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Original Article

Effects of major OMPs and LPS of *Brucella* on the control of activation of bone marrow-derived dendritic cells and proliferation of T-lymphocytes in mice

Xu, Z.[1*]; Tong, Z.[1***]; Zhang, H.[1]; Deng, X.[1]; Yang, N.[1]; Wang, Z.[2**] and Chen, C.[2*]**

[1]Graduated from School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China; [2]Department of Basic Veterinary Medicine, School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China

[*]***Correspondence:*** *C. Chen, Department of Basic Veterinary Medicine, School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China. E-mail: chuangfu_chen@163.com*

[**]***Co-correspondence:*** *Z. Wang, Department of Basic Veterinary Medicine, School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China. E-mail: 530469380@qq.com*

[***] These authors contributed equally to this work and were considered as the first authors

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Abstract

Background: *Brucella* outer membrane proteins (OMPs) are highly immunogenic, and lipopolysaccharides (LPS) are also considered significant antigens, making them potential candidates for subunit vaccines. **Aims:** To investigate the effects of *Brucella* OMPs and LPSs on mouse bone marrow-derived dendritic cells (BMDC) activation and T-lymphocyte proliferation. **Methods:** BMDC were isolated and cultured *in vitro*, and subsequently co-cultured with *Brucella* recombinant proteins (rOMP10, rOMP19, rBP26, rOMP25, and rOMP31), as well as smooth LPS (S-LPS) or rough LPS (R-LPS). The expression of maturation markers on the surface of BMDCs was determined using flow cytometry, while the expression of TLR receptors was determined using RT-PCR. The levels of inflammatory cytokines were measured using iELISA, and the impact on the proliferation of mouse T-lymphocytes was assessed using the MTT method. **Results:** The impact of LPS on BMDC maturation, TLRs-mediated cytokine secretion, and antigen presentation was found to be limited. In contrast, rOMP10, rOMP19, and rBP26 were observed to promote BMDC maturation, increase the expression of TLR-2 and TLR-4 mRNA, and activate T-lymphocyte proliferation by significantly increasing the expression of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-12) and antigen-presenting molecules. However, rOMP25 and rOMP31 did not promote BMDC maturation, inhibited the expression of MHCI and MHCII antigen-presenting molecules, and increased the expression of inflammation-suppressing cytokines (IL-10 and IL-4), resulting in the inhibition of T-lymphocyte proliferation. **Conclusion:** *Brucella* OMP10, OMP19, and BP26 play an important role in activating the host immune response, while OMP25 together with OMP31 may play a role in *Brucella* immune escape.

Key words: Antigen presentation, *Brucella* OMPs, Dendritic cell maturation, LPS, T-lymphocyte proliferation

Introduction

Brucellosis is one of the most common zoonotic diseases in the world and is endemic in more than 170 countries and regions around the globe, and this chronic infectious disease can affect the reproductive function of animals and may lead to abortion and infertility, resulting in huge economic losses (D'Anastasio *et al*., 2010; Hou *et al*., 2019). Currently, vaccination of susceptible animals with live attenuated vaccines is one of the effective means of preventing brucellosis, but such vaccines still have a number of drawbacks such as: lower safety, risk of virulence recovery and invisible infection, and inability to distinguish between natural infection and immunisation (Heidary *et al*., 2022; Elbehiry *et al*., 2023). The importance of developing a protein-based vaccine that can be easily detected serologically for the prevention and control of brucellosis cannot be overstated, and the research and development of novel vaccines require in-depth studies on the mechanisms of infection by pathogenic microorganisms and host immune response.

The main components of the *Brucella* outer membrane include lipopolysaccharide (LPS), outer membrane proteins (OMPs), and phospholipids (Ducrotoy *et al*., 2016; Vassen *et al*., 2019). *Brucella*

LPS can be categorized into smooth type (S-LPS) and rough type (R-LPS) (Vassen *et al*., 2019). LPS is a crucial molecule that is initially recognized by the innate immune system upon *Brucella* infection, leading to a strong immune response. It serves as the main component that stimulates the production of antibodies and induces a protective immune response in the body (ManLi, 2020). *Brucella* OMPs not only contribute to its structure and function, but also play a significant role in evading host immunity and promoting intracellular survival. Additionally, they exhibit good immunogenicity and antigenic protection (Kim *et al*., 2013b). The main outer membrane proteins of *Brucella*, including OMP10, OMP19, BP26, OMP31, and OMP25, have been extensively studied and shown to elicit immune responses and serve as targets for serodiagnostics in host organisms. Dendritic cells (DCs) are crucial initiators of the immune response and play a key role in regulating both adaptive and innate immunity (Nagai *et al*., 2006; Cabeza-Cabrerizo *et al*., 2021). Immature DCs possess strong endocytic activity, which decreases upon maturation. This maturation process involves upregulation of relevant surface molecules (CD40, CD80, CD86, MHC-I, MHC-II-like molecules) (Watts *et al*., 2010), as well as secretion of inflammatory cytokines (IL-12, IFN-γ, etc.) (Waithman *et al*., 2007). Mature DCs have the ability to activate T-lymphocyte proliferation (Langhorne *et al*., 2004). Given the crucial role of dendritic cells (DCs) in initiating and directing the acquired immune response, it may be more suitable to model the interactions between *Brucella* and DCs instead of relying on the traditional macrophage model. This approach can help us better comprehend the connection between *Brucella*'s pathogenic mechanism and the specific immune response of the infected host.

