



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Polysaccharide isolated from seeds of *Plantago asiatica* L. induces maturation of dendritic cells through MAPK and NF- κ B pathway

LeMing Jiang, DanFei Huang, ShaoPing Nie, MingYong Xie*

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

ARTICLE INFO

Article history:

Received 1 August 2017

Revised 25 September 2017

Accepted 27 September 2017

Available online 29 September 2017

Keywords:

Dendritic cell

Polysaccharides

Plantago asiatica L.

Toll-like receptor

MAPK

NF- κ B

ABSTRACT

Plantago species are used as traditional medicine in Asian and Europe. Polysaccharide isolated from the seeds of *Plantago asiatica* L. could stimulate maturation transformation of bone-marrow derived dendritic cells (DCs). We found that blocking p38, ERK1/2 and JNK MAPK signal transduction could significantly decreased the PLP-2 induced expression of MHC II, CD86 surface molecules on DCs. Blocking p38 and JNK signal also significantly inhibited the cytokine secretion of TNF- α and IL-12p70 as well, while blocking ERK1/2 signal only decreased the secretion of TNF- α . Meanwhile, DCs in the three MAPK signal-blocking groups showed dramatically attenuated effects on stimulating proliferation of T lymphocytes. Similarly, blocking signal transduction of NF- κ B pathway also significantly impaired the phenotypic and functional maturation development of DCs induced by PLP-2. These data suggest that MAPK and NF- κ B pathway mediates the PLP-induced maturation on DCs. Especially, among the three MAPK pathways, activation of JNK signal transduction is the most important for DCs development after PLP-2 incubation. And PLP-2 may activate the MAPK and NF- κ B pathway by triggering toll-like receptor 4 on DCs. © 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Polysaccharides are considered as effective biological response modifiers (BRM) that exhibit a lot of biological functions (Tzianabos, 2000; Leung et al., 2006), such as immunoenhancement (Bhunia et al., 2011), anti-oxidant (Wang et al., 2013; Samad et al., 2017) and anti-tumor activity (Zong et al., 2012). Especially, Lentinan has been developed as an adjuvant to cancer chemotherapy and clinically used in Japan and China (Chen et al., 2013).

Plantago asiatica L. is a perennial plant that belongs to the Plantaginaceae family. It is used as a traditional herb in many areas of

Asia and Europe (Samuelsen, 2000). Mucilage derived from the seed coat of *Plantago asiatica* L. are mainly composed of polysaccharides, and the extraction conditions of the seed polysaccharides were well studied (Ye et al., 2011). In our previous works, the *Plantago asiatica* L. polysaccharide (PLP) was separated into three fractions (PLP-1, PLP-2 and PLP-3) and the primary structure was characterized. PLP-2 was highly branched heteroxylyan containing a β -1,4-linked Xylp backbone, and it composed of rhamnose, arabinose, xylose, mannose, glucose and galactose in a molar ratio of 0.05:1.00:1.90:0.05:0.06:0.10 (Yin et al., 2012a,b), PLP-3 is arabinoxylyan consisted of Xylp backbone with 1,2,4-linked Xylp attached to O-2 position and 1,3,4-linked Xylp attached to O-3 position (Yin et al., 2012a,b; Zaidi et al., 2017).

Dendritic cells were considered playing indispensable roles in innate and adaptive immunity, and the maturation of DCs plays a crucial role in the immune activation and regulation (Banchereau and Steinman, 1998; Mortellaro et al., 2008). We have demonstrated that the *Plantago asiatica* L. polysaccharide is a strong immunoregulatory adjuvant that could induce the maturation of bone-marrow derived dendritic cells (DCs) *in vitro* (Huang et al., 2009a,b). But the underlying mechanism is still unclear.

In the present study, MAPK pathway (p38, ERK1/2, JNK pathway) and nuclear factor κ B (NF- κ B) were investigated to probe into the molecular mechanism underlying the immunoregulation effect of PLP-2 in DCs.

Abbreviations: CD, cluster of differentiation; DCs, dendritic cells; ERK, extracellular signal regulated kinases; TNF, tumor necrosis factor; IL, interleukin; JNK, c-Jun amino-terminal kinases; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor κ B; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognition receptors; PDTC, pyrrolidine dithiocarbamate; PLP, polysaccharide from the seeds of *Plantago asiatica* L.; TLR, toll-like receptor.

* Corresponding author.

E-mail address: Jianglemingnc@163.com (M. Xie).

Peer review under responsibility of King Saud University.



<https://doi.org/10.1016/j.sjbs.2017.09.011>

1319-562X/© 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2. Materials and methods

2.1. Mice

4- to 6-week-old BALB/c (H-2K^d and I-A^d) and C57BL/6 (H-2K^b and I-A^b) female mice were purchased from Hunan SJA Laboratory Animal Co. (Hunan Province, China), and maintained in a specific pathogen-free environment for at least 1 week prior to the experiment. All conditions and handling of the animals were conducted according to the international guiding principles for biomedical research involving animals.

