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ORIGINAL ARTICLE

Bursopentin (BP5) from chicken bursa of fabricius attenuates the immune function of dendritic cells

Yinyan Yin · Tao Qin · Qinghua Yu · Qian Yang

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Abstract Bursopentine (BP5), a novel pentapeptide isolated from chicken bursa of fabricius, has been proved to have immunomodulatory effects on B and T lymphocytes, anti-oxidative stress on macrophages, and antiproliferation on tumor cells. However, the effects of BP5 on the immune function exhibited by dendritic cells (DCs), which are regarded as a major target for immunomodulators, remain unknown. In this study, we examined the effects of BP5 on the activation and maturation of murine bone marrow-derived DCs. Our results showed that BP5 significantly suppressed the secretion of lipopolysaccharide (LPS)-induced pro-inflammatory (TNF-α, IL-1β, IL-6 and IL-12p70) and anti-inflammatory (IL-10) cytokines by DCs, and this impact was not due to its cytotoxicity. Besides, BP5 reversed the morphological changes and attenuated the expression of phenotypic markers (MHC II, CD40, CD80 and CD86 molecules) in LPS-induced DCs. Furthermore, BP5 restored the decreased FITC-dextran uptake in LPS-treated DCs, arrested the LPS-induced migration of DCs and abrogated the promoting ability of LPS-induced DCs for allogeneic T cell proliferation. These findings show a new immunopharmacological capability of BP5 and provide a novel approach in the prevention and therapy of chronic inflammation and autoimmunity via abolishing the immune function of DCs.

Keywords Bursopentin · Bursa of fabricius · Dendritic cells · Immunosuppression

Y. Yin · T. Qin · Q. Yu · Q. Yang (✉)
Key Lab of Animal Physiology and Biochemistry of China's Department of Agriculture, College of Veterinary Medicine, Nanjing Agriculture University, Nanjing 210095, People's Republic of China
e-mail: zxbyq@njau.edu.cn

Introduction

Dendritic cells (DCs) are a potent kind of antigen-presenting cells (APCs) with the unique ability to induce primary immune responses (Banchereau and Steinman 1998). DC-mediated modulation of immune responses depends on the DC maturation status after encountering microbial products (Steinman et al. 2003). Mature DCs which up-regulate the expression of major histocompatibility complex (MHC) molecules and costimulatory molecules migrate to T cell areas of organized lymphoid tissues, where they activate naïve T cells (Banchereau and Steinman 1998). Meanwhile, DCs also have the ability to regulate inflammatory responses through promoting the generation of cytokines and chemokines (Iwasaki and Medzhitov 2010; Manicassamy and Pulendran 2009). Thus, it can be seen that DCs play a crucial role in connecting the induction of innate immunity and the subsequent development of the adaptive immune response. However, DCs act as the double-edged sword in immunity as well, namely they are potent inducers in various detrimental chronic and acute inflammations except for their well-characterized capacity of beneficial immunoprotection. Previous studies in patients indicated that aberrant activations or functions in DCs were associated with different autoimmune diseases such as rheumatoid arthritis (RA) (Santiago-Schwarz 2004), multiple sclerosis (MS) (Pashenkov et al. 2002), systemic lupus erythematosus (SLE) (Seitz and Matsushima 2010) and inflammatory bowel disease (IBD) (te Velde et al. 2003). In addition, DCs were also the key factors in sepsis, which are characterized by an initial intense inflammatory response or "cytokine storm" that occured during severe infection (Efron and Moldawer 2003). Sepsis is triggered by bacteria and bacterial components, such as lipopolysaccharide [LPS

(endotoxin)]. The LPS-initiated TLR4 signaling in myeloid DCs leads to the activation of nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs) and interferon regulatory factor 3 (IRF3), as well as the subsequent secretion of a variety of inflammatory cytokines and chemokines (Akira and Takeda 2004; O'Neill and Bowie 2007). Thus, it is not surprising that considerable researches in sepsis focus on the regulation of DC activation and maturation. Substances such as natural products which down-regulated DC activation could potentially be applied to the treatment of these diseases (Li and Vederas 2009).

The bursa of fabricius (BF) is known as a primary central humoral immune organ responsible for the establishment and maintenance of the B cell compartment in avian species (Mueller et al. 1962; Masteller et al. 1995). It has been approved that some small molecular weight peptides from BF could induce a variety of immunoregulation in vivo and in vitro. Bursin, the first peptide isolated from BF, has been shown to play a vital role in the induction and differentiation of B cells (Brand et al. 1976; Lassila et al. 1989). As reported recently, bursal-derived BSP-I (EPASGMM) and BPP-I (LGPGP) had antiproliferative effects on tumor cells (Feng et al. 2011, 2012b). Bursopentine (BP5, with an amino acid sequence of Cys–Lys–Asp–Val–Tyr) was a novel immunomodulatory peptide isolated from chicken BF, and primarily related to the immunomodulatory effects on B and T lymphocytes (Li et al. 2011) and the protection against oxidative stress for macrophages (Li et al. 2012). In a recent study, BP5 was identified as a potent activator of p53, which is one of the most important tumor suppressor proteins (Feng et al. 2012a). Until now, the cellular targets of BP5 in the immune system remain enigmatic, especially for the roles of BP5 in the cellular maturation and immunoregulatory activity of DCs.

In this study, we attempted to characterize the effects of BP5 on the activation and functional properties of BMDCs. Our findings showed for the first time that BP5 inhibited the phenotypic and functional maturation of BMDCs. Moreover, we also hope this readily available agent may provide a simple, inexpensive, and highly effective means for the manipulation of the immunostimulatory properties of DCs.

Materials and methods

Animals and BP5

Male 4- to 6-week-old C57BL/6 and BALB/c mice were purchased from the Animal Research Center of Yangzhou University (Yangzhou, China). The animals were housed in the specific pathogen-free environment within our animal facility for at least 1 week before use. All animal care and experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care & Use Committee (IACUC) of Nanjing Agriculture University.