Brucella is an intracellular parasite that elicits both innate and adaptive immune responses in the body. Among these responses, T-cell-mediated immune responses have been found to play a crucial role in resisting *Brucella* infections. However, the impact of *Brucella* major outer membrane proteins and LPS on dendritic cell activation and T-lymphocyte proliferation is still unclear. To address this, we conducted an experiment where mouse bone marrow-derived dendritic cells (BMDCs) were incubated with *Brucella* major outer membrane protein and LPS. Our study aimed to investigate the effects of this incubation on dendritic cell activation and T-lymphocyte proliferation. The findings from this study provide valuable data and theoretical support for understanding the host mechanism of *Brucella* infection and for the development of novel subunit vaccines.

Materials and Methods

Animals and feeding

All experimental procedures involving animals were approved by the Biological Ethics Committee of Shihezi University (approval No.: A2022-098). Sixty female BABL/C mice, aged 6-8 weeks and weighing an average of 20 ± 5 g, were obtained from the Animal Experiment Centre of Xinjiang Medical University. All experimental procedures and animal care were conducted following the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The mice were provided with appropriate and compassionate care during their rearing, as stipulated in the Guide for the Care and Use of Laboratory Animals, and remained in good health throughout the study.

Bacteria strains

Prokaryotic recombinant expression strains of *Escherichia coli* PET-30a-OMP10 (Tiansen, 2017), PET-32a-OMP19 (Ningning *et al*., 2020), PET-28a-OMP25 (Jianxin, 2004), PET-28a-BP26 (Liyan *et al*., 2009), and PET-30a-OMP31 (Hongxing *et al*., 2009) have been pre-constructed and preserved in the zoonotic laboratory of Shihezi University. *Brucella* lipopolysaccharide (R-LPS, S-LPS), *Escherichia coli* lipopolysaccharide (E-LPS) were preserved in the zoonotic laboratory of Shihezi University.

Acquisition of OMPs

E. coli prokaryotic expression strains PET-30a-OMP10, PET-32a-OMP19, PET-28a-OMP25, PET-28a-BP26, and PET-30a-OMP31 were cultured in LB liquid medium at 37°C until the OD_{600nm} value was 0.6-0.8, and then added with final concentration of 1 mmol/L IPTG for about 6 h. Bacteria were collected by centrifugation at 12000 r/min for 10 min, and rOMP10, rOMP19, rBP26, rOMP25, rOMP31 were further obtained by the His-Tagged Protein Purification Kit (inclusion body proteins) (Kangwei, China). All the proteins were endotoxin free (ToxinEraserTM Endotoxin Removal Kit, GenScript Biotechnology, Nanjing, China).

Isolation and cultivation of DCs

The mouse DC isolation method of Inaba *et al*. (1992) was used in this study (Boes *et al*., 2003). Briefly, cells were flushed and purified from the tibias and femurs of BABL/C mice, which had been euthanized by cervical dislocation. The cells were plated at 1×10^6/well in 2 ml of RPMI 1640 and then cultured at 37°C in 5% CO_2 for 3 h. The supernatant was then discarded to remove non-adherent cells, and fresh 10% fetal bovine serum (10% FBS) with GM-CSF (10 ng/ml) and IL-4 (10 ng/ml) (all PeproTech, USA) were added. Fresh medium was added on days 2 and 4 and cells were collected on day 6. Flow cytometry was used to identify PE-anti-mouse CD11c, PE-anti-mouse CD80, PE-anti-mouse CD83, PE-anti-mouse CD86, PE-anti-mouse CD40, PE-anti-mouse MHC-I, and PE-anti-mouse MHC-II (1 μL/tube) (BioLegend, California, USA).

Flow cytometry analysis

DCs treated with OMPs, R-LPS, S-LPS, and E-LPS were analyzed via flow cytometry for expression of multiple markers of maturation on the cells surface. The following antibodies and appropriate isotype controls (all BD Biosciences, USA) were used. DCs were stimulated

by purified OMPs (50 ug/ml) (endotoxin removed), PBS was used as a control. After 24 h, the cells were collected and washed three times and the cell concentration was adjusted to 1×10^6 cells/tube using PBS. All antibodies (PE-anti-mouse CD11c, PE-anti-mouse CD80, PE-anti-mouse CD83, PE-anti-mouse CD86, and PE-anti-mouse CD40 (1 µL/tube)) were added to each tube. The DCs and antibodies were mixed and incubated at 4°C for 20 min and then washed three times and the unbound antibodies were removed. The cells were resuspended in PBS (300 ml) and cell phenotype changes were detected by flow cytometry. Data were collected on a FACSCalibur (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Cytokine detection

Cytokine measurements were done at 24 h. The cell culture supernatant was collected and the expression of cytokines TNF-α, IFN-γ, IL-6, IL-12, IL-10, and IL-4 were quantified according to ELISA kit instructions (BD Biosciences).

Quantitative real-time PCR

Expression of transcriptional factors including TLR2, TLR4, and TLR9 mRNA were evaluated using real-time PCR (RT-PCR). Briefly, cellular RNA was extracted using the Trzol method and its concentration was measured. cDNA was synthesized by reverse transcription using the HiFiScript cDNA first strand synthesis kit. Cells from the extracted the PBS negative control group were used as templates using a Roche LightCycler480® fluorescence real-time quantitative PCR. cDNA was used as template and GADPH as internal reference gene to detect the effect of *Brucella* outer membrane proteins on the transcript levels of BMDC cytokines, TLRs and the expression levels of MHCI and MHCII-like molecules. Primers are shown in Table 1. Reaction conditions: 95°C for 30 s, 95°C for 5 s, 60°C for 10 s, and 72°C for 15 s, 40 cycles. Relative quantification was performed by the $2^{-\Delta\Delta Ct}$ method. Three test replicates were performed under the same conditions.

