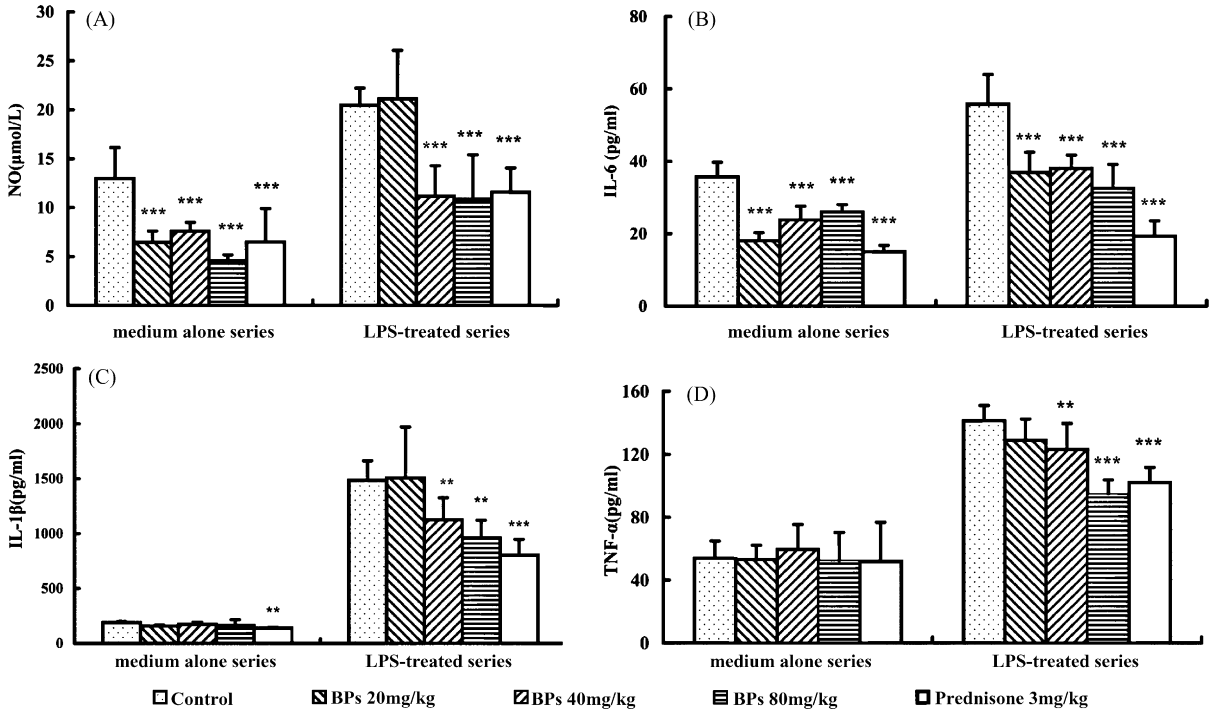

Fig. 3. Effect of BPs on peritoneal macrophages NO, IL-6, IL-1β and TNF-α production. Mice were treated with BPs 20 mg kg^{-1} day^{-1}, 40 mg kg^{-1} day^{-1}, 80 mg kg^{-1} d^{-1}, and prednisone 3 mg kg^{-1} d^{-1} from day 0 to day 6 and were intraperitoneal injected with 1 mL 5% sodium thioglycollate on day 2. Murine peritoneal macrophages were incubated for 24 h with medium alone or 1 μg/mL LPS. NO production was quantified by measuring nitrite in the cell-free supernatants. IL-6, IL-1βand TNF-α production in the cell-free supernatants was quantified with ELISA kits based on extrapolation from corresponding standard curve, according to the manufacturer's protocol. Data were expressed as means ± S.D.; $n = 6$ mice for each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, tested by ANOVA and Fisher's PLSD.

containing LPS (1 μg/mL) from *Escherichia coli* O127:B8 (Sigma) for 24 h. After that 200 μL of supernatants were removed and stored at 80 °C for measurement of NO and cytokines.

Determination was performed according to the colorimetric method described previously (Schepetkin et al., 2005), using nitrite ion concentration as an indication of NO production and $NaNO_2$ as a standard substance. Briefly, 100 μL cell culture supernatant were transferred to flat-bottom microtiter plates, mixed with an equal volume of Griess reagent [0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride and 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid]. The samples were incubated for 15 min at room temperature. The optical densities were measured at 540 nm using a well scanner ELISA reader (Labsystems Dragon) and nitrite concentration was determined using a standard curve generated with known concentrations of $NaNO_2$.

2.8. Determination of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)

IL-1β, IL-6 and TNF-α enzyme-linked immunosorbent assay (ELISA) kits (R&D, USA) were used to detect IL-1β, IL-6 and TNF-α in macrophages supernatants. Cytokine concentrations were determined by extrapolation from IL-1β, IL-6 and TNF-α standard curves, according to the manufacturer's protocol.