Synthetic BP5 was obtained from Shanghai Biotech Bioscience and Technology Co., Ltd (Shanghai, China). The sequence of the synthetic peptide was confirmed by electrospray ionisation tandem mass spectrometry (ESI–MS/MS), and the purity of the synthetic peptide was >98 % by reversed phase high pressure liquid chromatography (RP-HPLC).

Reagents and antibodies

RPMI 1640 medium, penicillin, and streptomycin were bought from Invitrogen (Grand Island, NY, USA). Fetal bovine serum (FBS) was from Hyclone (Thermo, Melbourne, Australia). Recombinant GM-CSF, IL-4, and CCL19 were purchased from Peprotech (Rocky Hill, NJ). LPS (from *Escherichia coli* 026:B6) was obtained from Sigma-Aldrich (St Louis, MO, USA). Fluorescent-labeled anti-mouse mAbs CD11c-APC, CD40-FITC, CD80-FITC, CD86-FITC, MHC II-FITC, or respective isotype controls were purchased from eBioscience (San Diego, CA, USA). Rabbit anti-mouse CCR7 was from Abcam (Cambridge, USA). Rabbit anti-mouse GAPDH and goat anti-rabbit IgG-HRP were from Bioworld (St. Louis Park, MN, USA).

Isolation and culture of BMDCs

BMDCs were generated from bone marrow progenitor cells as previously described with some modifications (Inaba et al. 1992; Shi et al. 2011). Briefly, bone marrow was extracted from the femurs and tibias of C57BL/6 mice and treated with red blood cell lysing buffer (Beyotime, China). Then the cells were resuspended in complete medium (RPMI1640 supplemented with 10 % heat-inactivated FBS, 1 % streptomycin and penicillin, 10 ng/ml GM-CSF, and IL-4) and plated at 1×10^6 cells/ml in 6-well plates (Corning, Cambridge, MA). After about 3 days of culture, medium was gently discarded for removing non-adherent granulocytes. On day 6, the clusters were harvested and subcultured overnight for removing adherent cells. Non-adherent cells were collected on day 7, washed, only cultures with >90 % cells expressing CD11c by flow cytometry (FACS) were used as immature DCs for the studies.

Cell viability assay

Viability of DCs treated with BP5 (dissolved in RPMI 1640 medium) at the indicated concentrations in the absence or presence of LPS for 24 h was measured using the WST-1 cell proliferation and cytotoxicity assay kit (Beyotime,

China) in accordance with the manufacturer's instruction. Briefly, 5×10^3 cells were cultured in 96-well plate. After treatment of cells, 10 μl WST-1 was added to each well and the cells were incubated for an additional 2 h. The plate was shaken gently for 1 min before the absorbance of samples was measured under a wavelength of 450 nm using a microplate reader, and the results were expressed as percentage of the control group.

Cytokine assay

Levels of TNF-α, IL-1β, IL-6, IL-12p70, and IL-10 in culture supernatants were measured by ELISA using commercial immunoassay kits from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China) and performed according to manufacturer's instructions.

Phenotype assay

BMDCs were harvested and washed twice with phosphate buffered saline (PBS, 0.01 M, pH7.4), and resuspended in ice-cold FACS washing buffer (2 % FBS and 0.1 % sodium azide in PBS). Then the cells were incubated with APC-labeled CD11c and FITC-labeled MHC II, CD40, CD80 and CD86, or the respective isotype controls at 4 °C for 30 min as per manufacturer's guidelines. After washing three times with PBS, DCs were phenotypically analyzed by FACS.

Endocytosis assay

Endocytic activity was assessed as previously described (Lutz et al. 1996). Briefly, 4×10^5 BMDCs were incubated at 37 °C for 45 min with 1 mg/ml FITC-dextran (mol wt 40,000; Sigma-Aldrich). After incubation, cells were washed twice with cold washing PBS and stained using APC-conjugated anti-CD11c antibody. Double-stained DCs were analyzed by FACS. In addition, parallel experiments were performed at 4 °C to determine the nonspecific binding of FITC-dextran to DCs (Kim et al. 2009).

Migration assay

Migration assay was performed as described previously (Liu et al. 2007). BMDCs in serum-free medium were placed in a 24-well transwell migration chamber (pore size, 5 μm; Corning, NY, USA). 0.1 ml RPMI 1640 medium containing BMDCs (1×10^5 cells) was loaded onto the upper wells. 0.6 ml RPMI 1640 medium containing CCL19 (200 ng/ml) was added to the lower chambers to induce cell chemotaxis. After incubation for 4 h at 37 °C, the migrated cells were collected from the lower wells, and the number of $CD11c^+$ cells was determined by FACS.

Western blotting

DCs were collected and washed twice with ice-cold PBS and directly lysed with RIPA buffer (1 % Triton X-100, 1 % deoxycholate, 0.1 % SDS, 150 mM NaCl, 50 mM Tris–HCl pH 7.4, 10 mM EDTA pH 8.0 dissolved in PBS) containing phosphatase and protease inhibitors (1 mM PMSF, 1 mM Na_3VaO_4, 25 mM NaF) on ice for 30 min. The cell lysates were centrifuged at 14,000*g* for 10 min at 4 °C and the protein concentrations were determined via BCA protein assay (Pierce, Rockford, IL) according to the manufacturer's recommendation. The normalized amounts of protein were separated on 10 % SDS–polyacrylamide gels and then transferred to the polyvinylidene fluoride membrane. The membranes were blocked for 2 h with 5 % skim milk in PBS-T (blotting in PBS with 0.1 % Tween-20) and then incubated with anti-mouse against CCR7 (1:5,000), or rabbit anti-mouse against GAPDH (1:5,000) at 4 °C overnight. After five washes with PBS-T, the membranes were incubated with HRP-conjugated secondary antibody (1:8,000) for 2 h at room temperature. The membranes were then developed for visualization of protein by adding an enhanced chemiluminescence reagent (Pierce, Rockford, IL). Autoradiograms were scanned and analyzed with Quantity One (Bio-Rad, Hercules, CA) to quantify band densities.