Table 1: Primers and sequences of TLRs

Primer	Primer sequences (5´-3´)
TLR-2-F	GCGACATCCATCACCTGACTCTTC
TLR-2-R	GCCTCGGAATGCCAGCTTCTTC
TLR-4-F	CACAGAAGAGGCAAGGCGACAG
TLR-4-R	GACTGGCACTAACCACATAGAGAACTG
TLR-9-F	ACCTCAGCCACAACATTCTCAAGAC
TLR-9-R	TGCCACACTTCACACCATTAGCC
GAPDH-F	GGTGAAGGTCGGTGAACG
GAPDH-R	CTCGCTCCTGGAAGATGGTG

Proliferation of spleen T-lymphocytes

BMDCs were cultured for 6 d and subsequently stimulated with OMPs and LPS for 48 h. The cell concentration was adjusted to 1×10^5 cells/ml; mitomycin C was added at a final concentration of 25 µg/ml, and incubated at 37°C for 30 min. Following incubation, the cells were centrifuged at 1500 r/min for 5 min, after which the supernatants were discarded and the cells were washed twice with RPMI-1640 medium. The cells were then resuspended and inoculated into 96-well cell culture plates at a volume of 100 µL per well as stimulus cells. Mouse spleens were removed under sterile conditions and mouse splenic lymphocytes were isolated according to the instructions of the Mouse Splenic Lymphocyte Isolation Kit (TBD, Tianjin, China). The final concentration of lymphocytes was adjusted to 5.0×10^6 cells/well, and 96-well cell culture plates were prepared with 100 µL per well as reaction cells. The ratios of stimulated cells to reaction cells were set at 1:25, 1:50, and 1:100, with three replicate wells for each ratio. After incubation for 72 h at 37°C with 5% CO_2, 20 µL of MTT was added to each well, and incubation continued for an additional 4 h. Microscopic observation was conducted to examine the intracellular punctate particles. The plate was then centrifuged at 1500 r/min for 5 min, and the supernatant was discarded using a pipette gun. Subsequently, 100 µL of DMSO was added to each well and incubated in the dark for approximately 4 h. Once the intracellular purple crystals were completely dissolved, the OD value was measured at 570 nm. The stimulation index (SI) = OD value of the test wells/OD value of the control group.

Statistical analysis

Data are presented as mean ± standard deviation and were analyzed using Graph Pad Prism software (Graph-Pad Software Inc., San Diego, CA, USA). The differences between groups were examined through the analysis of variance using SPSS 17.0 software (SPSS, Inc. Chicago, IL, USA). All experiments were independently performed at least three times.

Results

Purification and identification of *Brucella* OMPs

After prokaryotic expression and purification of *Brucella* OMP10, OMP19, OMP25, BP26, and OMP31, the SDS-PAGE results showed specific bands appeared near 10KD, 19KD, 26KD, 28KD, and 25KD, respectively. This was in line with the expected size of the target band (Figs. 1A and B), indicating that *Brucella* major outer membrane proteins were well expressed and purified.

Isolation and culture of mouse bone marrow-derived dendritic cells

After stimulating bone marrow primary cell culture with RPMI-1640 containing GM-CSF and IL-4, we observed the growth of cells close to the wall on the first day of induction (Fig. 2A). A small number of cell colonies appeared on the third day of induction, and some cells were gently detached and semi-suspended by