2.2. Preparation of polysaccharide

The seeds of *Plantago asiatica* L. were purchased from Ji'an County, Jiangxi Province, China. The polysaccharide derived from the seeds of *Plantago asiatica* L. was prepared as previously reported (Yin et al., 2012a,b). Briefly, water-soluble polysaccharide was extracted with boiling water for 3 h. Then the aqueous extract was deproteinized, dialyzed (8000–14,000 kDa) and finally precipitated by 80% (v/v) ethanol for more than 12 h to obtain crude polysaccharide. Then the crude polysaccharide was redissolved and purified using a Sephacryl™ S-400 HR column (2.6 cm × 60 cm). The column was eluted with 15 mM NaCl at 1.2 mL/min. Polysaccharide content of each fraction was determined using phenol-sulfuric acid method and the main component (PLP-2) was collected.

The bacterial endotoxin content of PLP-2 was determined by tachypleus amebocyte lysate reagent (Chinese Horseshoe Crab Reagent Manufactory Co., Fujian Province, China). The quantity of endotoxin was estimated to be ≤ 0.015 endotoxin unit (EU) per mg of PLP-2.

2.3. Generation of dendritic cells and functional analysis

BALB/c (H-2K^d and I-A^d) mice were sacrificed to obtain the bone-marrow derived DCs. Like a previous report (Inaba et al., 1992), bone marrow cells from femurs and tibias were flushed out and cultured with basic RPMI1640 medium (Solarbio, Peking, China) for 3 h. Then the basic medium was replaced by complete RPMI1640 medium, which was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Hyclone), 10 ng/ml recombinant mouse (rm) GM-CSF and 10 ng/ml rmlL-4 (R&D systems, USA). 75% medium was replaced with fresh complete medium every 2 days. On the 5th day, the newly generated non-adherent cells and loosely adherent cells were harvested with vigorous pipetting and used as immature DCs, the CD11c⁺ cells ratio of which was higher than 75%.

After PLP-2 incubation, DCs were collected and the phenotypic and functional maturation was investigated according to previous method (Huang et al., 2014). Briefly, expression levels of surface molecules and endocytosis activity of DCs were determined by flow cytometry (fluorescent antibodies were all from ebioscience, USA; dextran, 40,000 Da, Sigma-Aldrich, USA) using FACSCalibur flow cytometer (BD Biosciences, USA). Besides, T lymphocytes isolated from C57BL/6 mice using Pan T cell isolation kit II (Miltenyi Biotec Inc, Germany) were co-cultured with DCs at a ratio of 10:1 to determine the T cells proliferation stimulating activity of DCs. In addition, the quantity of TNF- α , IL-12p70 cytokines in the cell culture supernatant were determined using commercial ELISA kits (Boster, Wuhan, China).

2.4. Western analysis

DCs treated with PLP-2 at 100 μ g/mL or LPS at 1 μ g/mL for 24 h were collected by centrifugation and washed three times with pre-

cold phosphate buffer (PBS). Then total proteins of DCs were prepared using KGP950 Phosphorylated Protein Extraction kit (KeyGEN Biotech, Jiangsu Province, China). Besides, the nuclear and cytoplasmic proteins were prepared using Nuclear and Cytoplasmic Protein Extraction kit (Beyotime, China). Protein concentration was determined using BCA assay kit (Beyotime, China).

Total proteins were separated by electrophoresis using a 10% SDS polyacrylamide gel and transferred onto a nitrocellulose membrane. Membrane was blocked using Tris-buffered-saline with Tween-20 (TBST) containing 5% BSA (Solarbio) at 25 °C for 1 h, and incubated with primary rabbit anti-phospho-p38, anti-phospho-ERK or anti-phospho-JNK antibody (All from Cell Signaling Technology, USA) for 12 h at 4 °C. The membrane was then incubated with secondary horse radish peroxidase (HRP)-conjugated anti-rabbit antibody (ZSGB-BIO, Beijing, China) for 1 h at 37 °C. Finally, membrane was developed with enhanced ECL kit (Beyotime, China). Chemiluminescence detection was performed on ChemiDoc XRS+ imaging system (BIO-RAD). Similarly, I κ B- α in the cytoplasmic extracts were also determined by Western blot analysis with anti-I κ B- α antibody (Cell Signaling Technology, USA).

2.5. Signal pathway blocking on DCs

Immature DCs were pre-treated with 20 μ M SB203580, 30 μ M PD98059 or 30 μ M SP600125 (All from Sigma-Aldrich, USA) respectively for 40 min to block p38, ERK1/2 and JNK pathway signal transduction. To block NF- κ B pathway signal transduction, the cells were pre-treated with 30 μ M pyrrolidine dithiocarbamate (PDTIC, Sigma-Aldrich, USA) for 40 min. Besides, DCs were pre-treated with 30 μ M anti-TLR4/MD2 monoclonal antibody (ebioscience, USA) for 1 h to block toll-like receptor-4 (TLR-4) signal recognition. After incubation, the cells were thoroughly washed with PBS three times and stimulated with PLP-2 (100 μ g/mL) for another 48 h. At the same time, groups of DCs only treated with PLP-2 (100 μ g/mL) or medium were set as control.

2.6. Statistical analysis

The results were shown as mean \pm standard deviation (S.D.) of the indicated number of experiments. The statistical significance was determined using a Student's *t*-test. $p < .05$ was considered as statistically significant, $p < .01$ as highly significant.

