Effects and mechanisms of Portulaca oleracea L. polysaccharides on the activation of dendritic cells derived from mice immunized with FMD vaccine

Rui Zhao (zr601@163.com)
Heilongjiang Bayi Agricultural University https://orcid.org/0000-0001-8794-8299
Xiechen Zhou
Heilongjiang Bayi Agricultural University
Hui Zhao
Centers for Disease Control and Prevention
Guiyan Jia
Heilongjiang Bayi Agricultural University
Liyang Li
Heilongjiang Bayi Agricultural University
Tao Li
Heilongjiang Bayi Agricultural University
Yan Li
Heilongjiang Bayi Agricultural University
Xin Qi
Heilongjiang Bayi Agricultural University

Research Article

Keywords: Portulaca oleracea L, Polysaccharides, DCs, immunostimulant, cytokines

Posted Date: January 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1249268/v1

Abstract

Background

Our previous study has showed that *Portulaca oleracea* L. (POL-P), as an immunoenhancer, could increase the IgG and isotypes antibody titers in mice immunized with foot and mouth disease (FMD) vaccines. However, the structural features and the mechanism of action are still unclear. Enhancing antigen presentation is one of the main ways that immunoenhancer boost immune response. Dendritic cells (DCs) are the most potent antigen presenting cell (APC), which stimulate the initial T cells directly and initiate the specific immune responses. In addition to extracellular factors and intracellular genetic factors, epigenetics plays a major role in the regulation of DCs. In this study, we obtained POL-P, and structural features and monosaccharide composition were analyzed. We evaluated the effect of POL-P on functional maturation of DCs derived from mice immunized with foot and mouth disease (FMD) vaccine and explored the related mechanism responsible for immunoenhancer. The levels of protein and gene related to IL-12p35 and IL-12p40 were determined by western blot and chromatin immunoprecipitation (ChIP) assays. The expressions of TLR2, TLR4 receptors and the downstream molecules of MyD88 and NF-κB were examined using immunohistochemistry.

Results

The average molecular weight (Mw) of the POL-P was 4×10^4 Da. The monosaccharide composition of the POL-P was mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose and arabinose with a relative mass of 1.2%, 13.2%, 33.5%, 1.2%, 3.3%, 32.2% and 15.4%, respectively. We concluded that co-administration of POL-P with the FMD vaccine could significantly promote DCs maturation of phenotype and the immune function. In addition, the acetylation level of histone H3 of IL-12 was closely connected with the immune activity of DCs. Moreover, POL-P induced immune response was related to up-regulating protein expression of TLR2, TLR4, MyD88 and NF-κB in DCs.

Conclusions

Our evidence suggested that POL-P could be a potential immunostimulant in the regulation of DCs maturation for FMD vaccine.

Background

Vaccination is a more powerful measure to prevent and control infectious disease, but it is not very satisfactory to induce a sufficient immune response due to weak immunogenicity and suppressive maturation/function of dendritic cells (DCs)[1]. Immunostimulants are vital substance to boost immunity and alter immune responses to coadministered antigens. Unfortunately, as agents that enhance immune responses, existing immunostimulants have potential disadvantages, such as allergic reactions,

granulomas and failing to effectively enhance the cellular immune response[2]. Therefore, it becomes of practical significance and urgency to find safe, efficient and convenient new immunostimulants.

Traditional Chinese medicines (TCM) have become the research focus of immunomodulators in recent years. TCM enhance specific immunogenicity nonspecifically through binding with antigens, and stimulate the body's immune response or change the type of immune response, which has a unique effect on vaccines[3]. Polysaccharides, one of the main active ingredients of TCM, can be used as immune enhancers and virus inhibitors[4]. Moreover, as opposed to injectable adjuvants, oral polysaccharides play a special role in antiviral immune because they have the characteristics of promoting cellular immunity, mucosal immunity, biodegradability and low toxicity[5]. *Portulaca oleracea* L., which belongs to the *Portulacaceae* family, as the folk medicine and food nutrient supplements, has been widely used around the world for thousands of years[6]. *Portulaca oleracea L.* polysaccharides (POL-P) have many pharmacological activities, such as antitumor, antioxidation and antiviral[7–9]. Our published paper have showed that POL-P could increase the IgG and isotypes antibody titers, promote SIgA secretion of intestinal mucosa in mice immunized with foot and mouth disease (FMD) vaccines[10]. However, the structural features and the mechanism of action are still unexplored.

Enhancing antigen presentation is one of the main ways that immunostimulants boost immune response. DCs are the most potent antigen presenting cells (APC), which stimulate initial T cells directly and initiate the specific immune responses. Mature DCs are mainly involved in cellular immunity and T cell-dependent humoral immunity, connecting innate immunity and adaptive immunity, and participating in the regulation of immune function by secreting cytokines to mediate the chemotaxis of immune cells[11]. The efficiency of antigen presentation by DCs is very high. Antigen and DCs activate T cells, stimulate the proliferation and maturation of B cells, and enhance the activity of helper T cells (Th) and natural killer cells (NK) to participate in the organism anti-infection immunity[12]. Polysaccharides, as the broad spectrum biological response modulator, play a major role in immune regulation. In the present study, we explored that POL-P induced functional maturation of DCs derived from mice immunized with FMD vaccine, which may explain the underlying mechanism associated with immunoenhanced activity.

Results

Characterizations of POL-P

The spectral characteristics of POL-P were illustrated in Figure1. The purified POL-P displayed a typical polysaccharide IR spectrum. The broadly stretched intense peak at 3400.00 cm^{-1} corresponded to the O-H stretching vibrations, and a weak C-H peak at around 2926.60 cm^{-1}. The relatively strong absorption peak at around 1597.52 cm^{-1} was attributed to the C=O asymmetric stretching vibration. Moreover, POL-P showed a specific absorption band in 1000-1200 cm^{-1}, which indicated ring vibrations overlapped with the (C-O-C) glycosidic band vibrations and (C-OH) stretching vibrations of side groups. Absorption peaks at 1024.58 cm^{-1}, 1079.37 cm^{-1} and 1153.21 cm^{-1} showed C-C and C-O link band positions, which

indicated POL-P was a pyranose form of sugar[13]. Absorptions peaks at 843.68 cm^{-1} and 930.69 cm^{-1} suggested the existence of α- and β-glycosidic linkage in POL-P structure[14].

Based on gel column chromatography, the average molecular weight of POL-P was 4×10^4 Da (Fig. 2A, B, C). The single symmetric peak indicated that POL-P was homogeneous polysaccharide component (Fig. 2B, C). The content of uronic acid was 57.20% by M-hydroxybiphenyl method. The total sugar content of POL-P was 64.90%, using Phenol-sulfuric acid method. Analysis monosaccharide composition of POL-P indicated that polysaccharides contained mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose and arabinose with a relative mass of 1.2%, 13.2%, 33.5%, 1.2%, 3.3%, 32.2% and 15.4%, respectively (Fig. 3).

Effect of POL-P on DCs morphology

Flow cytometry detection for positive rate of CD11c (Fig. 4). To evaluate the roles of POL-P in DCs maturation, we first observed the state and ultrastructure of DCs by light microscope and SEM. The microscopical observations showed that the DCs were cultured for 5 days, became round and irregular dendrites in the presence of POL-P. POL-P dose-dependently increased the number and volume of DCs, and the protruding protuberance of the cell membrane became more obvious, showing a typical DC shape. TEM observation also showed that the cell surface was not smooth, accompanied with short dendritic structure, and fewer organelles were in the cytoplasm between the control group and the low-dose group. A significant number of branch like projections on the cell surface and more mitochondria, rough endoplasmic reticulum and other organelles were visible in the POL-P(M) and POL-P(H) groups. In particular,, the cells in the high-dose group had different electron densification depth, and the nucleus were a tendency to one side. There were a large number of vesicles in the cells and phagocytic foreign bodies could be seen in some of the vesicles (Fig. 5A).

Effect of POL-P on maturation of DCs

In addition to morphological observation, we also detected the maturation surface markers CD80, CD83 and CD86. As showed in Figure 5B, C, POL-P significantly promoted the expression of CD80, CD83 and CD86 on the DCs surface, and the effect was more noticeable in POL-P (H) group. mRNA expression levels of MHC-Ⅰ and MHC-Ⅱ of DCs in vaccinated mice were detected by qRT-PCR. The results showed that POL-P pretreatment dose-dependently increased the expression levels of MHC-Ⅰand MHC-Ⅱ (Fig. 5D). It was suggested that POL-P could stimulate DCs maturation of mice immunized with FMD inactivated vaccine.