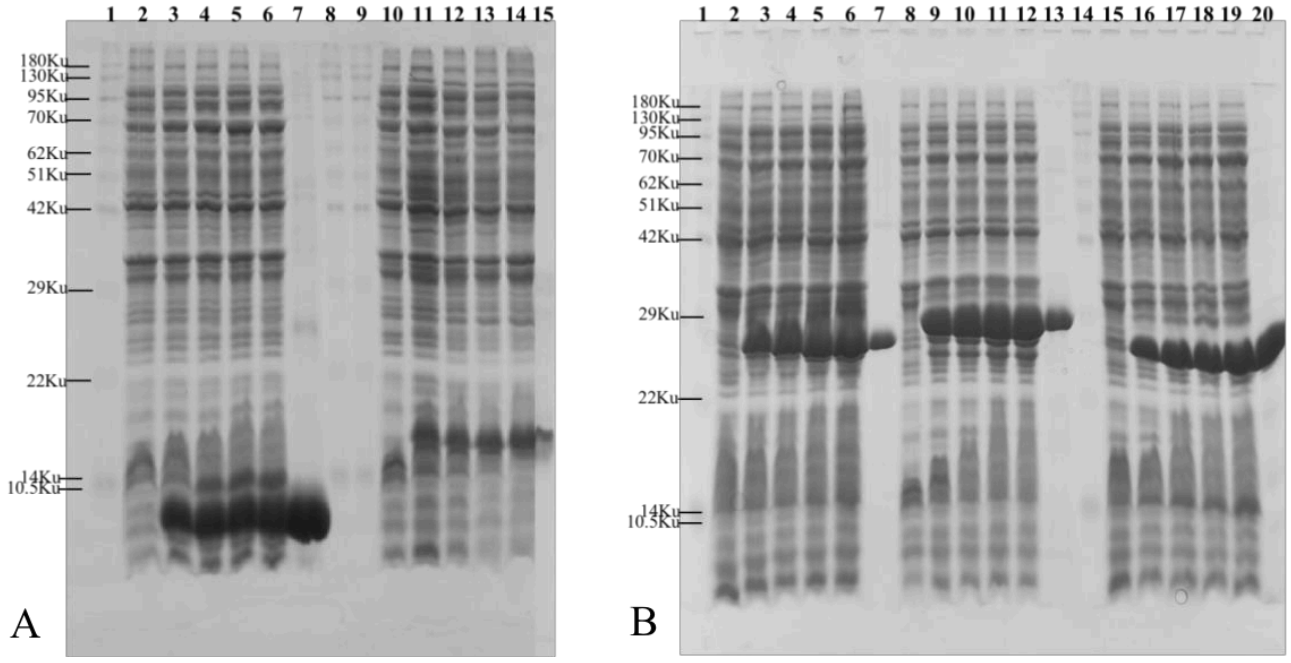

Fig. 1: Expression and purification of OMPs from *Brucella abortus* by SDS-PAGE. (**A**) Lanes 1, 8, 9: 180 KD protein marker, Lanes 2-7: Induction of 0h, 2h, 4h, 6h, 8h and purified outer membrane protein rOMP10, Lanes 10-15: Induction of 0h, 2h, 4h, 6h, 8h and purified outer membrane protein rOMP19, and (**B**) Lanes 1, 14: 180 KD protein marker, Lanes 2-7: Induction of 0h, 2h, 4h, 6h, 8h and purified outer membrane protein rOMP31, Lanes 8-13: Induction of 0h, 2h, 4h, 6h, 8h and purified outer membrane protein rBP26, and Lanes 15-20: Induction of 0h, 2h, 4h, 6h, 8h and purified outer membrane protein rOMP25

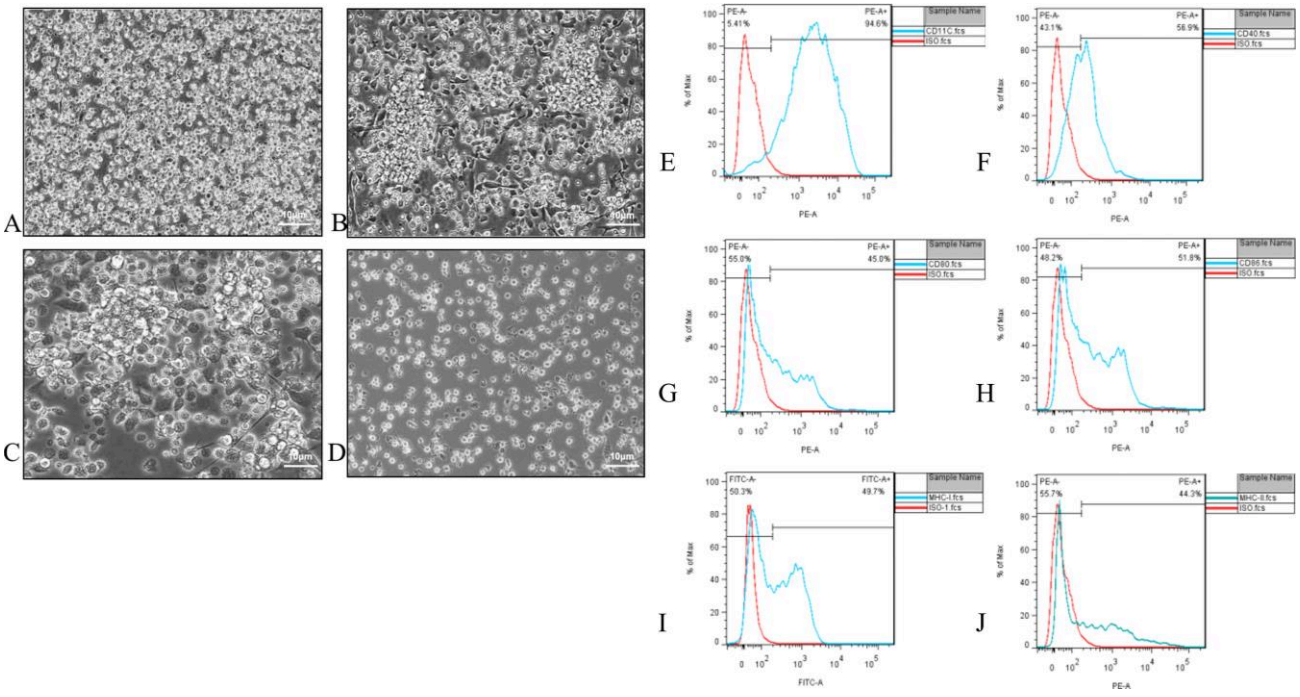

Fig. 2: BMDC morphology and cellular phenotype identification. (**A-D**) The morphology of mouse BMDC on day 1, day 3, day 6 (×100) and day 8 (×200) of culture, respectively, (**E**) The expression of CD11c molecule, (**F-H**) The expression of cell surface co-stimulatory molecules CD40, CD80, CD86, and (**I, J**) Expression of MHC-I and MHC-II antigen-presenting molecules

shaking the culture plate (Fig. 2B). By the sixth day of induction, the cell colonies increased and loosely adhered to the cells, indicating the presence of immature BMDC cells (Fig. 2C). On the eighth day of induction, most of the cells became suspended and exhibited obvious dendritic projections on the cell surface, indicating the presence of mature BMDCs (Fig. 2D). Flow cytometry was then used to identify the presence of CD11, CD40, CD80, and CD86 on the surface of the immature BMDC cells. The results showed that the

isolated cells contained 94.6% CD11c cells, expressing lower levels of CD40, CD80, and CD86 cell surface co-stimulatory molecules (Figs. 2E-J). These findings were consistent with the phenotypic characteristics of typical immature BMDC cells, confirming their suitability for subsequent experiments.