2.9. Statistical analysis

Quantitative variables were expressed as means ± S.D. One-way analysis of variance (ANOVA) was used. If any significant change was found, post hoc comparisons were performed using Fisher's PLSD.

3. Results

3.1. Effects of BPs on phagocytic activities

For phagocytosis of apoptotic thymocytes, both the phagocytic rate and phagocytic index increased significantly in BPs 80 mg kg^{-1} day^{-1} when compared with control group ($P < 0.001$, $P < 0.01$) (Fig. 2A). BPs 20, and 40 mg kg^{-1} day^{-1} had no significant effect on rate and index of phagocytosis of apoptotic thymocytes. Administration of 3 mg kg^{-1} day^{-1} prednisone inhibited the rate of phagocytosis ($P < 0.05$).

As shown in Fig. 2B, a considerable enhancement of both rate and index of phagocytosis of IgG-SRBCs was observed in BPs as compared with control group ($P < 0.001$). Treatment with prednisone 3 mg kg^{-1} day^{-1} suppressed the rate and index of phagocytosis of IgG-SRBCs ($P < 0.01$, $P < 0.05$).

When it comes to phagocytosis of CRBCs, treatment with BPs and lavamisole increased the index of phagocytic rate and index significantly (Fig. 2C).

3.2. Effects of BPs on NO

In the culture supernatants, a minimum amount of NO production was determined when macrophages incubated with medium alone; whereas, treatment with 1 μg/mL LPS resulted in a great increase in NO production ($P < 0.001$) (Fig. 3A).

NO production in the medium alone was reduced by BPs and prednisone significantly ($P < 0.001$) as compared with its control group.

Compared with its control group, BPs (40 and 80 mg kg^{-1} day^{-1}) and prednisone reduced the NO production stimulated by LPS ($P < 0.001$).

3.3. Effects of BPs on IL-1β, IL-6 and TNF-α

TNF-α, IL-1β and IL-6 are inflammation related factors. Compared with medium alone, IL-1β, IL-6 and TNF-α productions were significantly increased by LPS stimulation ($P < 0.001$, $P < 0.01$, $P < 0.001$).

IL-6 production was reduced by BPs (20, 40, and 80 mg kg^{-1} day^{-1}) and prednisone (3 mg kg^{-1} day^{-1}) significantly with and without LPS stimulation as compared with control group ($P < 0.001$) (Fig. 3B).

In the absence of LPS treatment, macrophages generated a low level of IL-1β. In macrophages treated with 1 μg/mL LPS, administration of 40 and 80 mg kg^{-1} day^{-1} BPs suppressed IL-1β production as compared with its control group ($P < 0.01$) (Fig. 3C).

Both BPs and prednisone had no significant effect on TNF-α secretion when cultured without LPS. Reduction of TNF-α production was observed in BPs (40 and 80 mg kg^{-1} day^{-1}) with LPS stimulation (Fig. 3D).

4. Discussion

In recent decades, numerous polysaccharides isolated from plants, epiphytes and animals have been proven to be fewer side effects and possess a wide range of biological functions such as antitumor, immunomodulation and anti-oxidation properties (Li et al., 2009). Overall, the primary effect of botanical polysaccharides is to enhance and/or activate macrophage immune responses. In particular, these compounds have been shown to increase macrophage cytotoxic activity against tumor cells and microorganisms, activate phagocytic activity, increase reactive oxygen species and NO production, and enhance secretion of cytokines and chemokines, such as TNF-α, IL-1β, IL-6, IL-8, IL-12, IFN-γ and IFN-β_2 (Schepetkin and Quinn, 2006). However, a few polysaccharides showed suppressive effects, for example, the polysaccharides isolated from *Phellinus baumii* suppressed the LPS-induced NO production in RAW264.7 cells (Chang et al., 2007). In this study, the crude polysaccharides from *Bupleurum smithii* var. *parvifolium* enhanced phagocytic activity of macrophage but inhibited LPS-induced NO and inflammatory cytokines production. These characteristics were quite different from most published polysaccharides.

Macrophage is the most important professional phagocyte and is able to clear large amounts of various materials (Katsiari et al., 2009). Besides, phagocytes express a broad array of receptors that participate in particle recognition and internalization when they play their scavenger role in the clearance of none-self materials such as microorganisms and altered-self materials such as apoptotic cells, immune complexes, senescent erythrocytes, and inflammatory products (Thomas et al., 2008). As for the three kinds of phagocytosis determined in our present work, the first was correlated with scavenger receptor (Licht et al., 1999); Fcγ receptor was involved in phagocytosis of IgG-SRBCs (Song et al., 2008); and the rate and index of phagocytosis of CRBCs were notably positively correlated with the expression of toll-like receptor 4 (Li et al., 2004).