Allogenic mixed lymphocyte reaction (MLR)

Responder T cells were purified from BALB/c mesenteric lymph node using T cell isolation kit (Miltenyi Biotech, Germany) and labeled with carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Then these cells (5×10^5/well) were co-cultured in duplicate with BMDCs (DC: T cell ratios of 1:1, 1:10). MLR was conducted in a 24-well plate in 5 % CO_2 incubator at 37 °C for 5 days and then detected by FACS.

Statistical analysis

Results were expressed as the mean ± SD. Statistical significance was determined by Student's *t* test with value of $P < 0.05$ considered to be statistically significant.

Results

BP5 efficiently inhibited BMDCs activation

The effects of a new biological active factor bursal-derived BP5 on BMDCs activation and function are still unknown. To define the safe working concentration of BP5 for the BMDCs, we performed WST-1 assays. The viability of

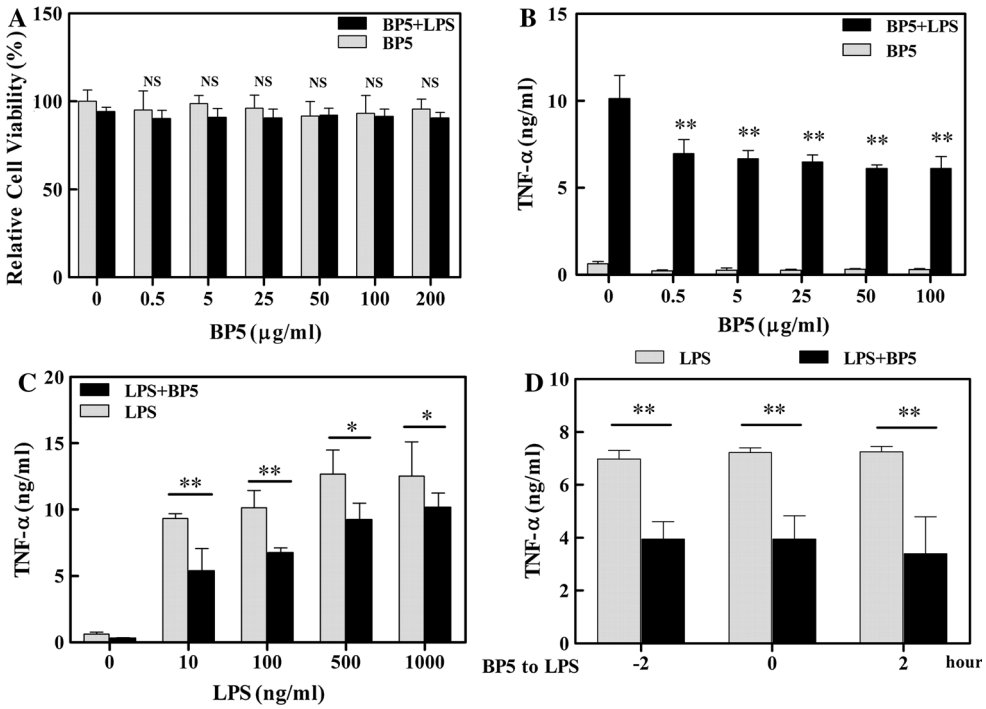

Fig. 1 BP5 efficiently suppressed BMDCs activation. BMDCs (1×10^6 cells) were incubated with the indicated concentrations of BP5 for 2 h, and then incubated with or without LPS (100 ng/ml) for 22 h. Supernatants were collected and TNF-α production was measured by ELISA. **a** The cytotoxicity of BP5 in BMDCs without or with LPS was analyzed by the WST-1 assay. **b** The inhibition of the TNF-α secretion by BP5. **c** Suppressive effect of pre-BP5(50 μg/ml) on BMDCs after treatment with the indicated doses of LPS. **d** DCs stimulated with LPS before or after BP5 (50 μg/ml) incubation. Data shown are the mean ± SD of three samples. NS, $P > 0.05$; $^*P < 0.05$; $^{**}P < 0.01$ (Student's *t* test) are comparisons between BP5-treated and non-treated LPS-stimulated DCs. All results are representative of three independent experiments