Effects of *Brucella* OMPs and LPS on BMDC phenotypes and antigen-presenting molecules

Flow cytometry was used to assess the effect of *Brucella* OMPs and LPS on BMDC maturation, compared with the PBS group, the *E. coli* LPS group (E-LPS) caused a significant increase in all cell surface markers and promoted the maturation of BMDC (Figs. 3A-F); *Brucella* R-LPS and S-LPS increased the expression of the cell surface markers CD86 and MHC-II only (Fig. 3F), while rOMP19, rOMP10, and rBP26 would significantly up-regulate the secretion of dendritic cell surface maturation markers CD40, CD80, CD83, CD86, MHC-I, and MHC-II (Figs. 3A-F), whereas rOMP25 and rOMP31 significantly inhibited the secretion of CD40, MHC-I, and MHC-II from dendritic cells (Figs. 3A, D, F).

Effect of *Brucella* OMPs and LPS on mRNA transcript levels of BMDC TLRs

Using GAPDH as an internal reference gene, E-LPS significantly increased TLR2 and TLR4 mRNA transcripts compared with the PBS group, and S-LPS, rOMP10, and rOMP19 significantly increased TLR2, TLR4 mRNA transcripts, R-LPS significantly increased the level of TLR4 mRNA transcripts, rOMP25, rOMP31, and rBP26 significantly increased the level of TLR2 mRNA transcripts, whereas incubation with OMPs and LPS did not affect the level of TLR9 mRNA transcripts (Fig. 4), probably due to the fact that TLR9 is localized in the endoplasmic reticulum of dendritic cells, which primarily recognize bacterial DNA containing non-methylated CpG sequences (CpG-containingoligo nucleotides).

Effects of *Brucella* OMPs and LPS on BMDC secretory cytokine secretion

The effects of *Brucella* OMPs and LPS on BMDC cytokine secretion were analyzed using ELISA. Compared with the PBS group, the E-LPS group significantly increased the secretion of pro-inflammatory factors TNF-α, IFN-γ, IL-6, and IL-12, and significantly decreased the secretion of anti-inflammatory factors IL-10 and IL-4; R-LPS and S-LPS promoted the secretion of IL-10 (Fig. 5C), whereas rOMP10, rOMP19, and rBP26 significantly induced BMDC secretion of cytokines TNF-α, IFN-γ, IL-6, and IL-12, and significantly inhibited the secretion of IL-10 and IL-4; on the contrary (Figs. 5A-F), OMP25 and OMP31 significantly induced the secretion of IL-10 and IL-4 (Figs. 5A, C), and significantly inhibited the secretion of cytokines TNF-α, IFN-γ, and IL-12 (Figs. 5D, E, F).