Effect of POL-P on cytokines levels

Secreting cytokines is another well-characterized immunological function for mature DCs. For further analysis of DCs maturation, we also investigated whether POL-P promoted cytokines secretion. As showed in Figure 6, compared to the control group, POL-P co-treatment increased the secretion of cytokines, including IL-12, IFN-r, TNF-α, IL-1β, IL-8 and IL-6 in a dose-dependent manner. The results

suggested that POL-P could induce the functional maturation of DCs, thereby enhancing the ability of regulating immunity in mice immunized with FMD inactivated vaccine.

Effect of POL-P on the expression of IL-12p35 and IL-12p40

IL-12 was a key cytokine for activating Th1 immune response, which related to the organism immunity. The intact IL-12p70 is composed of p35 and p40 subunits. The expression levels of IL-12p35 and IL-12p40 were detected by western blot. The results showed that POL-P could increase protein expression of IL-12p35 and IL-12p40 in DCs (Fig. 7). The above results indicated that POL-P promoted the maturation and enhanced the immune function of DCs by up-regulating the protein expression of IL-12 in DCs.

Effect of POL-P on histone H3 acetylation levels in the IL-12p35 and IL-12p40 promoter regions of DCs

To better clarify the molecular mechanisms underlying the promoting activity of POL-P on DCs maturation and activation in immunized mice, we detected POL-P-mediated histone modifications at the IL-12p35 and IL-12p40 promoter regions of DCs by CHIP assay. The results showed that the acetylation levels of histone H3 in the promoter of IL-12p35 and IL-12p40 genes in DCs were effectively up-regulated by POL-P treatment (Fig. 8). It was further verified that the transcription level of IL-12, the functional cytokine of DCs, was affected by epigenetic regulation, suggesting that the acetylation level of histone H3 at the IL-12 promoter locus in DCs was closely related to POL-P-induced immune activity.

Effect of POL-P on protein expression of TLR2, TLR4, MyD88 and NF-κB in DCs

Polysaccharides exert immune regulation ability by binding to the corresponding receptors of the cells surface and activating the downstream signaling pathway. Given the crucial role of TLRs in the immune response, we performed an immunohistochemical method to determine whether TLR/NF-κB pathway was involved in the process. Pretreatment with POL-P led to protein expressions increase including TLR2, TLR4, MyD88 and NF-κB in DCs in a dose-dependent manner (Fig. 9). These data suggested that POL-P significantly promote the phenotype and function maturation of DCs, and the mechanism may be related to activation of TLR2, TLR4 and downstream proteins.

Safety evaluation of POL-P in mice

To evaluate whether POL-P had toxic effects on immunized mice, tissue sections of liver, kidney and small intestine, the oral absorption organ were stained and analyzed. Compared with the control group, the central vein of the liver was in the middle, and the liver cords were arranged radially, without inflammatory cell infiltration and necrotic cell generation. The renal tissue structure was normal, and the glomerular and tubular structures were clear and complete. In addition, the intestinal mucosa was intact and the ratio of villi height/crypt depth did not change significantly (Fig. 10). Taken together, these

observation results showed that POL-P had no observable toxic effect on liver, kidney and small intestine of mice immunized with FMD inactivated vaccine.

Discussion

Our previous researches have showed that POL-P could increase the FMDV-specific antibody production in mice immunized with FMD vaccines[10]. In the present study, the structural features of POL-P and the molecular mechanism were revealed in detail. An ideal new immunostimulant not only enhances immune response, but also acts on specific immune cells to mobilize the whole immune function of the body, and changes the type of immune response caused by antigen, whereby regulating the balance between humoral immunity and cellular immunity[15]. APC is indispensable in inducing antiviral cellular immunity. DCs, the strongest known APC, are the main initiator of immune response and play a key role in antiviral immunity. High expression of MHC-I and MHC-II in mature DCs activates T cells and mediates the immune response. DCs activate MHC-□ and then help T cells differentiate into Th1 and Th2 types, which mediate cellular immunity and humoral immunity, respectively[16].

The regulation of dynamic balance between Th1 and Th2 plays an important role in maintaining the normal immune function. Mature DCs secrete a variety of cytokines. Thl and Th2 cytokines are key regulators of Ig homeotype conversion. On the one hand, Thl cytokines, such as IFN-γ, not only stimulate the cytotoxic lymphocyte (CTL) response, but also directly promote the secretion of IgG2a. On the other hand, IgGl is preferentially regulated by Th2 cytokines, which match our previous findings. In this study, co-administering POL-P with the FMD vaccine induced high levels of IL-12, IFN-γ, IL-1β, IL-8, TNF-α and IL-6 in DCs, which indicated that POL-P could modulate Thl and Th2 immune responses simultaneously[17]. IL-12 is considered to be an essential functional regulator for Th1/Th2 balance. IL-12, also known as cytotoxic lymphocyte maturation factor, is mainly produced and secreted by DCs. High level of IL-12 suggests that a large number of DCs are activated[18]. IL-12 is composed of p35 and p40 polypeptide chains. P35 subunit plays a crucial role in IL-12 synthesis, and p40 is mainly regulated at the transcriptional level[19, 20]. We found that POL-P increased protein expression of IL-12p35 and IL-12p40 in DCs. Moreover, POL-P significantly increased the mRNA expression of MHC-I and MHC-II in DCs of mice immunized with FMDV vaccine. We concluded that stimulating DCs maturation was one of the ways for POL-P used as an immunostimulant by above detection results and morphological analysis.

Recent studies have demonstrated that the differentiation, maturation and function of DCs are not only influenced by extracellular factors and intracellular genetic factors, but also regulated by epigenetics[21, 22]. Under the action of specific signals, including drug components, DCs could undergo epigenetic regulation to determine its maturation process and mechanism[23]. At present, there are no landmark studies on the regulation of DCs by epigenetic modification factors from the perspective of DCs specific function. It will enable us to understand the mechanism of TCM immunostimulants on DCs maturation and function from a new perspective and at a higher level if further studys are based on the establishment and maintenance of DCs biological functions at the chromatin level. Histone acetylation and deacetylation are related to the activation and inhibition of gene transcription, which has been the

focus of current research[24, 25]. These studies suggested that it had more significant scientific value to explore the effect of POL-P on the acetylation level of histone H3, the promoter of the functional cytokine IL-12. High histone acetylation is involved in gene activation, while low histone acetylation is associated with gene silencing. In this study, we detected the acetylation level of histone H3 at the promoter region of IL-12p35 and IL-12p40 genes of DCs. In support of this recommendation, we found that the transcription level of the functional cytokine IL-12 in DCs was affected by epigenetic regulation. And this confirmed that the acetylation level of histone H3 was closely connected with the immune activity of DCs. These results identified targets of DCs for POL-P. It laid a good foundation for the further study on POL-P as a new vaccine immunostimulant.

Toll-like receptors (TLRs) are the most representative type of pattern recognition antigen, which plays an essential role in the recognition of invading pathogens. They are also an essential bridge between innate immunity and adaptive immunity[26]. TLR2 and TLR4 are two prominent members of the TLRs family and are widely expressed on various cell membranes. TLR4 can promote the production of cytokines, increase the expression of immune-related molecules on the surface and enhance the maturation and activation of DCs[27]. TLR2 is a transmembrane receptor, and the TLR2 pathway is able to induce DCs in favor of Th2 immune response. Both TLR2 and TLR4 signaling pathways involve the activation of the adaptor protein MyD88, which ultimately leads to activation of NF-κB[28]. Numerous studies have showed that polysaccharides obtained from TCM could act on DCs through TLR2 or/and TLR4, thus achieving the immune-enhancing effect[29–31]. It was further speculated that TLR2/4 might be the specific binding receptor of POL-P regulating DCs. POL-P affected the secretion of cytokines by recognizing TLR2/4 receptor on the surface of DCs membrane, and regulating the immune response of DCs. In this study, we further analysed the protein expressions of TLR2, TLR4, MyD88 and NF-κB in mature DCs stimulated by POL-P, as well as the secretion of cytokines, the final products of the pathway. The results showed that POL-P could dramatically up-regulate the protein expressions of TLR2, TLR4, MyD88 and NF-κB in DCs, and promote the secretion of cytokines. In general, both TLR2 and TLR4 related proteins were involved in POL-P regulation of DCs, thus performing its biological function and participating in the immune response.