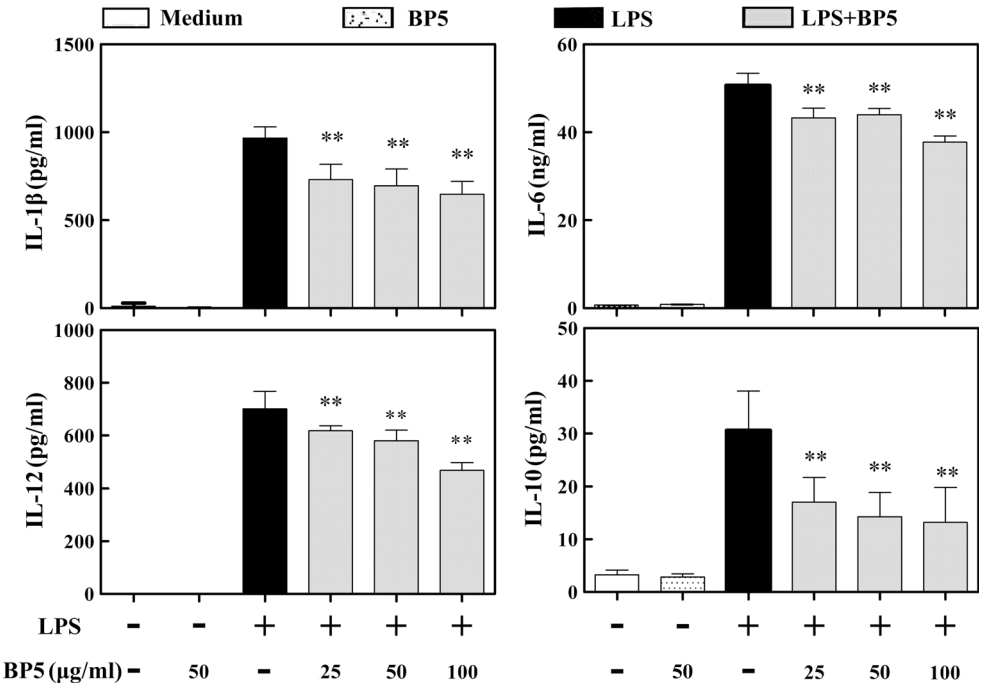

Fig. 2 BP5 impaired the secretion of cytokines by LPS-stimulated BMDCs. BMDCs were incubated with the indicated concentrations of BP5 for 2 h, and then incubated with or without LPS (100 ng/ml) for 22 h. Supernatants were collected and tested for IL-1β, IL-6, IL-12p70, and IL-10 by ELISA. Data shown are the mean ± SD of three samples. $^*P < 0.05$; $^{**}P < 0.01$ (Student's *t*-test) are comparisons between BP5-treated and non-treated LPS-stimulated DCs. All results are representative of three independent experiments

BMDCs in the absence or presence of various concentrations of BP5 plus LPS or not is shown in Fig. 1a. The results showed that cell viability was not changed by 24 h treatment with up to 200 μg/ml BP5 or plus 100 ng/ml LPS. Next, we tested whether BP5 affected the production of TNF-α in LPS-stimulated BMDCs, which is a characteristic of BMDC activation. Significantly, TNF-α was down-regulated by BP5 (Fig. 1b), suggesting that BP5 inhibited BMDC activation. BP5 also blocked such process by LPS at high doses (up to 1 μg/ml) (Fig. 1c). In addition, the amount of TNF-α was declined in all tests, regardless of whether the BP5 treatment was administered to BMDCs before or after LPS stimulation (Fig. 1d). Taken together, these data demonstrated that BP5 may be a very efficient inhibitor of BMDC activation.

BP5 impaired the cytokines secreted by LPS-stimulated BMDCs

Excepting for TNF-α, activated DCs secrete a variety of pro- and anti-inflammatory cytokines which play vital roles in regulation of immune responses. To determine whether there is a similar inhibitory effect of BP5 on other cytokines produced by LPS-stimulated BMDCs, we assessed the secretion of IL-1β, IL-6, IL-12p70, and IL-10 by ELISA assays. As expected, BP5 appreciably decreased the production of these cytokines (Fig. 2). Surprisingly, IL-10 production was not increased, which implied that the suppressive effect of BP5 probably was not mediated through anti-inflammatory cytokine. These results suggested that BP5 impaired the cytokines secreted by LPS-activated BMDCs.

The morphological changes of BMDCs upon LPS activation were reversible by BP5

We further examined the suppressive function of BP5 on morphological changes of BMDCs during the inflammatory response upon LPS activation. Mature DCs easily form larger clusters and longer extensions, compared with immature DCs (Zeng et al. 2012; Bakri et al. 2005; Lin et al. 2011). LPS treatment alone, BMDCs showed increased size of clusters and extension morphologies, compared with the untreated

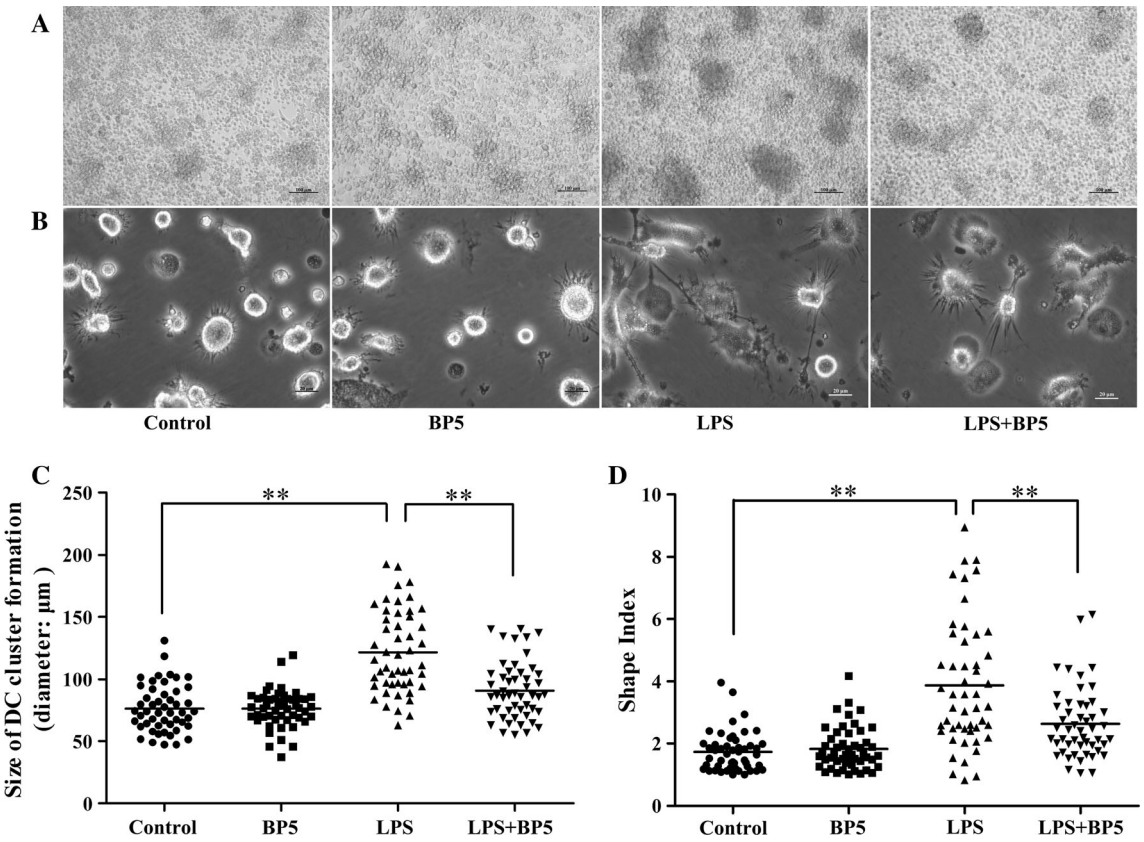

Fig. 3 BP5 reversed the morphological changes of LPS-stimulated BMDCs. BMDCs were pretreated with or without BP5 (50 μg/ml) and then exposed to LPS (100 ng/ml) or not for additional 22 h, followed by microscopic observations. **a** DC aggregation was examined by microscopy. *Scale bar* = 100 μm. **b** Morphology of DC dendrites was observed by microscopy. *Scale bar* = 20 μm. **c** Statistical results on the size of DC cluster formation in each group. **d** The statistical results of the cellular shape indices. *Horizontal lines* across the scatter diagram represent mean values. $^{*}P < 0.05$; $^{**}P < 0.01$, $n = 50$ (50 clusters or dendritic cells randomly selected from 3 separate experiments)