3. Results

3.1. PLP-2 activates the phosphorylation of MAPK proteins and nuclear translocation of NF- κ B in DCs

To determine whether MAPK pathway was activated by PLP-2, immature DCs were treated with PLP-2 (100 μ g/mL) and LPS (1 μ g/mL) respectively for 24 h, and then phosphorylation of MAPK proteins was determined by western blotting. As shown in Fig. 1 (a), a low level of phosphorylated p38, ERK1/2 or JNK protein was found in control group, but much higher levels of the phosphorylated proteins were observed in the PLP-2 group. LPS is a typical ligand of TLR-4 and it is a strong inducer of DC-maturation. As a positive control, LPS dramatically increased the level of phosphorylated proteins. No difference was found in the quantity of MAPK among the groups. These data indicated that ratio of phosphorylated MAPK to MAPK in DCs was increased by PLP-2. The effect of PLP-2 on the activation of NF- κ B pathway was also determined. As shown in Fig. 1(b), the level of I κ B- α in the cytoplasm was decreased by PLP-2 stimulation, which indicates a degradation of I κ B was enhanced. These results showed that PLP-2 strongly induced the nuclear translocation of NF- κ B p65 subunit in DCs.

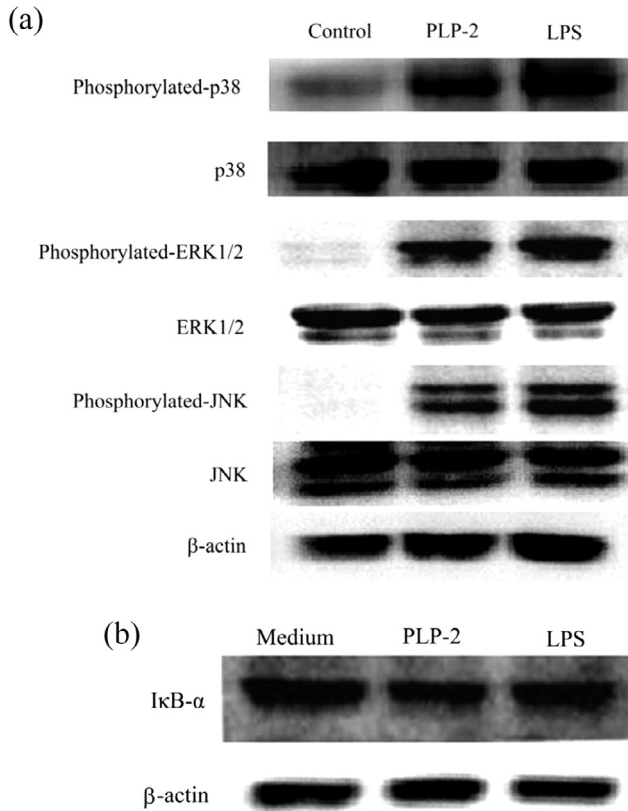


Fig. 1. Activation of MAPK and NF-κB pathway signaling in PLP-2 treated DCs.

On the 5th day, immature DCs were stimulated with 100 μg/mL PLP-2 or 1 μg/mL LPS for 24 h. Then the protein expression levels were determined by western blot analysis. (a) Phosphorylated-p38, ERK1/2 and JNK proteins were detected in the total protein extracts. The ratio of phosphorylated MAPK protein to MAPK protein was increased by PLP-2 treatment. (b) Activation of NF-κB was determined as nuclear translocation of NF-κBp65 subunit. Level of IκB-α in the cytoplasm was decreased, but level of NF-κBp65 subunit in the nucleus was increased, suggesting that PLP-2 strongly induced the NF-κBp65 translocation in DCs.

3.2. Blocking of MAPK and NF-κB pathway inhibit expression of surface molecules on DCs

Development of maturation plays a crucial role for DCs to initial immune response. During this process, large number of surface molecules are synthesized and expressed on DCs, with which DCs could activate lymphocyte, especially naïve T lymphocyte.

DCs were treated with PLP-2 at 100 μg/mL for 24 h, and then the expression of surface molecules was determined by flow cytometry. As shown in Fig. 2, compared with control group, expression levels of MHC II and CD86 molecules were significantly enhanced in PLP-2 group. However, expression of surface molecules was dramatically suppressed in the anti-TLR-4 group. Similarly, in the downstream pathway block groups, expression of surface molecules was also significantly inhibited, especially in the NF-κB pathway blocked group.

3.3. Blocking of MAPK and NF-κB pathway attenuate functional maturation of DCs

DCs undergo profound changes in morphology and function once the maturation is triggered. Their lymphocyte activation capacity is greatly enhanced, while the endocytosis is dramatically decreased. As shown in Fig. 3, functional maturation was observed in PLP-2 treated DCs that showed a lower uptake of FITC-dextran. But in TLR-4 blocking group, the reduction of endocytosis is significantly attenuated. Besides, blocking NF-κB pathway as well as MAPK pathway also suppressed this PLP-2 induced degradation of endocytosis.