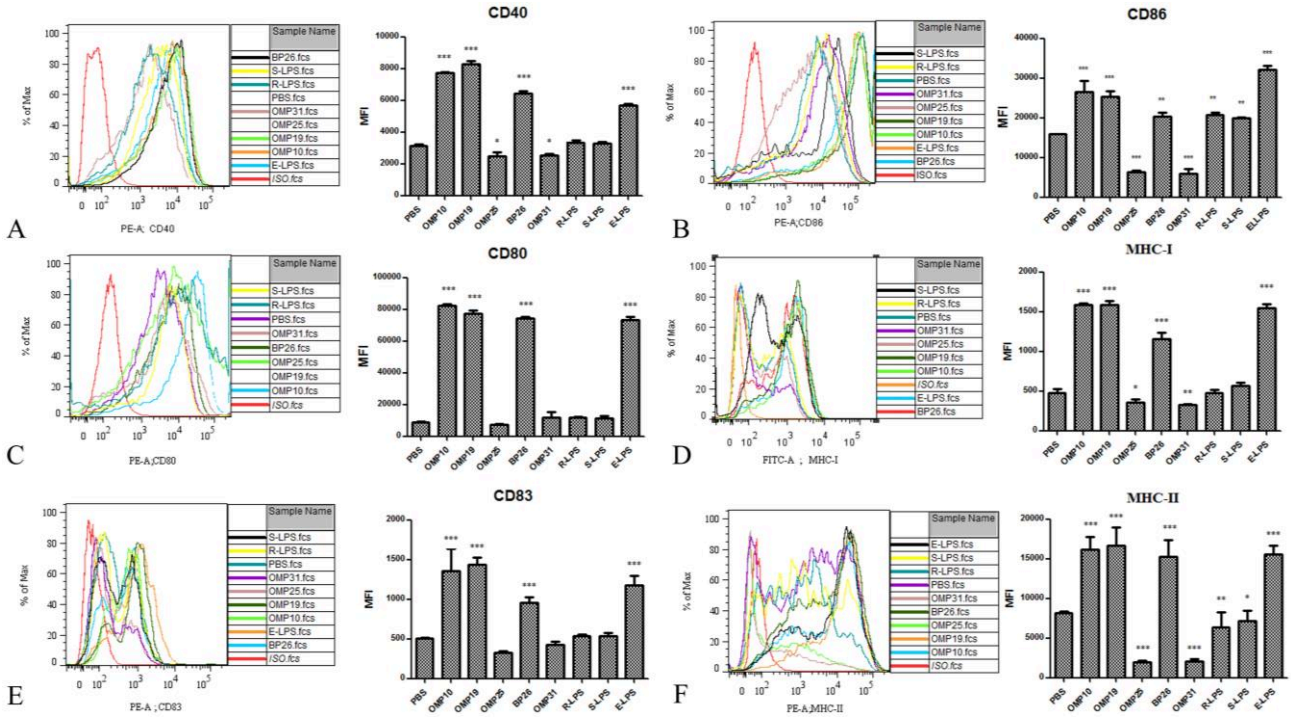

Fig. 3: Effects of *Brucella* OMPs and LPS on BMDC phenotypes and antigen-presenting molecules. (**A**) CD40 mean fluorescence intensity (MFI) flow and bar graphs, (**B**) CD86 mean fluorescence intensity (MFI) flow and bar graphs, (**C**) CD80 mean fluorescence intensity (MFI) flow and bar graphs, (**D**) MHC-I mean fluorescence intensity (MFI) flow and bar graphs, (**E**) CD83 mean fluorescence intensity (MFI) flow and bar graphs, and (**F**) MHC-II mean fluorescence intensity (MFI) flow and bar graphs. * P<0.05 difference significant, ** P<0.01 difference highly significant, and *** P<0.001 difference highly significant

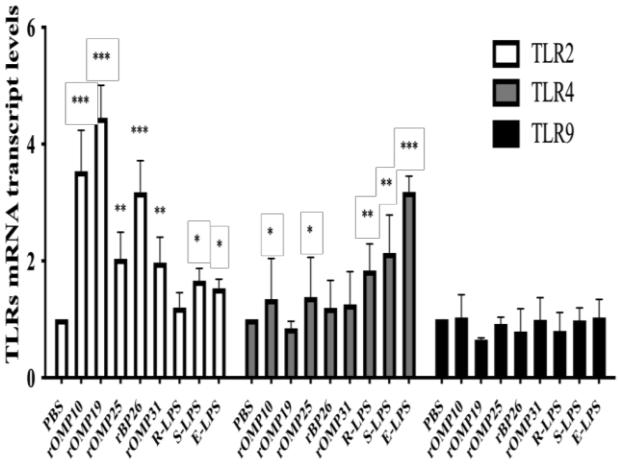

Fig. 4: Effect of *Brucella* OMPs and LPS on mRNA transcript levels of BMDC TLRs. * P<0.05 difference significant, ** P<0.01 difference highly significant, and *** P<0.001 difference highly significant

Effect of *Brucella* OMPs and LPS on BMDC-triggered proliferation of T-lymphocytes in allogeneic mice

The proliferation efficiency of T-lymphocytes after co-incubation of OMPs and LPS pretreated BMDC with T-lymphocytes was detected using the MTT method. Compared with the PBS pretreated group, the E-LPS group was able to effectively stimulate the proliferation of T-lymphocytes in all three DCs: T cells ratios, R-LPS and S-LPS could not effectively stimulate the proliferation of T-lymphocytes, and the BMDC pretreated with OMP10, OMP19 and BP26 were able to significantly promote the proliferation of T-lymphocytes, and at a DCs: T cells ratio of 1:50, T-lymphocyte proliferation was the best, and the stimulation index (SI) of the OMP10, OMP19, and BP26 pretreated group was more than 2-fold higher than that of the PBS group; whereas the BMDC pretreated with OMP25 and OMP31 significantly inhibited T-lymphocyte proliferation (Fig. 6). It was shown that rOMP10, rOMP19, and rBP26-mediated BMDC activation promoted the proliferation of mouse T-lymphocytes, and rOMP25, rOMP31 could effectively inhibit the proliferation of mouse T-lymphocytes by inhibiting BMDC activation.

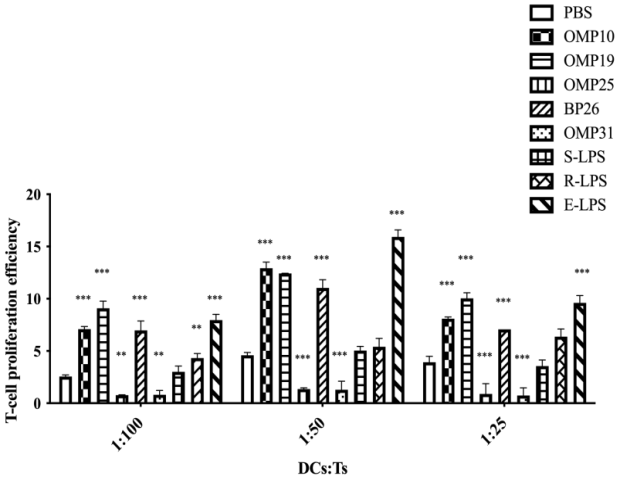

Fig. 6: Effects of *Brucella* OMPs and LPS on the proliferative capacity of T-lymphocytes in BMDC-activated mice. * P<0.05 difference significant, ** P<0.01 difference highly significant, and *** P<0.001 difference highly significant