and the BP5 alone group. However, these processes were suppressed after addition of BP5 (Fig. 3a, b). We next calculated the size of clusters and the cell shape index (major axis/minor axis) of each group (Green et al. 2009). LPS treatment increased such two indexes, compared with the untreated group, respectively ($P < 0.01, n = 50$; Fig. 3c, d). But in the LPS + BP5 group, both of them were significantly reduced to the level close to the untreated group ($P < 0.01$, $n = 50$; Fig. 3c, d). These observations indicated that BP5 was able to reverse the morphological changes of LPS-activated BMDCs.

BP5 attenuated the phenotypic maturation of LPS-induced BMDCs

DC phenotypic maturation is also characterized by increased expression of MHC II, CD40, CD80, and CD86 (Higgins et al. 2003). To investigate whether BP5 modulated the DC maturation, the expressions of MHC II and costimulatory molecules on BMDCs were analyzed by FACS. After exposure to LPS (100 ng/ml) for 22 h, the expressions of MHC II, CD40, CD80, and CD86 were up-regulated, whereas they were significantly inhibited with

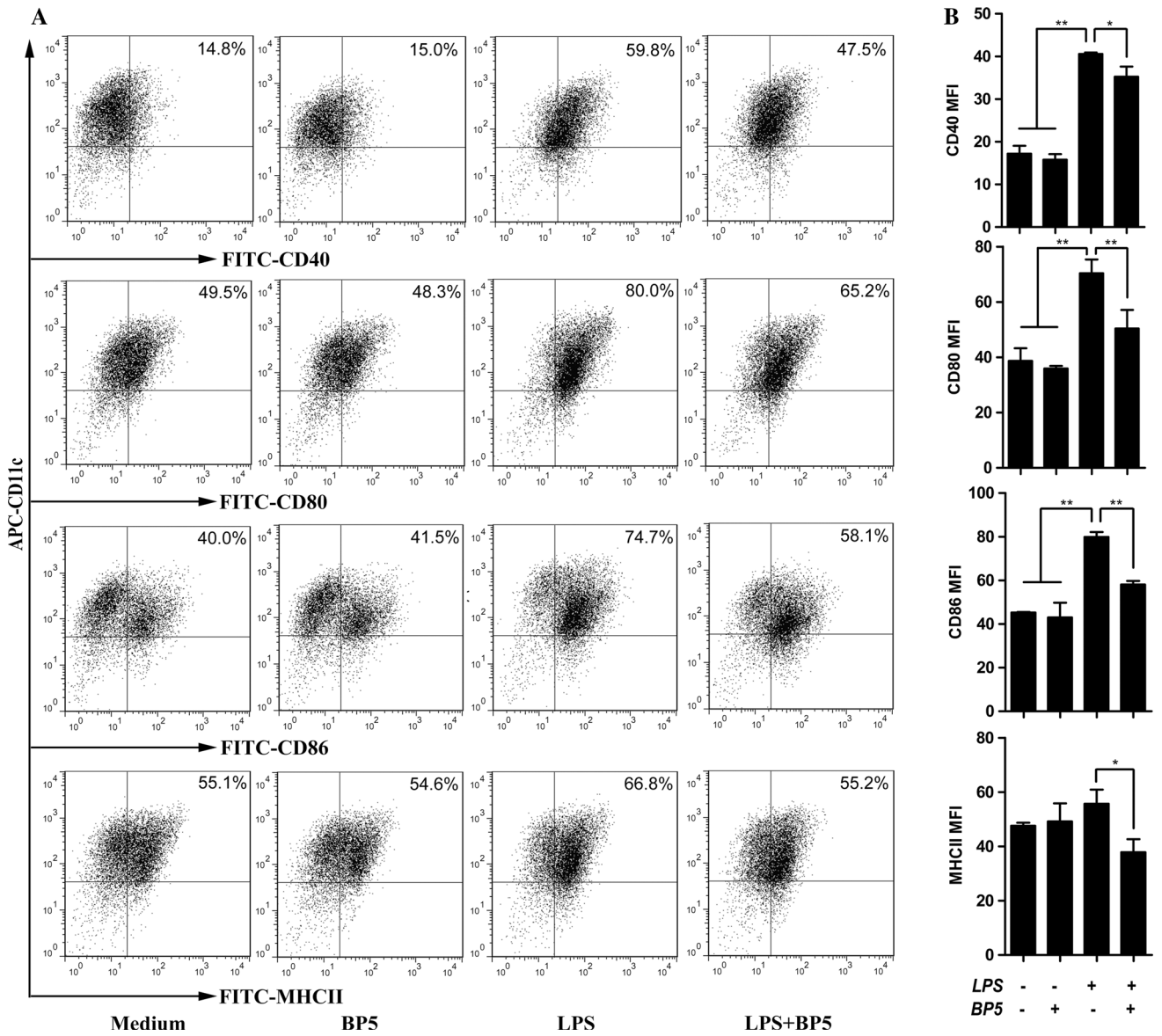

Fig. 4 BP5 suppressed the expression of MHC II, CD40, CD80, and CD86 by LPS-stimulated BMDCs. BMDCs were pretreated with or without 50 μg/ml BP5. After 2 h of incubation, the cells were stimulated with 100 ng/ml LPS or not for 22 h. The expressions of CD11c, MHC II, CD40, CD80, and CD86 were determined by staining and FACS. **a** Proportions of double-positive cells are shown in dot plots. **b** The mean fluorescence intensity (MFI) values are shown as the mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$, $n = 3$. Representative results out of three independent experiments were shown

pretreatment of BP5 (CD40 and MHC II, $P < 0.05$; CD80 and CD86, $P < 0.01$; Fig. 4a, b). BP5 alone did not change the expression of these molecules compared with that in the control group. These data suggested that BP5 attenuated the LPS-induced phenotypic maturation of BMDCs and limited the immunostimulatory activity of BMDCs.