Immature DCs were treated with 100 μg/mL PLP-2 for 48 h. Then the cells were harvested and incubated with 1 mg/mL FITC-dextran for 40 min. Endocytosis activity of DCs was determined by flow cytometry. PLP-2 significantly reduced uptake of dextran of DCs, indicating that functional maturation of DCs was induced. However, PLP-2 induced maturation of DCs was suppressed by pre-treatment with anti-TLR-4 mAb, MAPK pathway inhibitors or NF-κB pathway inhibitor. A representative of three independent experiments is shown and the results are shown as MFI.

3.4. Blocking of MAPK and NF-κB pathway inhibit Th1 cytokine secretion of DCs

Besides expressing MHC II and co-stimulatory molecules to direct T cell, producing cytokines as the third signal is also consid-

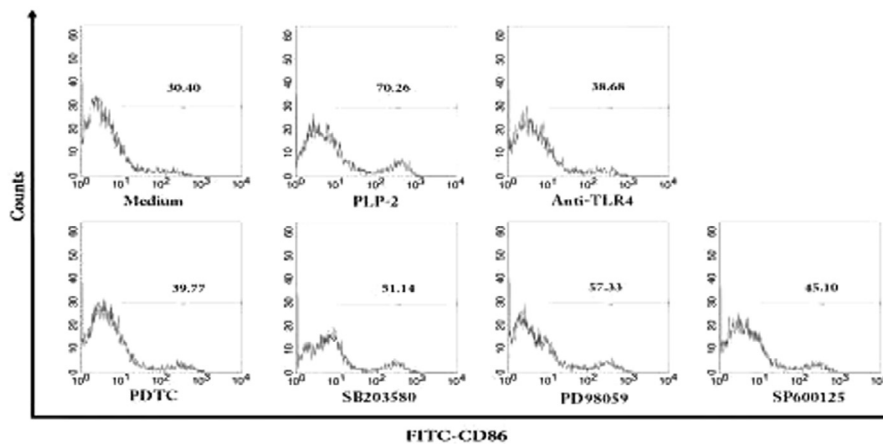


Fig. 2. DCs stimulated with PLP-2 are impaired in the up-regulation of MHC II and CD86 molecules by blocking signal transduction with anti-TLR-4 mAb, MAPK pathway inhibitors and NF-κB pathway inhibitors. DCs were pre-incubated with 30 μM anti-TLR4/MD2 monoclonal antibody for 1 h to block TLR-4. Similarly, NF-κB pathway, p38 MAPK, ERK1/2 and JNK pathway were blocked using 30 μM PDTC, 20 μM SB203580, 30 μM PD98059 and 30 μM SP600125 special inhibitors respectively. PLP-2 induced up-regulation of MHC II (a) and CD86 (b) molecule expression was significantly attenuated in the signal transduction blocking groups. A representative of three independent experiments is shown and the results are shown as mean fluorescence intensity (MFI).

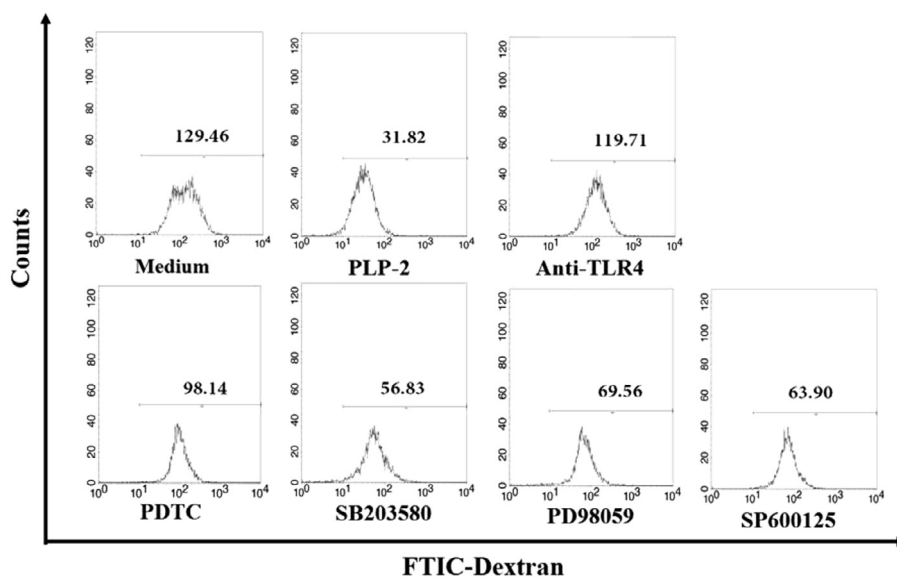


Fig. 3. PLP-2 induced functional maturation of DC was inhibited by blocking TLR-4 signal transduction.

ered as an important immune modulation mechanism of DCs (Roses et al., 2008). As shown in Fig. 4, secretion of both TNF- α and IL-12p70 in DCs was enhanced by PLP-2 remarkable. Compared with PLP-2 group, the secretion of TNF- α was much significantly suppressed in TLR-4-blocking group. Moreover, inhibition of MAPK and NF- κ B pathways could also dramatically inhibit the secretion of TNF- α . Like TNF- α , the secretion of IL-12p70 enhanced by PLP-2 stimulation was also inhibited by blocking TLR-4 and NF- κ B pathway. However, in the MAPK inhibited groups, the suppression phenomena were only observed in p38-inhibited or JNK-inhibited group.