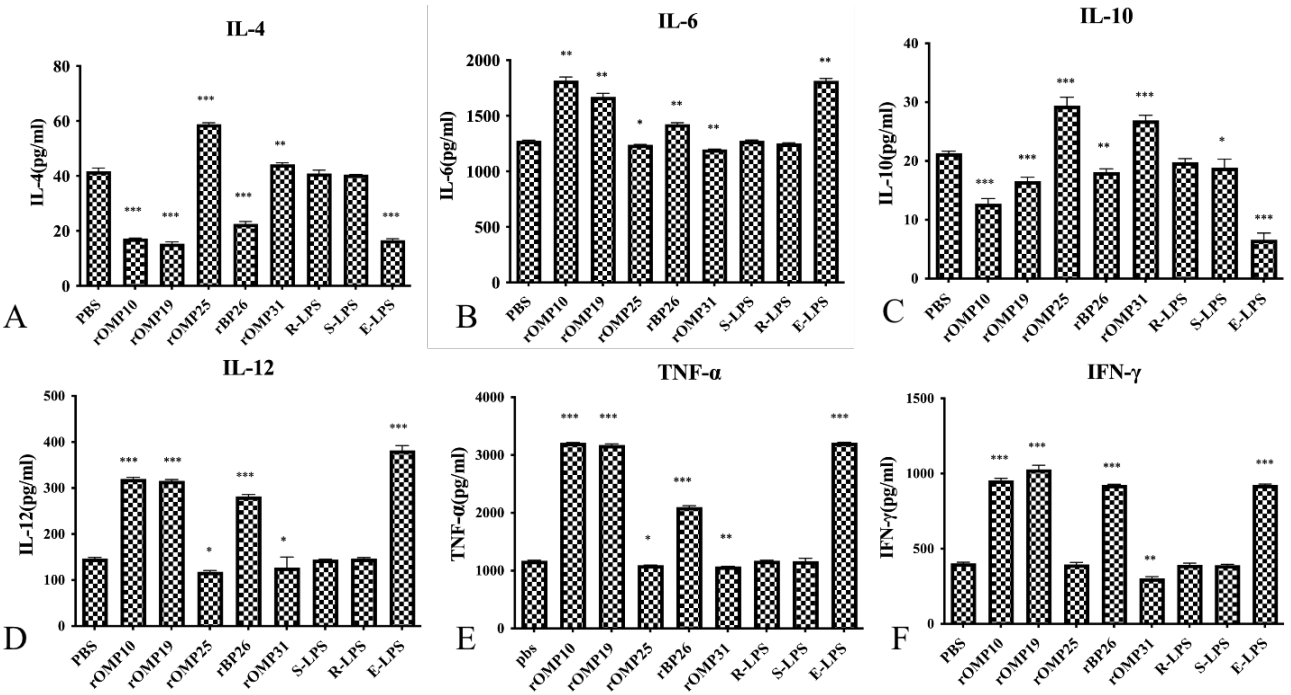

Fig. 5: Effects of *Brucella* OMPs and LPS on BMDC secretory cytokine secretion. (**A**) IL-4 expression levels, (**B**) IL-6 expression levels, (**C**) IL-10 expression levels, (**D**) IL-12 expression levels, (**E**) TNF-α expression levels, and (**F**) IFN-γ expression levels. * P<0.05 difference significant, ** P<0.01 difference highly significant, and *** P<0.001 difference highly significant

Discussion

Previous studies have demonstrated that *Brucella abortus* OMP10, OMP19, and BP26 induce increased secretion of cellular IL-6 and TNF-α when co-incubated with RAW264.7 cells (Bin *et al*., 2018). In addition, immunization of mice with recombinant outer membrane proteins showed that rOMP19 increased the number of IgG-secreting cells, leading to a Th1/Th2 immune response (Bin *et al*., 2018). On the other hand, OMP25 and OMP31 inhibited the production of TNF-α and IL-12 by RAW264.7 cells and suppressed the activation of the NF-κB signaling pathway (Shojaei *et al*., 2018). Both dendritic cells and macrophages play crucial roles in the host adaptive immune response. Mature dendritic cells, as antigen-presenting cells, effectively stimulate the proliferation of initial T-lymphocytes (Langhorne *et al*., 2004). In this study, we observed that *Brucella* rOMP10, rOMP19, and rBP26 significantly induced activation of bone marrow-derived dendritic cells (BMDCs) and upregulated the expression of key marker molecules CD40, CD80, CD83, and CD86. Immature BMDCs exhibited strong endocytosis function, and their maturation was gradually induced after antigen uptake. The phagocytosis of *Brucella* rOMP10, rOMP19, and rBP26 by BMDCs may be the main reason for this activation, indicating an increase in antigen-presenting ability. Furthermore, the high expression of MHC-I and MHC-II, which are the key factors for antigen presentation, was significantly enhanced in BMDCs after co-incubation with rOMP10, rOMP19, and rBP26. In contrast, rOMP25 and rOMP31 significantly inhibited BMDC maturation and reduced the secretion of CD40, MHC-I, and MHC-II. In contrast, rOMP25 and rOMP31 significantly inhibited the maturation of BMDC, and the secretion of CD40, MHC-I and MHC-II was significantly reduced. After phagocytosis and uptake of exogenous antigens by DCs, the MHC-I and MHC-II molecules were highly expressed, and the antigen was delivered to the T-lymphocytes in the form of complexes, which induced the T-lymphocytes to proliferate, differentiate, and generate cellular immune responses. It suggests that *Brucella* OMP25 and OMP31 may play an immunosuppressive function during the establishment of persistent infection by *Brucella*. In addition, we found that *Brucella* S-LPS and R-LPS only significantly increased the associated marker molecule CD86 and the antigen-presenting molecule MHC-II molecule, and did not effectively affect the activation of BMDC. After that, we further explored the effects of rOMP10, rOMP19, rBP26, rOMP25, rOMP31 as well as S-LPS and R-LPS on the transcript levels of mRNAs of TLR2, TLR4, and TLR9. Toll-like receptor (TLR) is an immune receptor that can directly make an immune defense response when the body is invaded by a pathogenic microbe. When TLRs are activated, a signaling cascade can occur, inducing the release of cytokines, which in turn initiates various immune responses (Fitzgerald and Kagan, 2020). Among them, TLR2, TLR4 and TLR9 are important immune mode receptors in the family of TLRs, which are thought to be associated with *Brucella* interactions (Kim *et al*., 2013a), the present study confirms that rOMP10, rOMP19, rBP26, rOMP25, and rOMP31 can activate the transcription of TLR2 mRNA, and rOMP10, rOMP25, S-LPS, and R-LPS could activate the transcription of TLR4 mRNA. In addition, TLR9 did not show significant changes after incubation with rOMPs and LPS, possibly due to the fact that TLR9 is present only in the endoplasmic reticulum of DC and mainly recognizes non-methylated CpG (CpG-containingoligo nucleotides) sequences in bacterial DNA (Weihua and Li, 2011). Cellular signal transduction is complex and diverse, and TLRs may indirectly regulate *Brucella* outer membrane protein-mediated BMDC activation by activating their downstream pathways.