SLE patients have an impaired ability to clear apoptotic material via cells of mononuclear phagocyte system (Kavai and Szegedi, 2007) and autoimmune-prone mice show genetic defects in the promoter of the mouse FcγRII b gene (Hogarth, 2002). Toll-like receptors, which are broadly expressed on immune cells as macrophages, play crucial roles in innate immunity and pathogenesis of autoimmune diseases (Wang et al., 2009c).

Our data demonstrated BPs 80 mg kg^{-1} day^{-1} had a significant effect on rate and index of phagocytosis of apoptotic thymocytes. A considerable enhancement of both rate and index of phagocytosis of IgG-SRBCs and CRBCs was observed in BPs (20, 40, and 80 mg kg^{-1} day^{-1}) as compared with control group ($P < 0.001$). Since our data suggested that BPs could enhance the phagocytic capability of macrophages, the benefit effect of BPs on immune and inflammatory diseases might be partly attributed to the improvement of defective or deficient phagocytosis of macrophage.

NO is a critical mediator of a variety of biological functions, however production of excessive amounts of NO will lead to a different range of pathological outcomes and important pathologies. It is demonstrated that NO is overproduced in the setting of lupus activity (Oates and Gilkeson, 2006).

Cytokines are essential molecules involved in the differentiation, maturation and activation of cells and thus, by nature, have a significant influence on the immunoinflammatory response (Aringer and Smolen, 2005). But excessive secretion of cytokines has harmful effect on human health. Numerous abnormalities of the cytokine network have been described in patients suffering from inflammatory diseases, such as SLE (Alaa et al., 2006b). TNF-α plays a major role in propagating the inflammatory processes responsible for tissue damage in SLE and is over-expressed both systemically and locally in this disease (Uppal et al., 2009). Murine data have suggested that IL-1β, would be of importance in SLE (Lemay et al., 1996). Several data suggest that IL-6 plays a critical role in the B-cell hyperactivity and immunopathology of SLE, and that it may have a direct role in mediating tissue damage (Chun et al., 2007).

According to our data, without LPS treatment, a small quantity of NO, IL-1β, IL-6 and TNF-α could be detected in the supernatant. BPs administration inhibited NO and IL-6 production but did not suppress IL-1β and TNF-α. That means BPs had various influences on different proinflammatory mediators when they were in physiologic level. However, with the stimulation of 1 μg/mL LPS, productions of NO, IL-1β, IL-6 and TNF-α were significantly elevated and BPs administration for 6 days exerted suppressive effect on the overproduced NO and proinflammatory cytokines in various degrees. Therefore the suppressive effects of BPs on the elevated NO and proinflammatory cytokines productions might play an important role in releasing symptoms of inflammatory diseases, such as SLE.

As a typically immunosuppressive drug, prednisone inhibited the physiologic and LPS-induced productions of NO and cytokines and exerted suppressive effects on phagocytic activities. Long-term use of immunosuppressive drug always associates with the risk of infectious and malignant diseases, which may be partly attributed to the suppression of normal tissues (Crispin and Tsokos, 2007). However, BPs decreased the LPS-induced excessive production of NO and cytokines but had various effects on proinflammatory cytokines when they were in physiologic levels. Plus, BPs promoted the phagocytic capability. This divergence characteristic of BPs is unique and could be of potential benefits in treatment of SLE and other inflammatory diseases.

TLR-4 is the ligand of LPS. Stimulation of TLR-4 with LPS resulted in a significant increase in cytokines production (Lorne et al., 2009). Besides, it is reported the rate and index of phagocytosis of CRBCs were notably positively correlated with the expression of TLR-4 (Li et al., 2004). Our present data suggested the suppressive effect of BPs on the production of NO and cytokines might be closely related to TLR-4 pathway. That is really a paradox. As Imai et al. (2008) had mentioned, TLR-4 stimulation was relayed to cellular responses via different adaptors such as MyD88 and TRIF, the specific mechanisms of BPs on macrophages via different signal transduction tunnel need to be investigated deeply in our laboratory in the near future.

Some polysaccharides enhanced the expression of MHC molecules II in macrophages (Gi et al., 2004; Liu et al., 2006), however, others showed down-modulation of MHC II (Gi et al., 2003). As the role of macrophages to present antigens via MHC is very important and complicate in immune responses, whether BPs have effects on the expression of MHC and the following antigen-presenting

process will also be investigated deeply in our laboratory in the near future.

In conclusion, our study demonstrated that BPs had potent immunomodulatory activity on macrophages and at least part of the beneficial effects of *Bupleurum* polysaccharide on inflammatory diseases, such as SLE, might due to modulation of macrophages immune functions.

Acknowledgements

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