3.5. Proliferative response of allogeneic naïve T cells

Only few DCs are necessary to provoke a strong T lymphocyte response *in vitro* or *in vivo*. The T lymphocyte proliferation stimulating activity was determined using MTT method. As shown in Fig. 5, DCs treated with PLP-2 showed a strong T lymphocyte proliferation stimulating activity. But compared with DCs in the PLP-2 group, DCs pre-treated with TLR-4 monoclonal antibody showed a

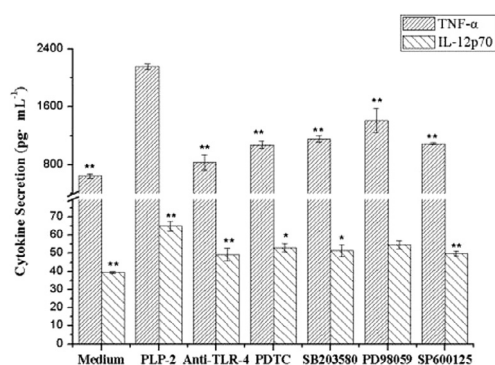


Fig. 4. PLP-2 induces secretion of TNF- α and IL-12p70 in DCs through TLR-4 signal transduction. Supernatants of DCs stimulated with 100 μ g/mL PLP-2 were collected and the TNF- α and IL-12p70 contents were determined. PLP-2 significantly enhanced the secretion of both TNF- α and IL-12p70. Blocking TLR-4 and the cascade pathways remarkably inhibited the secretion of TNF- α . Similar inhibiting effect on IL-12p70 secretion was observed in the signal transduction blocking group except ERK1/2 blocking group. * $P < .05$, ** $P < .01$ compared to PLP-2 group.

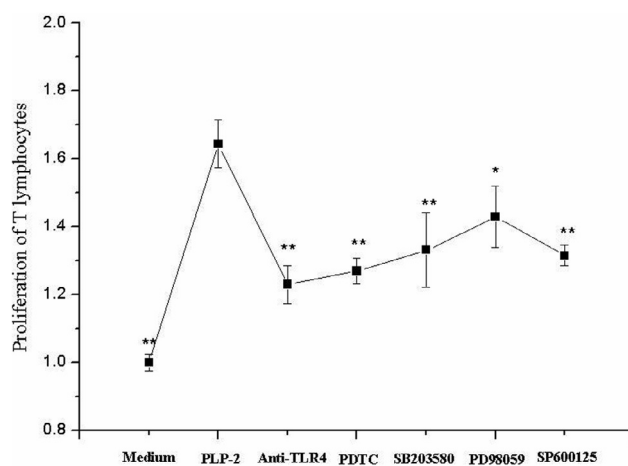


Fig. 5. T cells proliferation stimulating activity of DCs is enhanced by PLP-2 through TLR-4 signal transduction.

significant decrease in T lymphocyte activation. Likewise, blocking MAPK and NF- κ B also suppressed the enhancement of PLP-2-induced T lymphocyte initiation activity in DCs.

Responder mononuclear lymphocytes were isolated from the spleen of C57BL/6 mice using Pan T cell isolation Kit II and cultured in 96-well plate at 1×10^5 /well. DCs treated with PLP-2 were co-cultured with T lymphocyte at 1×10^4 /well as stimulator. The proliferation of T cells was determined by MTT assay. The absorption values of untreated DCs served as control values in the calculation of proliferation. A significant enhanced T cells proliferation stimulating activity was observed in PLP-2 treated DCs. However, this enhanced activity was significantly suppressed by pre-treating DCs with TLR-4 signal transduction inhibitors. * $P < .05$, ** $P < .01$ compared to PLP-2 group.

4. Discussion

Dendritic cells are widely distributed in lymphoid tissues, mucosal epithelium and organ parenchyma. They are professional antigen-presenting cells that play important roles in innate

responses to infections, and linking innate and adaptive immune response. MAPK signaling pathways is a highly conserved pathway that is involved in diverse cellular functions, including cell proliferation, differentiation and apoptosis. Three major subfamilies of MAPK proteins have been defined: extracellular signal regulated kinases (ERK), c-Jun amino-terminal kinases (JNK) and p38MAP kinase. Activation of MAPK is mediated by sequential protein dual-phosphorylation (Krens et al., 2006; Huang et al., 2009a,b; Razali and Said, 2017). PLP-2 was a powerful stimulus that could induce the maturation of DCs. We found that phosphorylation of MAPK proteins was activated during the PLP-2-induced maturation in DCs, which indicates that MAPK signal pathway may mediate such immune-enhancement effect of PLP-2.