BP5 enhanced the endocytosis of FITC-dextran in LPS-treated BMDCs

The competence for antigen uptake in immature DCs is efficient but lost during the maturation stage (Reis e Sousa 2006). To examine the effect of BP5 on endocytosis of BMDCs, we

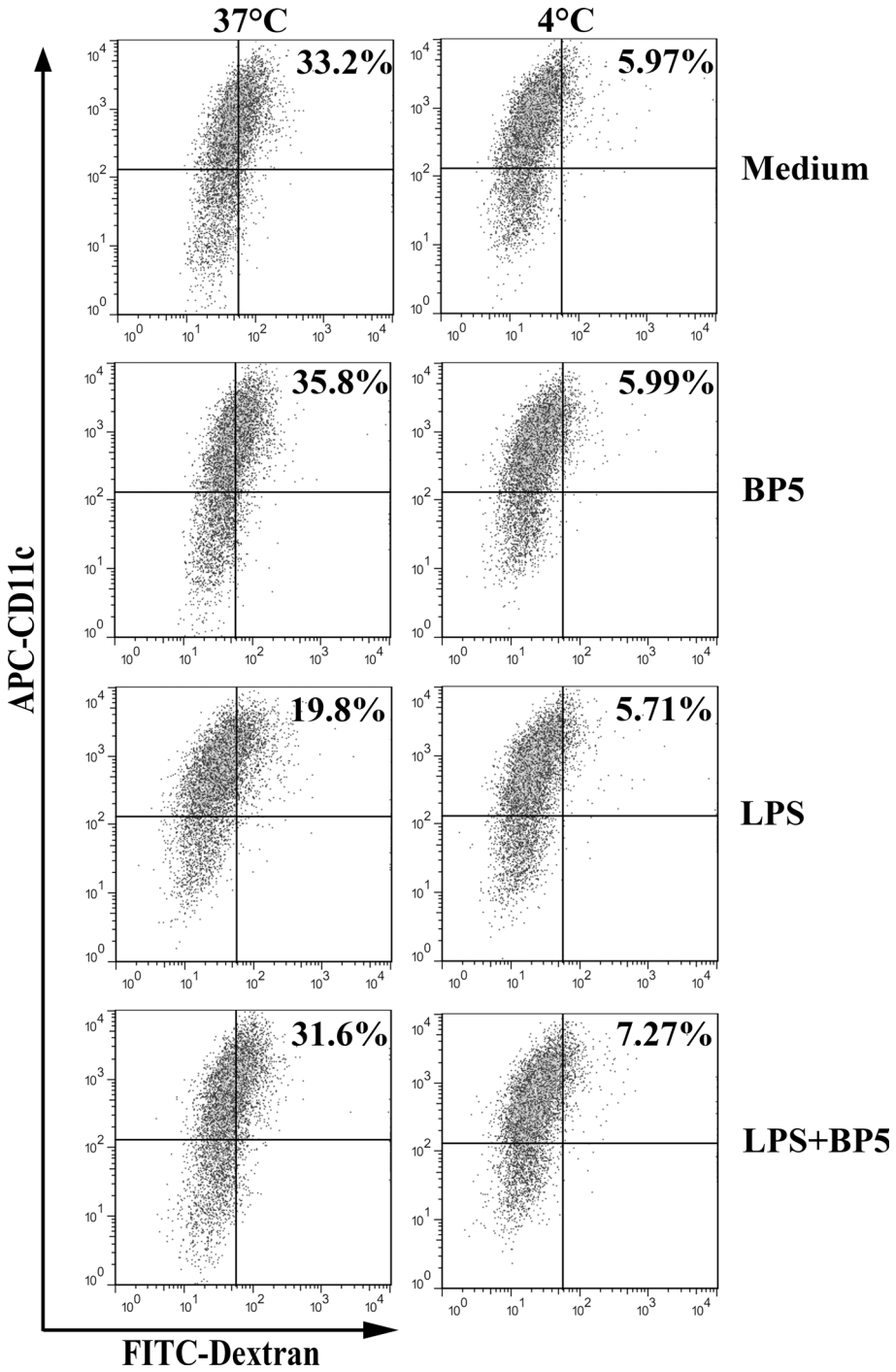

Fig. 5 BP5 enhanced the endocytosis of FITC-dextran in LPS-treated BMDCs. BMDCs were pretreated with 50 μg/ml BP5 for 2 h, followed by stimulation with 100 ng/ml LPS. After 22 h, DCs incubated with 1 mg/ml FITC-dextran at 37 °C for 45 min were compared with a negative control incubated at 4 °C for 45 min. Thereafter, the cells were washed twice with cold PBS and stained using the APC-conjugated anti-CD11c antibody. The numbers represent the percentages of cells. Medium represents untreated control group. To confirm the results, we repeated these experiment three times

used the fluorescent marker dextran-FITC, which is mainly taken up via the mannose receptor. The percentage of double-positive cells ($CD11c^+ \times$ dextran-FITC) was shown that there was no difference between the BP5-treated and untreated DCs. The percentage of LPS-stimulated BMDCs was less than that of untreated DCs. However, BP5 increased dextran uptake in LPS-stimulated BMDCs (Fig. 5). These results showed that BP5 partly recovered the endocytosis capability of LPS-stimulated BMDCs.