The safety of POL-P in animal must be considered although we had already verified that POL-P could improve the immune activity of FMD vaccine. POL-P was administered orally as the immunostimulant, so we wondered whether it was harmful to the intestines. Therefore, the alexipharmic tissue-liver, detoxifying tissue-kidney and small intestine were observed and analyzed. Morphological analysis revealed that POL-P was non-toxic and safe. The experimental results provided a scientific basis for toxicological safety evaluation of POL-P and laid a theoretical foundation for its development and application in clinical practice.

Conclusion

In summary, we demonstrated that the POL-P could induce DCs functional maturation and the mechanism involved in TLRs related proteins. Therefore, we concluded that POL-P was a safe and

effective immunostimulant in the regulation of DCs maturation and the enhancement of the immune responses for FMD vaccine.

Methods

Materials

The whole plant of *Portulaca oleracea* L. used in the study was obtained from Daqing China Freibon Co., Ltd. The plant was authenticated by Dr. Sun Yue-Chun at Heilongjiang Bayi Agricultural University, where the herbarium voucher has been kept (ID: 334475). Type O FMD inactivated vaccine was provided by the Inner Mongolia Jinyu pharmaceutical factory (Mongolia, China). The vaccine contains an inactivated OHM/02 strain of FMD Type O virus, and the virus content before inactivation should be at least $10^{7.0}$ LD_{50}/0.2 mL. Escherichia coli LPS and mitomycin C were purchased from Sigma Chemical Co.. Recombinant mouse GM-CSF, IL-4 and IL-2 were purchased from NOVUSBIO; Micro Beads and MS separation column was purchased from Miltenyi Biotec. Antibodies against CD11c, CD80, CD83 and CD86 were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The ELISA kits of IL-12, IFN-γ, IL-1β, IL-8, TNF-α, IL-4 and IL-6 were purchased from Beyotime Biotechnology (Jiangsu, China). Antibodies against IL-12p35 and IL-12p40 were purchased from Cell Signaling (Beverly, MA USA). The ECL detection system was purchased from Santa Cruz Biotechnology (CA, USA).

Preparation and identification of POL-P

Dried *Portulaca oleracea* L. samples (100 g) were ground and refluxed with petroleum ether (1:5, v/v). The residue was extracted with 80% ethanol at 90℃ for 2 h. After drying at room temperature, the filtered residue was extracted with distilled water for reflux three times at 70℃. The filtrate was mixed, centrifuged at 5000 r/min for 10 min, and the resulting supernatants were concentrated. The concentrated extract was coagulated with anhydrous ethanol overnight at 4℃. The precipitates were washed twice with anhydrous ethanol, 95% ethanol and acetone respectively, and then dried in vacuum to obtain the crude polysaccharides. The protein was removed by trichloroacetic acid-n-butanol and the impurity was removed by dialysis. Deproteined polysaccharide samples (5 mg) were dissolved in 1 mL of 0.15 M NaCl aqueous solution and centrifuged at 10000 r/min for 5 min. Then, the supernatants were loaded on a gel chromatography column. The column was eluted with 0.15 M NaCl aqueous solution at a flow rate of 0.15 mL/min and collected by an automatic collector for 20 min/ tube. Next, the fractions were purified by Sephadex G-25, G-15, and G-10 columns to remove other impurities. Finally, the content of carbohydrate was determined by the phenol-sulfuric acid method and uronic acid was determined by m-Hydroxybiphenyl method. The protein content was determined by Bradford method.

The average molecular weight of POL-P was determined using gel permeation chromatography (GPC). POL-P was applied to a Sepharose CL-6B column (2.0 × 90 cm) and eluted with 0.15 M NaCl at the flow rate of 0.5 mL/min. The calibration curve was established using Dextran standards (known molecular weights: 2000,000; 200,000; 70,000; 40,000; Glc).

The organic functional groups of the POL-P were analyzed using infrared spectroscopy. The purified POL-P was combined with a certain amount of potassium bromide and ground into pressure film for infrared spectrum measurement.

The monosaccharide composition of POL-P was analysis. In short, 2 mg polysaccharide samples were added to 0.5 mL anhydrous methanol solution containing 1 M hydrochloric acid, and hydrolyzed at 80℃ for 16 hours. After drying under an air pump, 2 M trifluoroacetic acid was added, and hydrolyzed at 120℃ for 1 hour. Derivatization was performed using 0.5 mL 1-phenyl3-methyl-5-pyrazolone (PMP) in the sample obtained from complete acid hydrolysis. Subsequently, 1 mL trichloromethane was added, mixed, then the excess PMP reagent was extracted. The trichloromethane layerwas removed by centrifugation. The water layer was filtered by a 0.22 μm filtration membrane and diluted with distilled water. The monosaccharide composition of POL-P was determined using the HPLC with standard monosaccharides (xylose, galacturonic acid, glucose, arabinose, galactose, fucose, glucuronic acid, mannose, rhamnose.). The operation was performed using the Shimadzu HPLC System (LC-10ATVP Pump and SPD-10AVD Uv Detector) and DIKMA Inertsil ODS-3 column (4.6 × 150 mm). The mobile phase was PBS (0.1 M, pH 7.0) - acetonitrile 82:18 (V/V) at a rate of 1.0 mL/min, the injection volume was 20 μL, and the detection wavelength was 245 nm.

Administration and grouping

BALB/c mice were provided by the Experimental Animal Center of Changchun. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the National Health and Family Planning Commission of the People's Republic of China. The experiments were approved by the Animal Ethics Committee of the HeiLongJiang BaYi Agricultural University. The animals were randomly divided into four groups with 10 mice for each group, including control (normal saline), low dose of POL-P (2 μg/mL), medium dose of POL-P (10 μg/mL) and high dose of POL-P (50 μg/mL). All the experimental animals were orally administered with 0.2 mL/10 g body weight of a solution once daily for 4 days. Type O FMD vaccine (200 μL per mouse) was injected subcutaneously through the groin after the last administration for 24 h, and the same method was used again to strengthen immune on the 15 th day.

Isolation and purification of bone marrow-derived DCs

After the last immunization, all the mice were euthanized using ether anesthesia. Bone marrow was harvested from the femurs of experimental mice. The bone marrow cells were collected and the supernatant was discarded, then the precipitated cells were washed twice with PBS and resuspended with RPMI 1640 containing 20 ng/mL IL-4 and 50 ng/mL GM-CSF. The cell suspension was gently added to the fresh medium and non-adherent cells were removed on the third day. Thereafter the nutrient medium was altered every 3 days. On the seventh day, the morphologic and quantitative changes of DCs were observed under an inverted microscope. DCs were collected and the positive rate of CD11c was detected by flow cytometry. CD11c is a characteristic marker molecule on DCs surface, which represents the purity of DCs.

Phenotype analysis of DCs by Flow Cytometry

CD11c+ DCs were collected and seeded into 24-well flat bottom multiwell at a density of 1×10^5 cells per well for 24 h at 37℃. For surface molecules expression determination, DCs were stained with extra FITC-conjugated and PE-conjugated antibodies against CD80, CD83 and CD86, and analyzed using FACS Caliber multicolor flow cytometer.

Qualitative real-time PCR (qRT-PCR)

The mRNA expression levels of MHC-I and MHC-II were detected by qRT-PCR. RNA from DCs of experimental groups was extracted with Trizol® Reagent following the manufacturer's instructions (Invitrogen). After RNA quantification and purity identification were performed, the product was identified by agarose gel electrophoresis. Then, RNA was reverse-transcribed with a SuperScript® IV first-strand synthesis system into cDNA. The expression levels of MHC-I and MHC-II were detected by qRT-PCR (SYBR Green method) with cDNA as template under specific primers, and the housekeeping gene GAPDH was used as control. Target gene mRNA was analyzed using a Bio-Rad CFX384 real-time PCR detection system (Bio-Rad), and relative gene expression was calculated *via* the $2^{-\Delta\Delta CT}$ method. The amplification primers are as following: MHC-I (For. 5' ATCGCTGTCGGCTATGTG 3'; Rev. 5' CCCTTGGCTTTCTGTGTCTC 3'), MHC-II (For. 5' GGAAACTCGGATACTAAATAGG 3'; Rev. 5' ACTGAGGCAGAATTAACAAG 3'), and GAPDH (For. 5' CTGCCCAGAACATCATCC 3'; Rev. 5' CTCAGATGCCTGCTTCAC 3').