To initiate immune response, two signals are necessary to activate naïve T or B lymphocyte. The specific binding of major histocompatibility complex (MHC) molecule to T cell receptor (TCR) or B cell receptor (BCR) is the first signal. In immature DCs, abundant MHC II molecules are synthesized, but they are mainly sequestered intracellularly in late endocytic compartments. However, a dramatic cytoplasmic reorganization highlighted by a redistribution of MHC II from intracellular compartments to the plasma membrane occurs during the maturation process (Mellman and Steinman, 2001; Halim and Phang, 2017). And the half-life of the MHC II molecules would increase from about 10 h to over 100 h in mature DCs, which result in a powerful capability of the cells to stimulate T cells even after several days (Cella et al., 1997). Besides such MHC II signal, the second signal is delivered by costimulatory molecules to ensure a successful immune response activation but not clonal anergy (Iwashima, 2003; Schwartz, 1990). For T cells, CD86 and CD80 molecules are well defined costimulators, which can be recognized through CD28 molecule. Blocking of p38, ERK and JNK could inhibit the expression of cell surface molecules on DCs. Especially, the expression of MHC II molecules and CD86 co-stimulating molecule was profoundly inhibited by JNK blocking. Nakahara reported that the JNK inhibitor SP600125 also effectively down-regulated lipopolysaccharide-induced expression of CD86, CD80, CD83 and CD54 on human-monocyte-derived DCs (Nakahara et al., 2004). Accompany with suppressing the surface molecules, MAPK pathways inhibitors also attenuated the down-regulation effect on the endocytosis activity of DCs. These data suggest PLP-2 induces the immature DCs transform from antigen-capture cells to antigen-presenting cells through MAPK signal pathways.

Along with the two signals, cytokines secreted by DCs also participate in the regulation of immune response. Incubation with PLP-2 could significantly enhance the secretion of TNF- α and IL-12 cytokines on DCs. TNF- α is one well-studied cytokine involved in the development of in vivo immune response. It plays an important role in the maturation of DCs after pathogen or virus challenge (Trevejo et al., 2001). TNF- α deficient would result in lack of form organized follicular dendritic cell networks (Pasparakis et al., 1996). TNF- α could also mediate migration of DCs from skin (Eaton et al., 2015) or intestinal (Hägerbrand et al., 2015; Shamsudin et al., 2017) to local lymph node. Myeloid DCs are a key source of IL-12 for naïve T cells. Especially, the myeloid DCs secrete high amounts of IL-12p70, but required combinational toll-like receptor stimulation (Nizzoli et al., 2013). Lichtenegger et al. (2012) reported that Th1 polarization capacity of toll-like receptor (TLR)-triggered DCs was dependent on CD86 signaling and IL-12p70. Wonderlich found that diminishing of IL-12 and IFN- α secretion of myeloid DCs severely impacts their T cell-stimulating function. Particularly, DCs could regulate naïve CD4⁺ T cell differentiation by modulating IL-12-STAT4 and TGF- β 1-SMAD3 axes and cytokine receptor expressions at the DC-T-cell interface (Liu et al., 2015). Treatment of those three special pro-

teins significantly suppressed the PLP-2-induced secretion of TNF- α on DCs, while the secretion of IL-12 was mainly depended on p38 and JNK pathways. In accordance with the lower expression of surface molecules and cytokine secretion, DCs in the JNK-blocking and p38-blocking groups exhibited significant decreased effects on stimulating proliferation of T cells. Among the three MAPK pathways, JNK pathway is the most important pathway that mediates PLP-2 induced maturation on DCs. Similarly, many studies also suggest that p38 and JNK MAPK pathways coordinate to positively regulate the maturation of DCs, while ERK is more inclined to regulate DC survival (Nakahara et al., 2006; Rescigno et al., 1998).

Besides, the role of NF- κ B pathway in the PLP-2-induced maturation on DC was also assessed. The NF- κ B family includes RelA (p65), RelB, cRel, NF- κ B1 (p50/p105) and NF- κ B2 (p52/p100), activation of which would drive expression of target genes. In TLR signaling, the most frequently activated form of NF- κ B is a heterodimer composed of RelA and p50 (Gao et al., 2017). Under normal conditions, RelA-p50 heterodimer remains inactive in the cytoplasm through a direct interaction with the inhibitors of NF- κ B (I κ B) family. TLRs triggering could eventually lead to degradation of the inhibitory protein I κ B, thus liberating the NF- κ B complexes for transport to the nucleus to promote gene expression (Rahman and McFadden, 2011). Incubation of PLP-2 activated the NF- κ B pathway in DCs since a reduction of I κ B in the cytoplasm of DCs was observed. We use PDTC to block the NF- κ B signal transduction, and found that PDTC significantly inhibited the DCs maturation as decreasing the expression of both MHC II and CD86 molecules, as well as suppressing the cytokine secretion of TNF- α and IL-12. Meanwhile, PDTC also decreases the T cell stimulating effect of DCs. Yoshimura blocked the NF- κ B signal by overexpressing I κ B α in DCs, and found that it inhibited surface molecules expression including HLA-DR, HLA-DQ, CD86, CD80 and CD40, as well as proinflammatory cytokine TNF- α , IL-12 and IL-6, which finally lead to a reduced antigen-presenting effect on DCs (2001). Ouaz et al. (2002), reported that DCs from doubly deficient p50^{-/-}Rel^{-/-} DCs were significantly impaired, while DCs from p50^{-/-}cRel^{-/-} mice could develop normally, but its CD40 ligand- and TRANCE-induced survival and IL-12 production was abolished. Brendan found that NF- κ B pathway is essential for CD40 ligand-treated DCs to activate T cells, particularly this function is enhanced if NF- κ B activation is prolonged (O'Sullivan and Thomas, 2002).