BP5 diminished the migration of LPS-stimulated BMDCs

We next asked if BP5 modulated the chemotactic function of LPS-induced BMDCs. We evaluated DC migration using chemotaxis assay in transwell chambers on the basis of attraction of mature DCs for CCL19 or CCL21. The migration of LPS-stimulated BMDCs was remarkably enhanced in response to CCL19. However, this process seemed to be retarded with BP5 pretreatment ($P < 0.05$; Fig. 6a). Western blot analysis was used to examine what role C–C chemokine receptor type 7 (CCR7) might have in cell migration. As expected, BP5 inhibited CCR7 expression in LPS-stimulated BMDCs ($P < 0.05$; Fig. 6b, c). Thus, these data indicated that BP5 diminished BMDC migration which might subsequently interfere with antigen presentation to T cells.

Inhibitory effect of BP5 on allostimulatory capacity

The immunostimulatory properties of DCs to T cells are essential for adaptive immune responses. Here, allogenic mixed lymphocyte reaction (MLR) was performed to determine whether BP5 could modulate the ability of LPS-induced BMDCs to induce the proliferation of allogenic T cells. As shown in Fig. 7, BP5 alone at a concentration of 50 μg/ml did not significantly change the ability of BMDCs to induce the proliferation of T cells compared to untreated group, while BP5 inhibited the ability of LPS-induced BMDCs to stimulate allogenic T cells at all ratios of DC: T cell tests.

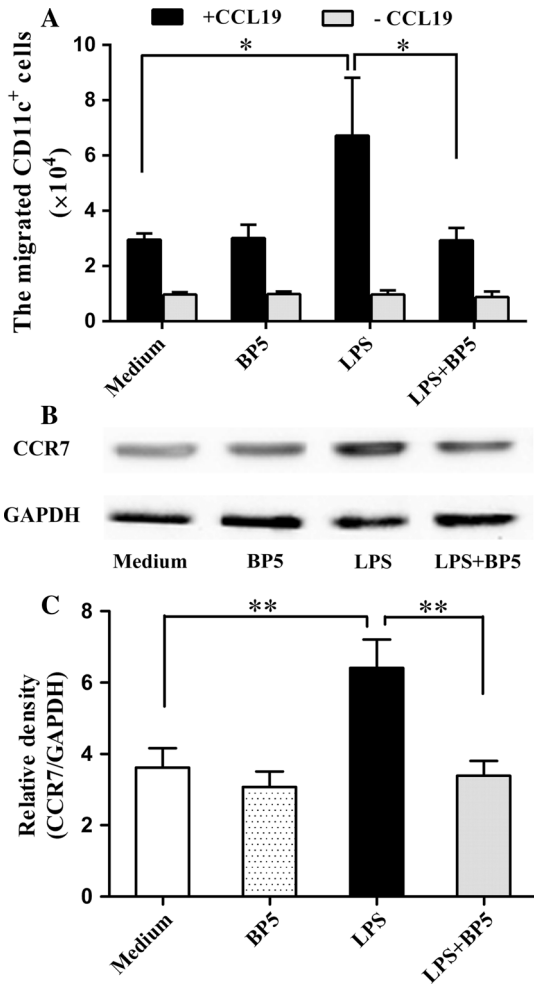

Fig. 6 BP5 diminished the migration of LPS-induced BMDCs. BMDCs were incubated with the indicated concentrations of BP5 (50 μg/ml) for 2 h, and then incubated with or without LPS (100 ng/ml) for 22 h, were assessed for the ability to migrate in vitro to a CCL19 concentration gradient. This was done using transwell cultures containing a polycarbonate filter with a pore diameter of 5 μm. **a** BMDCs were seeded into the upper wells of a 24-well transwell chamber, and CCL19 (200 ng/ml) was included in lower the wells. After 4 h, the number of cells that transferred from the upper to the lower wells was counted by FACS. The spontaneous migration of cells (absence of CCL19) was also shown. **b** Western blot of CCR7 in different groups is shown with GAPDH as control. **c** Quantification of the blots. Data shown are the mean ± SD of three samples. $*P < 0.05$; $**P < 0.01$. All results are representative of three independent experiments

Discussion

This study reported for the first time the immunosuppressive characteristics of BP5 on activation and maturation of DCs. Our investigational results demonstrated that BP5 reduced the secretion of LPS-induced pro-inflammatory (TNF-α, IL-1β, IL-6 and IL-12p70) and anti-inflammatory (IL-10) cytokines by BMDCs; reversed the morphological changes of LPS-activated BMDCs; attenuated the LPS-induced expression of MHC II, CD40, CD80, and CD86 molecules by BMDCs; enhanced the endocytosis of FITC-dextran in LPS-treated DCs; and arrested the LPS-induced migration of BMDCs. Moreover, BP5 abrogated the promoting ability of LPS-induced DCs for allogeneic T cell proliferation. These data powerfully indicated that BP5 may have potential application in the treatment of inflammatory diseases, especially for sepsis.

LPS, a cell wall component of gram-negative bacteria, and one of the most active ingredients of pathogen-associated molecular pattern (PAMP), specially binds to TLR4