Cytokine release detection

The obtained DCs were seeded into culture plates, and the negative control was set at the same time. After 48 hours of continuous culture, the cells were centrifuged, and the supernatants were collected. The cytokines levels of IL-12, IFN-γ, IL-1β, IL-8, TNF-α and IL-6 were detected by ELISA kit. The operation was conducted in strict accordance with the instructions. According to the standard curve, the contents of each index in the samples were calculated respectively.

Western blotting

The extracted protein was separated by SDS-PAGE and then transferred to PVDF membrane. After the membrane was blocked with 5% skim milk for 1 h at 37℃, IL-12p35 and IL-12p40 primary antibodies were incubated at 4℃ overnight. Subsequently, membranes were washed with TBST and incubated with the HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Protein signals were visualized by using an ECL detection system (SantaCruz, CA, USA).

Immunohistochemistry

The expressions of TLR2 and TLR4 receptors and the downstream molecules of MyD88 and NF-κB were identified by immunohistochemistry. DCs were routinely prepared and stained according to the instructions of SABC immunohistochemical kit. Five visual fields were randomly selected for each slide. Image analysis using Olympus image analyzer, and the expression intensity of TLR2, TLR4, MyD88 and NF-κB was represented by average optical density (OD).

Chromatin immunoprecipitation assay (ChIP assay)

DCs were incubated at 37℃ for 6 h, and then cells were collected. ChIP assays were operated according to the kit instructions. Briefly, DCs were treated with medium added 1% formaldehyde for 10 min at 37℃. After DCs were incubated with glycine for 5 min, the medium was discarded and the cells were washed twice with cold PBS. Chromatin was sonicated to an average size of 250 bp. Immune complexes were collected using protein-A magnetic beads (SantaCruz, CA, USA) and washed with ChIP dilution buffer, high salt buffer, LiCl buffer, and TE buffer, respectively. The samples were decrosslinked, and then DNA was purified using the Qiagen PCR purification kit (Qiagen, Mississauga, Ca). The PCR assay was performed with primers of the IL-12p35 and IL12-p40 promoter. In this experiment, positive and negative controls were set. The extracted DNA was used as a template, and the target genes were detected using the primer PCR showed in the table below.

Primer	Sequence	Size (bp)
p IL-12p35 F	5' ATCTGTATCCCAGCCTCCAC 3'	733
p IL-12p35 R	5' GCCAAGCCAATAAGAAGGAC 3'	
p IL-12p40 F	5' CAAGAAAACATGGGGAAAGG 3'	542
p IL-12p40 R	5' TTAGCGACAGGGAAGAGGAG 3'	

Transmission electron microscope (TEM) analysis

Ultrastructure of DCs was observed by TEM. The cultured DCs were fixed with 2% osmium acid for 1 h. After washing, dehydration, displacement, impregnation, embedding polymerization and slicing sequentially, the cells were stained finally with uranium acetate for 1 h. The samples were dried at room temperature, sealed, and the ultrastructure of DCs were observed under TEM.

Hematoxylin-hosin staining

For histopathological observation of liver, kidney and small intestine in mice treated with different doses of POL-P, the tissues were fixed in PBS buffer containing 10% formalin (pH 7.4) for over 24 h and then embedded in paraffin. Thin sections (5 μm) were prepared using a microtome (RM2235, Leica) and stained with hematoxylin and eosin. The sections were examined for morphology under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

All experiments were performed at least in triplicate. SPSS/13.0 software was applied to analyze all data, which were expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). Significant differences were considered at *P*-values of less than 0.05 ($p < 0.05$).

Abbreviations

POL-P
Portulaca oleracea L. Polysaccharides
DCs
Dendritic cells
APC
antigen presenting cell
FMD
foot and mouth disease
ChIP
chromatin immunoprecipitation
Mw
molecular weight
TCM
Traditional Chinese medicines
CTL
cytotoxic lymphocyte
PMP
1-phenyl3-methyl-5-pyrazolone
TEM
Transmission electron microscope.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

RZ conceived the study. XZ, HZ and RZ analyzed the data. XZ and HZ wrote the manuscript for submission. GJ, LL, TL ,YL and XQ participated in the design of the study, performed the data collection and analysis, and commented on the manuscript. All the authors approved the final manuscript.

Funding

This work is supported by the Chinese National Natural Science Foundation (31772789), the Innovation and Entrepreneurship Training Program for College Students of Heilongjiang Province of China (201810223005); the Innovative Research Project for Gaduate Students of Heilongjiang Bayi Agricultural University (YJSCX2021-Y103).

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Animal Experiments Committee of the Heilongjiang Bayi Agricultural University (registration protocol 201801007).

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author details

[1]College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China. [2]Centers for Disease Control and Prevention, Daqing, Heilongjiang Province, China. [3]College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing, China

References

1. Kim WS, Zhi Y, Guo H, Byun EB, Lim JH, Seo HS. Promotion of Cellular and Humoral Immunity against Foot-and-Mouth Disease Virus by Immunization with Virus-Like Particles Encapsulated in Monophosphoryl Lipid A and Liposomes. Vaccines. 2020;8:633–47.
2. Diaz-San Segundo F, Medina GN, Stenfeldt C, Arzt J, de Los Santos T. Foot-and-mouth disease vaccines. Vet Microbiol. 2017;206:102–12.
3. Qi F, Zhao L, Zhou A, Zhang B, Li A, Wang Z, et al. The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. Biosci Trends. 2015;9:16–34.
4. Feng H, Du X, Tang J, Cao X, Han X, Chen Z, et al. Enhancement of the immune responses to foot-and-mouth disease vaccination in mice by oral administration of a novel polysaccharide from the roots of Radix Cyathulae officinalis Kuan (RC). Cell Immunol. 2013;281:111–21.
5. Wang Y, Xu Y, Ma X, Liu X, Yang M, Fan W, et al. Extraction, purification, characterization and antioxidant activities of polysaccharides from Zizyphus jujuba cv. Linzexiaozao. Int J Biol Macromol. 2018;118:2138–48.
6. Iranshahy M, Javadi B, Iranshahi M, Jahanbakhsh SP, Mahyari S, Hassani FV, et al. A review of traditional uses, phytochemistry and pharmacology of Portulaca oleracea L. J Ethnopharmacol. 2017;205:158–72.

7. Zhao R, Gao X, Cai Y, Shao X, Jia G, Huang Y, et al. Antitumor activity of Portulaca oleracea L. polysaccharides against cervical carcinoma in vitro and in vivo. Carbohydr Polym. 2013;96:376–83.
8. Farkhondeh T, Samarghandian S. The therapeutic effects of Portulaca oleracea L. in hepatogastric disorders. Gastroenterol Hepatol. 2019;42:127–32.
9. Li YH, Lai CY, Su MC, Cheng JC, Chang YS. Antiviral activity of Portulaca oleracea L. against influenza A viruses. J Ethnopharmacol. 2019;241:112013.
10. Zhao R, Meng X, Jia G, Yu Y, Song B. Oral pre-administration of Purslane polysaccharides enhance immune responses to inactivated foot-and-mouth disease vaccine in mice. BMC Vet Res.2019;15(1):38.
11. Galamb O, B Molnár, Tulassay Z. Immunobiology of dendritic cells and their application in clinical practice. Orv Hetil. 2003;144(38):1873–879.
12. Ho NI, Huis In 't Veld LGM, Raaijmakers TK, Adema GJ. Adjuvants Enhancing Cross-Presentation by Dendritic Cells: The Key to More Effective Vaccines?. Front Immunol. 2018;9:2874.
13. Karmakar P, Pujol CA, Damonte EB, Ghosh T, Ray B. Polysaccharides from Padina tetrastromatica: Structural features, chemical modification and antiviral activity. Carbohydr Polym. 2010;80:513–20.
14. Li Q, Xie Y, Su J, Ye Q, Jia Z. Isolation and structural characterization of a neutral polysaccharide from the stems of Dendrobium densiflorum. Int J Biol Macromol. 2012;50:1207–11.
15. Cao Y, Zhu X, Hossen MN, Kakar P, Zhao Y, Chen X. Augmentation of vaccine-induced humoral and cellular immunity by a physical radiofrequency adjuvant. Nat Commun. 2018;9:3695.
16. Feng L, Ma L, Tian Y, Zhang Y. Alterations of dendritic cell subsets and TH1/TH2 cytokines in the peripheral circulation of patients with superficial transitional cell carcinoma of the bladder. J Clin Lab Anal. 2012;26:365–71.
17. Estes DM, Brown WC. Type 1 and type 2 responses in regulation of Ig isotype expression in cattle. Vet Immunol Immunopathol. 2002;90:1–10.
18. Bourque J, Hawiger D. Immunomodulatory Bonds of the Partnership between Dendritic Cells and T Cells. Crit Rev Immunol. 2018;38:379–401.
19. Gee K, Guzzo C, Che Mat NF, Ma W, Kumar A. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflamm Allergy Drug Targets. 2009;8:40–52.
20. Hasegawa H, Mizoguchi I, Chiba Y, Ohashi M, Xu M, Yoshimoto T. Expanding Diversity in Molecular Structures and Functions of the IL-6/IL-12 Heterodimeric Cytokine Family. Front Immunol. 2016;7:479.
21. Schultze JL, Aschenbrenner AC. Systems immunology allows a new view on human dendritic cells. Semin Cell Dev Biol. 2019;86:15–23.
22. Rodríguez-Ubreva J, Garcia-Gomez A, Ballestar E. Epigenetic mechanisms of myeloid differentiation in the tumor microenvironment. Curr Opin Pharmacol. 2017;35:20–9.
23. Boukhaled GM, Corrado M, Guak H, Krawczyk CM. Chromatin Architecture as an Essential Determinant of Dendritic Cell Function. Front Immunol. 2019;10:1119.