Brucella acute phase infection is dominated by a Th1-type cellular immune response (Vitry *et al*., 2012; Ghaznavi Rad *et al*., 2017), and the activation of TLR2 and TLR4 can lead to the production of various downstream inflammatory cytokines and the expression of co-stimulators to play the role of natural immunity, among which the release of cytokines, such as IFN-γ, TNF-α, and IL-12, is the key to the host cellular immunity mediated by mature BMDC. In this study, we found that BMDC phagocytosis of rOMP10, rOMP19, and rBP26 promoted the secretion of large amounts of Th1-type cytokines (IL-6, IL-12, TNF-α, and IFN-γ), and phagocytosis of rOMP25 and rOMP31 promoted the secretion of large amounts of Th2-type cytokines (IL-4 and IL-10), while phagocytosis of S-LPS and R-LPS only promoted IL-10 secretion. TNF-α is an important molecule for host defense against pathogen infection (Wohlleber *et al*., 2012); IL-6 is an important mediator for immune cell activation and production of amplified inflammatory responses (Santiago-schwarz *et al*., 1996); IL-12 is a key molecule for the organism to induce Th1-type immune responses, mediate cellular immunity, and promote IFN-γ release (Oliveira *et al*., 2008); IL-4 stimulates the differentiation of initial T-lymphocytes to Th2 cells, and the negative regulatory effect of Th2 cells is enhanced, which is not conducive to the effective clearance of *Brucella* by immune cells in the host (Spörri and Sousa, 2005; Alloatti *et al*., 2015; Pan *et al*., 2019). Previous experiments have demonstrated that rOMP10, rOMP19, and rBP26 can play a significant role in activating the humoral immunity of the organism. In our study, we co-incubated *Brucella* outer membrane proteins and LPS-pretreated BMDCs with T-lymphocytes. The results indicated that rOMP10, rOMP19, and rBP26 effectively stimulated the proliferation of T-lymphocytes. The activation of T-lymphocytes was conducive to the clearance of intracellular *Brucella*, and TNF-α was found to synergize with IFN-γ to eliminate *Brucella*, effectively inhibiting bacterial replication, thereby reducing the transmission of brucellosis. These findings suggest that these proteins could be potential candidates for a *Brucella* subunit vaccine. In contrast, rOMP25 and rOMP31 significantly inhibited BMDC activation and suppressed T-lymphocyte proliferation. It is possible that *Brucella* may

inhibit the maturation of dendritic cells through OMP25 and OMP31, thereby reducing T-lymphocyte proliferation and suppressing the host immune response. However, no significant effect of S-LPS and R-LPS on T-lymphocyte proliferation was found, probably because *Brucella* S-LPS and R-LPS do not affect the activation of DCs, resulting in limited cytokine secretion and antigen presentation mediated by TLRs, and poor induction of T-lymphocyte proliferation.

This study investigates the effects of *Brucella* rOMP10, rOMP19, rBP26, rOMP25, rOMP31, and LPS on the activation and antigen-presenting capacity of BMDC, as well as their influence on cytokine secretion and T-lymphocyte proliferation. The results show that rOMP10, rOMP19, and rBP26 enhance BMDC activation and antigen presentation, leading to the release of TLR-mediated Th1-type cytokines and promoting T-lymphocyte proliferation. On the other hand, rOMP25 and rOMP31 inhibit BMDC activation and antigen presentation, resulting in the release of Th2-type cytokines and suppressing T-lymphocyte proliferation. Interestingly, LPS appears to have a minimal effect on BMDC maturation, cytokine secretion, and antigen presentation mediated by TLRs. This study contributes to our understanding of the mechanisms underlying *Brucella* infection and host cellular immunity. Furthermore, the findings support the development of a new subunit vaccine for *Brucella*.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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