It is widely accepted postulation that activation of adaptive immunity relies on the perception of pathogen-associated molecular patterns (PAMPs) by the germ-line encoded pattern recognition receptors (PRRs) expressed on APCs. All TLRs except for TLR3 recruit MyD88, IRAKS and TRAF6 to activate the Ubc13/TAK1 pathway. The TAK1 complex not only activates the IKK complex to catalyze phosphorylation of I κ B protein, but also activate the MAPK pathway to induce the activation of the transcription factor AP-1 (Kawai and Akira, 2007a, 2007b). It has been confirmed that TLR-4 could mediate biological activities of many polysaccharides. For instance, polysaccharide from *Pueraria lobata* (2013), *Angelica gigas* (2007) and *Mori fructus* (2013) was found to induce the maturation of DCs derived from normal mice, but not from TLR-4-mutated mice. We found that DCs treated with anti-TLR-4 monoclonal antibody was hyporesponsive to PLP-2 incubation. And DCs in the TLR-4-blocking group showed more immature features than that of neither MAPK pathway-blocking nor NF- κ B pathway-blocking groups. It suggests that PLP-2 activates the MAPK and NF- κ B signal through triggering TLR-4. Rescigno et al. (2001) reported that DCs could open the tight junctions between intestinal epithelial cells, send dendrites outside the epithelium and directly take up bacteria, which may be a possible route for DCs to interact with PLP-2 in vivo.

5. Conclusion

In conclusion, MAPK and NF- κ B pathway mediates the PLP-induced maturation on DCs. Especially, among the three MAPK pathways, activation of JNK signal transduction is the most important for DCs development after PLP-2 incubation. And PLP-2 may activate the MAPK and NF- κ B pathway by triggering TLR-4 on DCs.