24. Wang L, Beier UH, Akimova T, Dahiya S, Han R, Samanta A, et al. Histone/protein deacetylase inhibitor therapy for enhancement of Foxp3+ T-regulatory cell function posttransplantation. Am J Transplant. 2018;18:1596–1603.
25. Anderson EM, Penrod RD, Barry SM, Hughes BW, Taniguchi M, Cowan CW. It is a complex issue: emerging connections between epigenetic regulators in drug addiction. Eur J Neurosci. 2019;50:2477–91.
26. Takeda K, Akira S. Toll-like receptors. Curr Protoc Immunol. 2007;Chapter 14 4:.
27. Takeda K, Akira S. TLR signaling pathways. Semin Immunol. 2004;16:3–9.
28. Majer O, Liu B, Barton GM. Nucleic acid-sensing TLRs: trafficking and regulation. Curr Opin Immunol. 2017;44:26–33.
29. Gan F, Liu Q, Liu Y, Huang D, Pan C, Song S, et al. Lycium barbarum polysaccharides improve CCl4-induced liver fibrosis, inflammatory response and TLRs/NF-kB signaling pathway expression in wistar rats. Life Sci. 2018;192:205–12.
30. Wu YG, Wang KW, Zhao ZR, Zhang P, Liu H, Zhou GJ, et al. A novel polysaccharide from Dendrobium devonianum serves as a TLR4 agonist for activating macrophages. Int J Biol Macromol. 2019;133:564–74.
31. Zhou L, Liu Z, Wang Z, Yu S, Long T, Zhou X, et al. Astragalus polysaccharides exerts immunomodulatory effects via TLR4-mediated MyD88-dependent signaling pathway in vitro and in vivo. Sci Rep. 2017;7:44822.

Figures

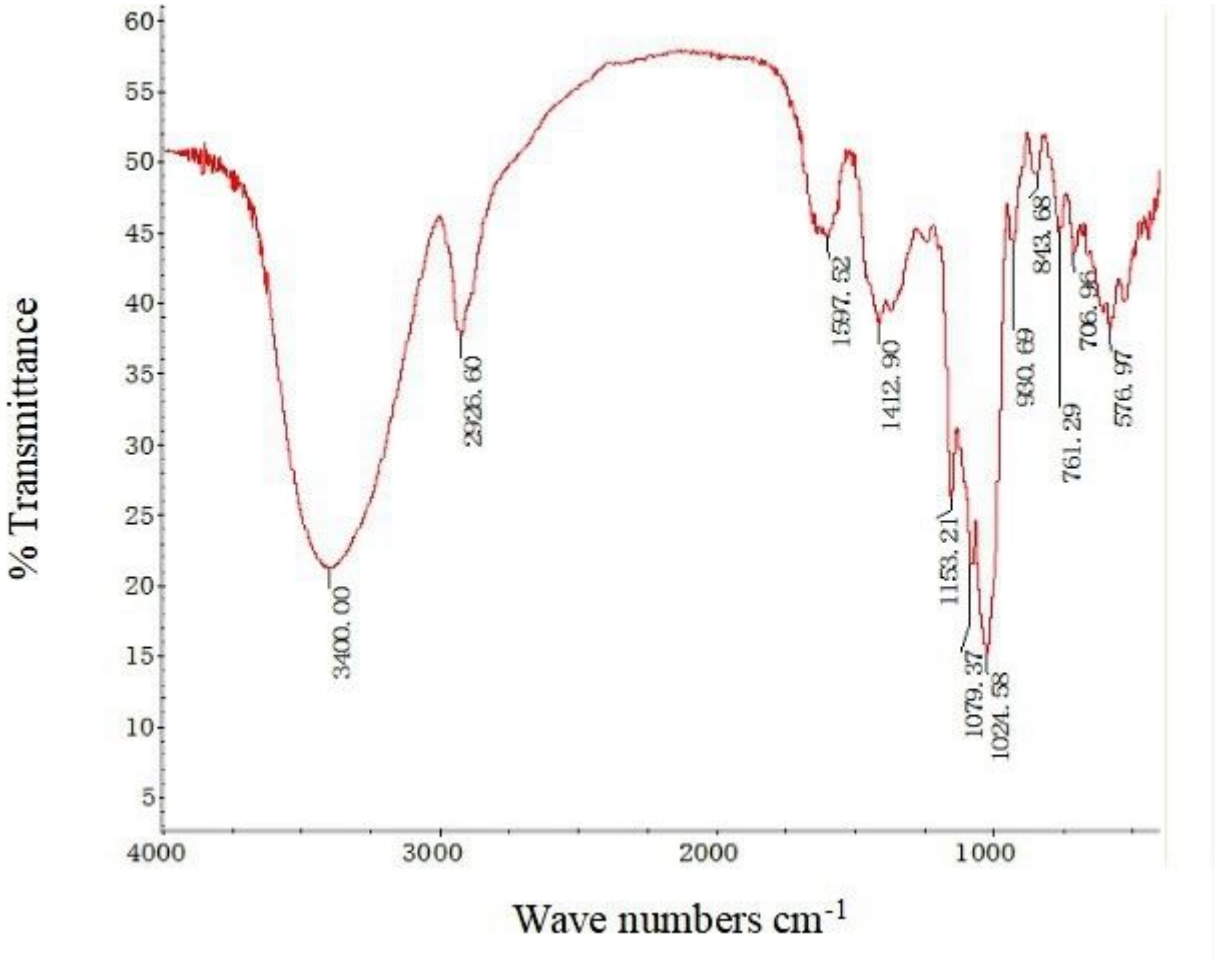

Figure 1

FTIR spectra of POL-P.