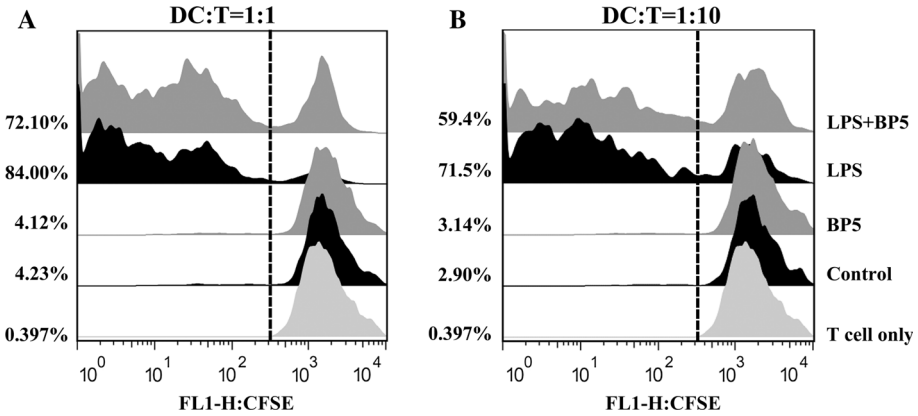

Fig. 7 BP5 abrogated the promoting ability of LPS-induced DCs for allogeneic T cell proliferation. BMDCs were incubated with the indicated concentrations of BP5 (50 μg/ml) for 2 h, and then incubated with or without LPS (100 ng/ml) for 22 h. The cells were washed extensively and then co-cultured with CFSE-labeled T cells at the indicated ratios. **a** DC: T = 1:1. **b** DC: T = 1:10. Control represents unstimulated BMDCs. Single T cell group was also shown. T cell proliferation was analyzed by CFSE dilution using FACS. Percentages refer to proportion of cells that proliferated within 5 days. All of the data are representative of at least three separate experiments

on DCs and then activates DC maturation. Subsequently, the matured DCs practise their antigen-presenting capability to start specific T cell immune responses (Reis e Sousa 2006). In this process, TNF-α is a rapid pro-inflammatory cytokine and it also can stimulate DCs to further mature (Ritter et al. 2003; Ding et al. 2011). Moreover, TNF-α plays a vital role in the modulation of other inflammatory cytokines such as induction of IL-1β and IL-6 production (Trevejo et al. 2001). These previous researches prompted us that BP5 might suppress the secretion of TNF-α, and then resulted in the down-expression of other cytokines. IL-1β is known to induce DCs to produce IL-12 (Wesa and Galy 2001; Eriksson et al. 2003), which also supports our data about reduction of IL-12 expression.

More interestingly, BP5 always impaired the LPS-induced TNF-α secretion in all the cases of pre-, combined or post-treatment. TLR4 recognizes LPS by forming a receptor complex with myeloid differentiation protein-2 (MD2) and cluster of differentiation antigen 14 (CD14), the latter of which also has an important role in the recognition of LPS (Moore et al. 2000). Likely, BP5 did not involve in the upstream inhibition of bacterial LPS/TLR4/CD14-mediated inflammation pathway, contrasting with a synthetic analogue of bacterial lipid A E5531 and a cyclic cationic polypeptide antibiotic PMB which inhibited TLR signaling via interactions with LPS (Means et al. 2001; Lien et al. 2001). In addition, since it has been well established that inflammatory responses may be resolved through the release of anti-inflammatory mediators such as IL-10 (Di Giacinto et al. 2005), we therefore evaluated the effects of pre-BP5 treatment in the LPS-induced IL-10 expression. However, this anti-inflammatory cytokine also decreased caused by BP5; thereby the role of BP5 was mediated through the inhibition of both pro- and anti-inflammatory cytokines. It was similar to quercetin which suppressed DC maturation though down-regulating the IL-10 secretion (Huang et al. 2010).

The development of DCs includes two major states. Immature DCs(iDCs) are considered to be specialized in antigen capture and processing, characterized by expression of low surface levels of MHC II and costimulatory molecules, while mature DCs(mDCs) present antigen and have an increased T cell stimulatory capacity (Michiels et al. 2005). Emerging evidence indicates that DCs are responsible for the establishment of tolerance as well as immunity. Especially, the iDCs are considered as an important factor in the induction of tolerance (Steinman and Nussenzweig 2002; Finkelman et al. 1996; Lu et al. 1995). Potential mechanism mainly involves that these DCs express low levels of T cell receptor ligand and costimulatory molecules, which are required for activation of immune functions in DCs (Nguyen et al. 2002). Alternately, iDCs are responsible for the formation of peripheral regulatory T cells, which are able to silence effector T cells including auto-aggressive diseases (Maloy and Powrie 2001; Levings et al. 2001; Piccirillo and Shevach 2001). Moreover, reduction of protrusions on DCs also is a phenotypic indicator of impaired antigen presentation (Kobayashi et al. 2001). Therefore, phenotypic switch and reversible morphological changes of BP5-treated BMDCs upon LPS activation suggested that BP5 might play a major role in the maintenance of the immature state of the BMDCs. This hypothesis was further supported by that BP5 suppressed functional maturation of BMDCs, involving the

cytokines production and MLR as above mentioned. In addition, the chemokine receptor CCR7 is crucial for the migration of mature DCs directing toward T cell-rich areas of the secondary lymph nodes which expressed CCR7 ligands CCL19 and CCL21, whereas the immature CCR7-negative DCs (Alvarez et al. 2008; Forster et al. 2008) do not. Our results demonstrated that BP5 probably could cut off the contact between DCs and T cells through down-regulating CCR7 expression, and lead to the reduced adaptive immune responses. This function would be consistent with several viruses such as vaccinia virus and herpes simplex virus type 1, which also described the inhibition of CCR7-driven migration (Humrich et al. 2007; Prechtel et al. 2005).

Previous reports have indicated that flavonoid extracts of traditional chinese medicine (TCM), including apigenin (Yoon et al. 2006), astilbin (Zou et al. 2010), epigallocatechin-3-gallate (Ahn et al. 2004), glabridin (Kim et al. 2010), kaempferol (Lin et al. 2011) and silibinin (Lee et al. 2007), had special immunosuppressive effect on DCs. Now researches show that BP5 is a new member with similar effects. Although these extracts of TCM are promising in clinical applications, they derive from plants after all and thus their safety has not been confirmed so far in human trials. In contrast, BP5, as a natural product originated from chicken bursa of fabricius has more advantages over TCM. However, further additional clinical trials are needed to assess the suitability of BP5 for disease control.

Taken together, our findings provide a new perspective on the roles of BP5 in the regulation of immune responses via their inhibitory effects on LPS-induced activation and maturation of DCs. And BP5 is a promising drug candidate that may display fewer side effects for immunosuppressive therapy.

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Conflict of interest The authors have no financial conflicts of interest.

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