Acknowledgements

This study is supported by the National Key Technology R & D Program of China (2012BAD33B06), National Natural Science Foundation of China (21062012, 31260364), and the Program for New Century Excellent Talents in University – China (NCET-12-0749), which are gratefully acknowledged.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. *Nature* 392 (6673), 245–252.
- Bhunia, S.K. et al., 2011. Isolation and characterization of an immunoenhancing glucan from alkaline extract of an edible mushroom, *Lentinus squarrosulus* (Mont.) Singer. *Carbohydr. Res.* 346 (13), 2039–2044.
- Cella, M. et al., 1997. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 388 (6644), 782–787.
- Chen, Y. et al., 2013. Quality evaluation of lentinan injection produced in China. *J. Pharm. Biomed. Anal.* 78, 176–182.
- Eaton, L.H. et al., 2015. Skin sensitization induced Langerhans' cell mobilization: variable requirements for tumour necrosis factor- α . *Immunology* 144 (1), 139–148.
- Gao, W. et al., 2017. Characteristics studies of molecular structures in drugs. *Saudi Pharm. J.* 25 (4), 580–586.
- Hägerbrand, K. et al., 2015. MyD88 signaling regulates steady-state migration of intestinal CD103+ dendritic cells independently of TNF- α and the gut microbiota. *J. Immunol.* 195 (6), 2888–2899.
- Halim, N.I.A., Phang, I.C., 2017. Salicylic acid mitigates pb stress in nicotiana tabacum. *Galeri Warisan Sains* 1 (1), 16–19.
- Huang, D. et al., 2014. A novel polysaccharide from the seeds of *Plantago asiatica* L. induces dendritic cells maturation through toll-like receptor 4. *Int. Immunopharmacol.* 18 (2), 236–243.
- Huang, D.-F. et al., 2009a. Effect of phenylethanoid glycosides and polysaccharides from the seed of *Plantago asiatica* L. on the maturation of murine bone marrow-derived dendritic cells. *Eur. J. Pharmacol.* 620 (1), 105–111.
- Huang, G., Shi, L.Z., Chi, H., 2009b. Regulation of JNK and p38 MAPK in the immune system: signal integration, propagation and termination. *Cytokine* 48 (3), 161–169.
- Inaba, K. et al., 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176, 1693–1693.
- Iwashima, M., 2003. Kinetic perspectives of T cell antigen receptor signaling. *Immunol. Rev.* 191 (1), 196–210.
- Kawai, T., Akira, S., 2007a. Signaling to NF- κ B by Toll-like receptors. *Trends Mol. Med.* 13 (11), 460–469.
- Kawai, T., Akira, S., 2007. TLR signaling. In: *Seminars in Immunology*. Elsevier.
- Kim, H.S. et al., 2013. A polysaccharide isolated from *Pueraria lobata* enhances maturation of murine dendritic cells. *Int. J. Biol. Macromol.* 52, 184–191.
- Kim, J.Y. et al., 2007. Angelan isolated from *Angelica gigas* Nakai induces dendritic cell maturation through toll-like receptor 4. *Int. Immunopharmacol.* 7 (1), 78–87.
- Krens, S., Spaank, H.P., Snaar-Jagalska, B.E., 2006. Functions of the MAPK family in vertebrate-development. *FEBS Lett.* 580 (21), 4984–4990.
- Leung, M. et al., 2006. Polysaccharide biological response modifiers. *Immunol. Lett.* 105 (2), 101–114.
- Lichtenegger, F.S. et al., 2012. CD86 and IL-12p70 are key players for T helper 1 polarization and natural killer cell activation by Toll-like receptor-induced dendritic cells. *PLoS One* 7 (9), e44266.
- Liu, G. et al., 2015. Dendritic cell SIRT1–HIF1 α axis programs the differentiation of CD4+ T cells through IL-12 and TGF- β 1. *Proc. Natl. Acad. Sci.* 112 (9), E957–E965.
- Mellman, I., Steinman, R.M., 2001. Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106 (3), 255–258.
- Mortellaro, A. et al., 2008. Dendritic cells as sensors of environmental perturbations. *Microbes Infect.* 10 (9), 990–994.
- Nakahara, T. et al., 2004. Role of c-Jun N-terminal kinase on lipopolysaccharide induced maturation of human monocyte-derived dendritic cells. *Int. Immunol.* 16 (12), 1701–1709.
- Nakahara, T. et al., 2006. Differential role of MAPK signaling in human dendritic cell maturation and Th1/Th2 engagement. *J. Dermatol. Sci.* 42 (1), 1–11.
- Nizzoli, G. et al., 2013. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* 122 (6), 932–942.
- O'Sullivan, B.J., Thomas, R., 2002. CD40 ligation conditions dendritic cell antigen-presenting function through sustained activation of NF- κ B. *J. Immunol.* 168 (11), 5491–5498.
- Ouaaz, F. et al., 2002. Dendritic cell development and survival require distinct NF- κ B subunits. *Immunity* 16 (2), 257–270.
- Pasparakis, M. et al., 1996. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J. Exp. Med.* 184 (4), 1397–1411.
- Rahman, M.M., McFadden, G., 2011. Modulation of NF- κ B signalling by microbial pathogens. *Nat. Rev. Microbiol.* 9 (4), 291–306.
- Razali, M.A.A., Said, F.M., 2017. Red pigment production by monascus purpureus in stirred-drum bioreactor. *Galeri Warisan Sains* 1 (1), 13–15.
- Rescigno, M. et al., 1998. Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med.* 188 (11), 2175–2180.
- Rescigno, M. et al., 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* 2 (4), 361–367.
- Roses, R.E. et al., 2008. Differential production of IL-23 and IL-12 by myeloid-derived dendritic cells in response to TLR agonists. *J. Immunol.* 181 (7), 5120–5127.
- Samad, N.S.A., Amid, A., Jimat, D.N., Shukor, N.A.A., 2017. Isolation and identification of halophilic bacteria producing halotolerant protease. *Galeri Warisan Sains* 1 (1), 07–09.
- Samuelsen, A.B., 2000. The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. *J. Ethnopharmacol.* 71 (1), 1–21.
- Schwartz, R.H., 1990. A cell culture model for T lymphocyte clonal anergy. *Science* 248 (4961), 1349–1356.
- Shamsudin, N.H., Wong, C.F., Abd. Rahman, R.N.Z.R., Ali, M.S.M., 2017. Tight repression of elastase strain K overexpression by Pt7 (A₁/O₄/O₃) shuttle expression system. *Galeri Warisan Sains* 1 (1), 20–22.
- Shin, B.R. et al., 2013. Promoting effect of polysaccharide isolated from *Mori fructus* on dendritic cell maturation. *Food Chem. Toxicol.* 51, 411–418.
- Trejejo, J.M. et al., 2001. TNF- α -dependent maturation of local dendritic cells is critical for activating the adaptive immune response to virus infection. *Proc. Natl. Acad. Sci.* 98 (21), 12162–12167.
- Tzianabos, A.O., 2000. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. *Clin. Microbiol. Rev.* 13 (4), 523–533.
- Wang, H. et al., 2013. An overview on natural polysaccharides with antioxidant properties. *Curr. Med. Chem.* 20 (23), 2899–2913.
- Ye, C.-L., Hu, W.-L., Dai, D.-H., 2011. Extraction of polysaccharides and the antioxidant activity from the seeds of *Plantago asiatica* L. *Int. J. Biol. Macromol.* 49 (4), 466–470.
- Yin, J. et al., 2012a. Structural characterization of a highly branched polysaccharide from the seeds of *Plantago asiatica* L. *Carbohydr. Polym.* 87 (4), 2416–2424.
- Yin, J.-Y. et al., 2012b. Methylation and 2D NMR analysis of arabinoxylan from the seeds of *Plantago asiatica* L. *Carbohydr. Polym.* 88 (4), 1395–1401.
- Yoshimura, S. et al., 2001. Effective antigen presentation by dendritic cells is NF- κ B dependent: coordinate regulation of MHC, co-stimulatory molecules and cytokines. *Int. Immunol.* 13 (5), 675–683.
- Zaidi, N.A., Hamid, A.A.A., Hamid, T.H.T.A., 2017. Lactic acid bacteria with antimicrobial properties isolated from the intestines of Japanese quail (*Coturnix coturnix japonica*). *Galeri Warisan Sains* 1 (1), 10–12.
- Zong, A., Cao, H., Wang, F., 2012. Anticancer polysaccharides from natural resources: a review of recent research. *Carbohydr. Polym.* 90 (4), 1395–1410.