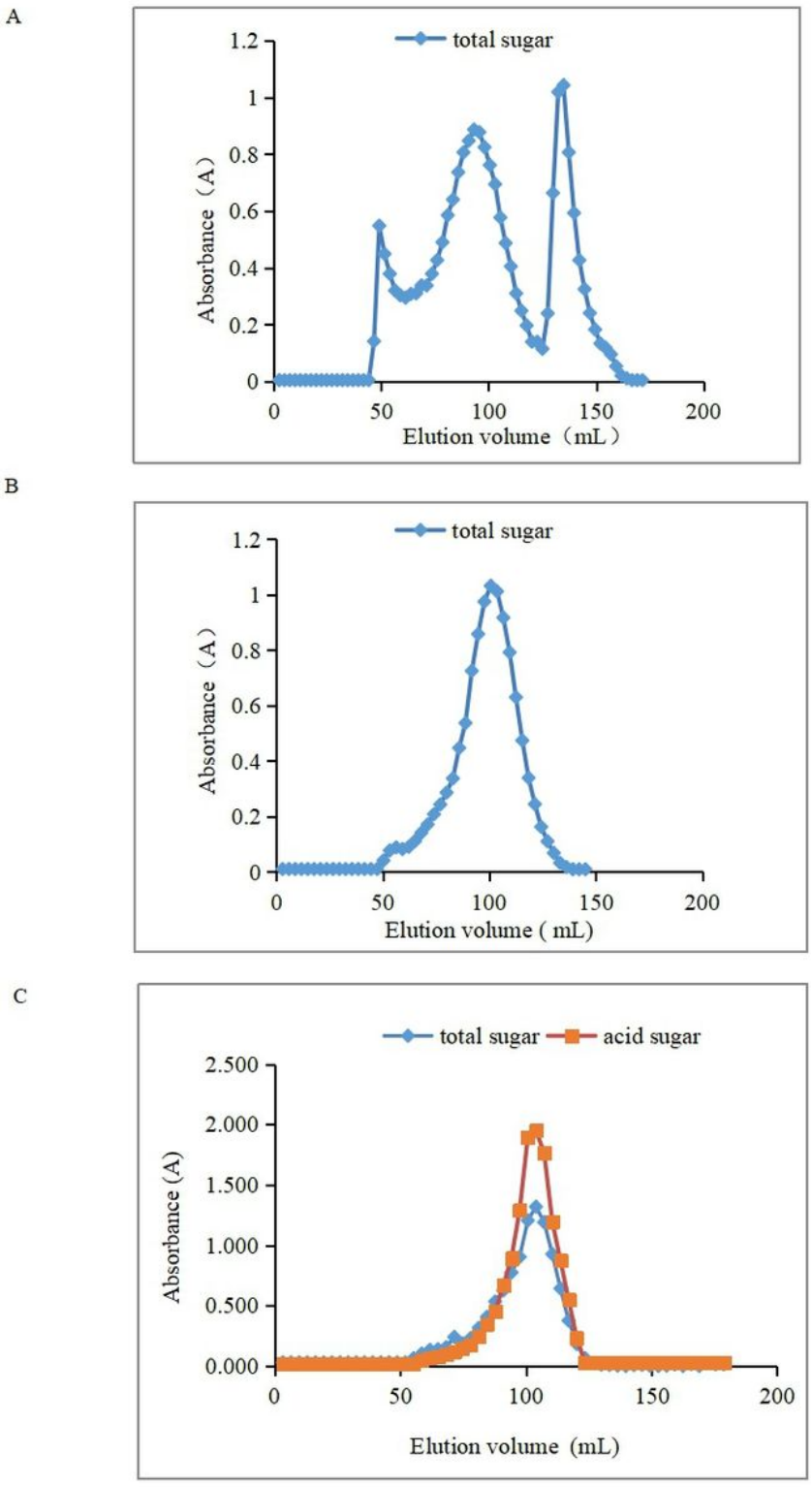

Figure 2

Chromatographic elution curve (A) Chromatographic elution curve of sepharose CL-6B for sugar standard (200 W, 7 W, Glc). (B) Chromatographic elution curve of sepharose CL-6B for sugar standard (20 W, 4 W). (C) Chromatographic elution curve of sepharose CL-6B for POL-P.

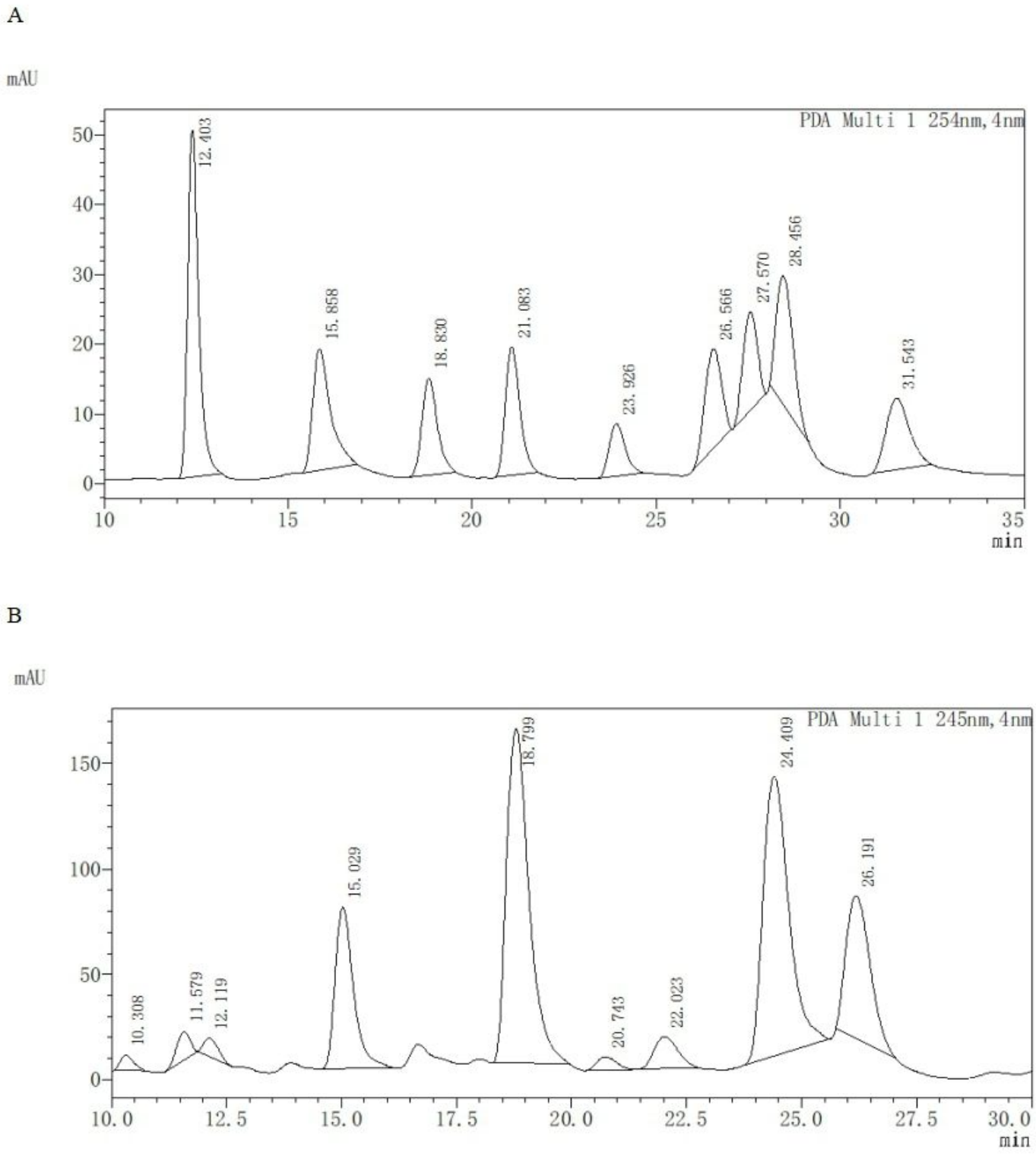

Figure 3

Analysis of the monosaccharide composition of POL-P (A) The monosaccharide reference substance. (B) The monosaccharide composition of POL-P.

Figure 4

Flow cytometry detection for positive rate of CD11c.

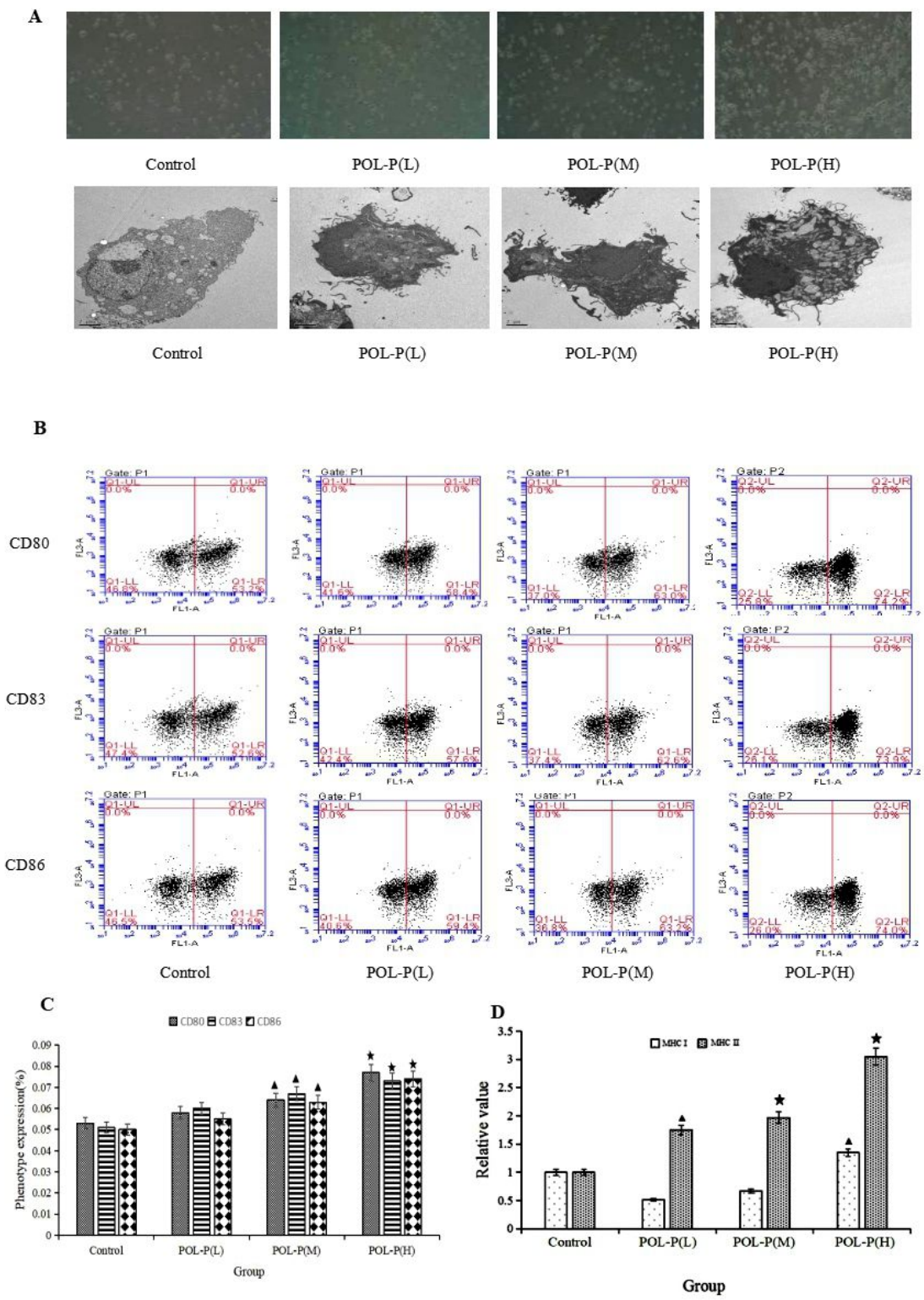

Figure 5

Effect of POL-P on DCs maturation obtained from mice immunized with FMD inactivated vaccine (A) Assessment of DCs maturation by light microscopy (10×20) and transmission electron microscope (5000 times). (B) Phenotypic characterization of DCs were analyzed by flow cytometry. (C) The quantitated

values represent the mean ± S.D. ▲P<0.05;P<0.01, compared with control. (D) Assessment of DCs maturation by the mRNA expression levels of MHC-I and MHC-II.

Figure 6

Effect of POL-P on cytokines levels of DCs obtained from mice immunized with FMD inactivated vaccine. The culture supernatants of DCs were collected and analyzed by ELISA to determine production levels of IL-12, IFN-r, TNF-α, IL-1β, IL-8 and IL-6 production. ▲*P*<0.05;*P*<0.01, compared with control.

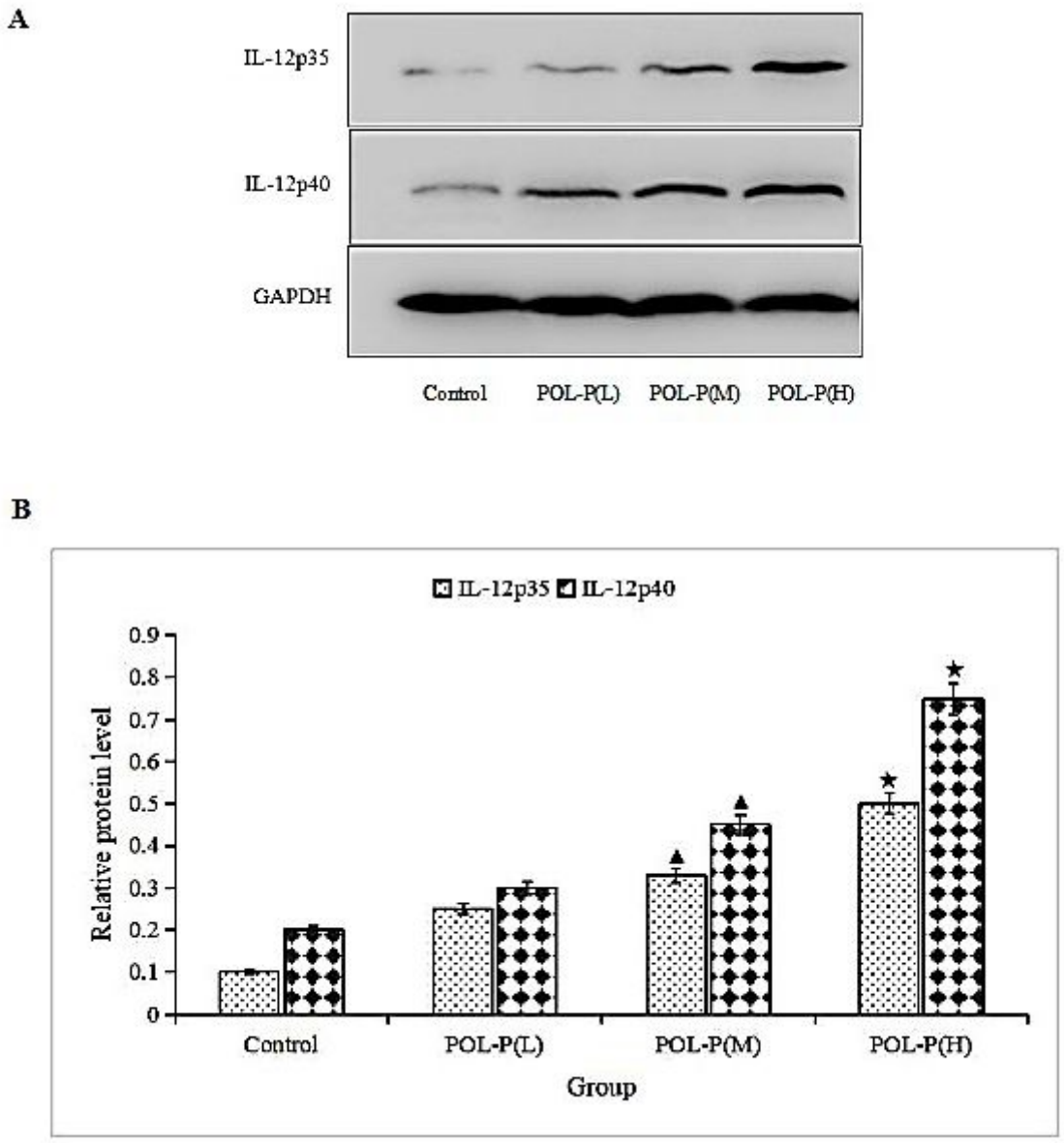

Figure 7

Effects of POL-P on the expression of IL-12p35 and IL-12p40 in DCs obtained from mice immunized with FMD inactivated vaccine. (A) Expression of IL-12p35 and IL-12p40 was analyzed by western blot. (B)

Statistical bar graph of expression of IL-12p35 and IL-12p40 protein. ▲$P<0.05$; $P<0.01$, compared with control.

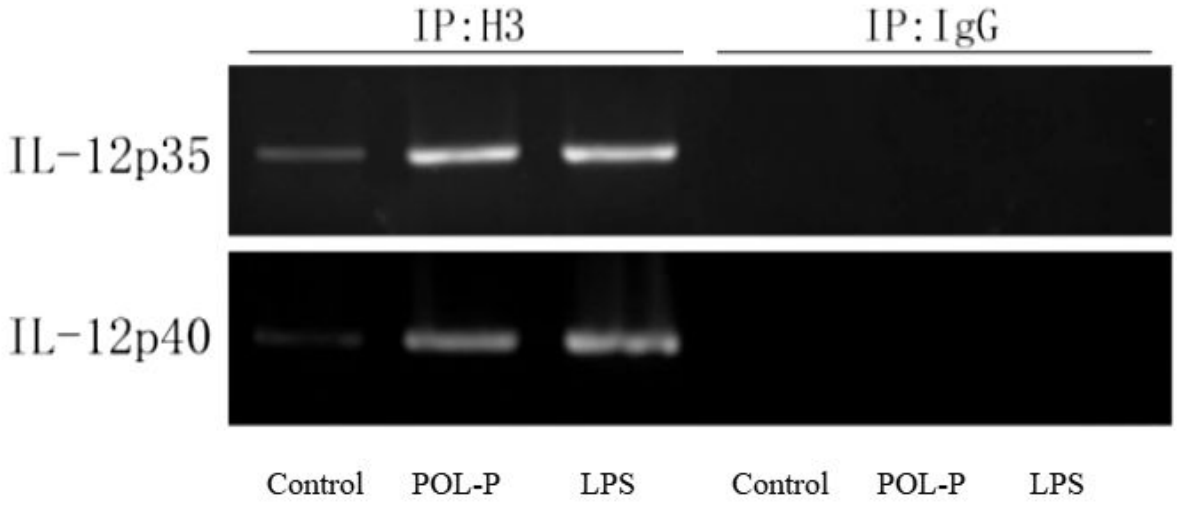

Figure 8

Effect of POL-P on histone H3 acetylation levels in the IL-12p35 and IL-12p40 promoter regions of DCs obtained from mice immunized with FMD inactivated vaccine by chromatin immunoprecipitation (ChIP) assay.

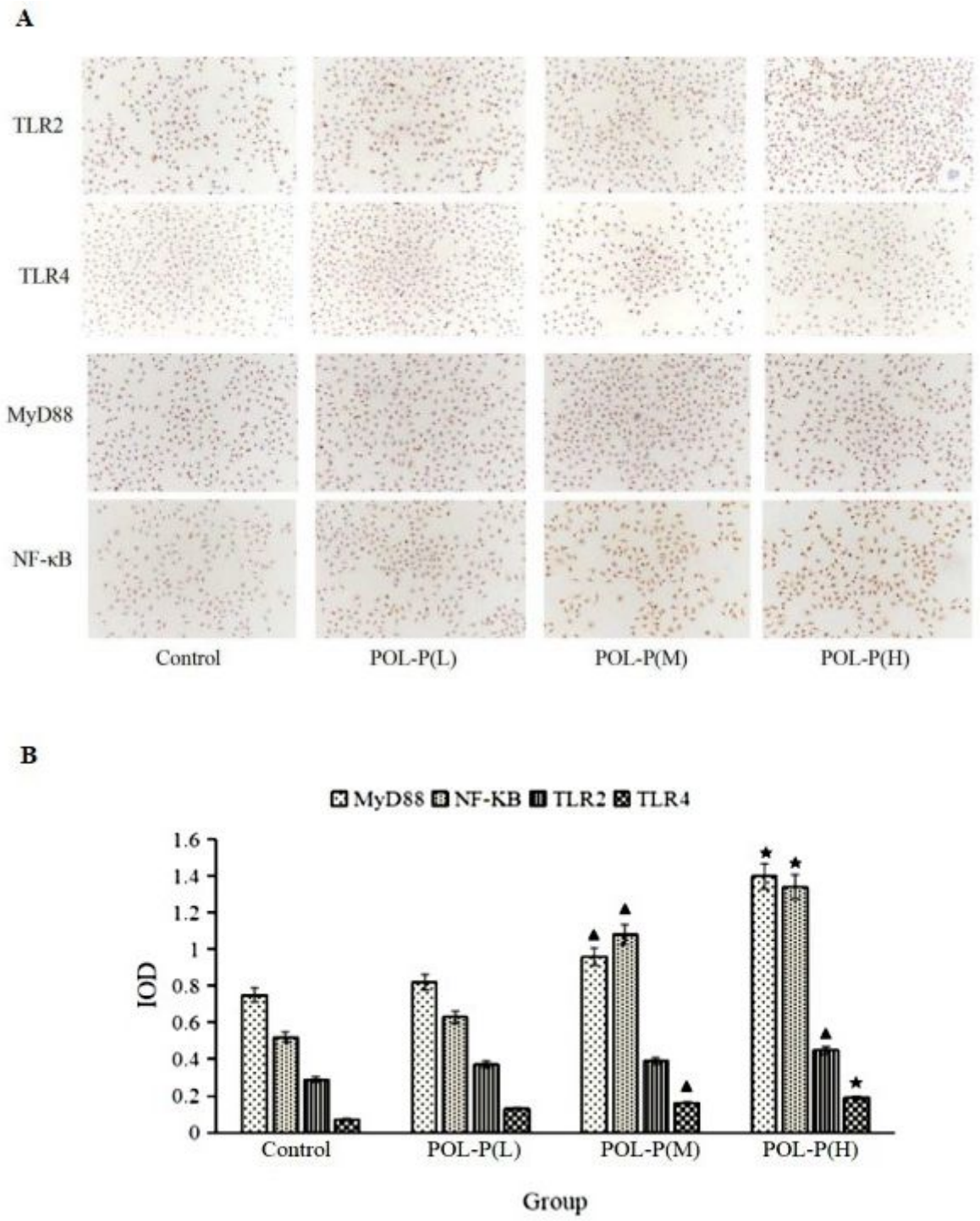

Figure 9

Effect of POL-P on TLRs/NF-κB related proteins (A) The protein expression levels of TLR2, TLR4, MyD88 and NF-kB in mature DCs were detected by immunohistochemistry. (B) Statistical bar graph of expression of TLR2, TLR4, MyD88 and NF-κB. ▲P<0.05;▯P<0.01, compared with control.

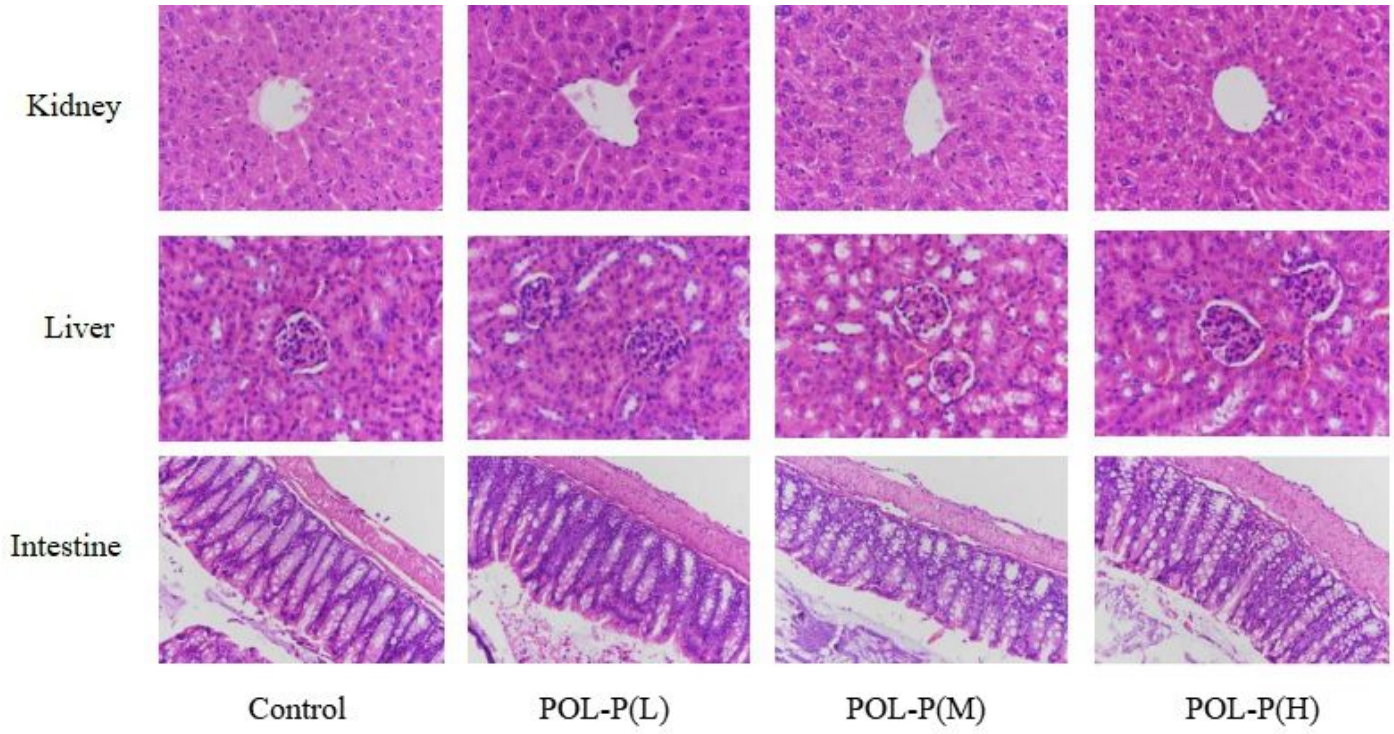

Figure 10

Histology of hematoxylin- and eosin-stained sections of kidney, liver and intestine in mice treated by oral administration with POL-P (20×10).