Carbohydrate Polymers 82 (2010) 680–686

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effects of astragalus polysaccharide on the immune response to foot-and-mouth disease vaccine in mice

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ARTICLE INFO

Article history:
Received 28 March 2010
Received in revised form 10 May 2010
Accepted 18 May 2010
Available online 24 May 2010

Keywords:
Astragalus polysaccharide
Oral administration
FMDV
Adjuvant

ABSTRACT

This study was conducted to evaluate the effects of astragalus polysaccharide (APS) on the immune responses of mice immunized with foot-and-mouth disease virus (FMDV) vaccine. BALB/c mice were randomly divided into five groups and 1 day before being immunized with FMDV vaccine, each group was orally administered APS at doses of 0, 0.125, 0.25, 0.5 and 1 mg, respectively. The cellular and humoral responses, including phagocytic capacity of peritoneal macrophage, the maturation of dendritic cells (DCs), splenic lymphocyte proliferation, cytokine production and antibody variation, were evaluated by a series of experiments after vaccination. The data showed that oral administration of APS significantly enhanced the phagocytic capacity of peritoneal macrophage, splenic lymphocyte proliferation, serum antibody titer, and the production of IL-4 and IL-10 in groups administered with 0.125–0.5 mg APS. The expression of CD40, CD86 and MHC-II on DCs was only slightly inhibited in mice given a low dose of APS, but was significantly enhanced in mice given higher doses. Furthermore, mice given the highest doses of APS showed markedly increased CD40 and MHC-II expression levels. These results suggest that orally administered APS could significantly enhance the efficacy of FMDV vaccination and has important implications for the further use of APS as a novel adjuvant.

1. Introduction

Foot-and-mouth disease (FMD) is a kind of acute, febrile, high contagious and sometime fatal viral disease of cloven-hoofed animals, and has spread throughout most of the world (Kitching, Hutber, & Thrusfield, 2005; Ward, Highfield, Vongseng, & Garner, 2009). Each outbreak of FMD usually results in severe economic losses and has a considerable impact on both national and international trade in live animals and animal products (Jamal et al., 2008; Zhang, Ahmed, Paton, & Bashiruddin, 2009).

To date, widespread vaccination with inactivated foot-and-mouth disease virus (FMDV) is the only practical means to control the epidemic in most developing countries (Shi, Wang, & Wang, 2007). Compared with other new generations of vaccines, due to the advantages of easy to prepare, low cost, and high immunogenicity, inactivated vaccine is still the best choice for FMD prevention. However, disadvantages such as genotoxic potential, short protecting duration and lacking of cellular immunity also exist (Jin et al., 2005; Shi, Wang, Zhang, & Wang, 2006; Song & Hu, 2009), and often result in failure to elicit effective immune responses by vaccination (Li et al., 2008). The use of adjuvant has solved this problem to some extent by exerting its immunomodulatory effect on the response of vaccination (McKee, Munks, & Marrack, 2007). But for the currently available FMDV vaccines, most of them use aluminum hydroxide or oil emulsions as adjuvant, and often cause side effects such as strong local stimulation and carcinogenesis. Also, they have been reported to induce poor immune response. Therefore, it is necessary to develop new generations of adjuvant with high efficiency, low toxicity and extensive resource.

Medicinal herbs have been used as adjuvant for thousands of years in eastern countries (Wang et al., 2009). Growing evidence has proved that medicinal herbs and ingredients possess immune-enhancing effects, and many investigators have tried to

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doi:10.1016/j.carbpol.2010.05.030

use medicinal herbs as adjuvant to vaccines and have made much progress (Gao et al., 2007; Kong, Hu, Rui, Wang, & Li, 2004; Yuan et al., 2008). As one of the widely used medicinal herbs, astragalus polysaccharide (APS) is well-known for its anti-inflammatory (Joshua & Cecilia, 2009), anti-virus (Wang et al., 2010), anti-tumor (Cho & Leung, 2007a) and renal protective actions (Li & Zhang, 2009). In recent years, attention has been focused on the immunomodulatory functions of APS both in humans and animals (Brush et al., 2006; Cho & Leung, 2007b; Huang et al., 2009; Li, Chen, Wang, Tian, & Zhang, 2009). Researchers have found that APS can stimulate T-cell proliferation (Shao et al., 2004), promote the express of cell surface antigens on lymphocytes (Song, Kobayashi, Xiu, Hong, & Cyong, 2000; Shao, Zhao, Zhi, & Pan, 2006), increase serum antibody titer, and enhance secretion of a broad range of cytokines (Chu, Yan, Li, & Hu, 2006; Lee et al., 2003). However, until this study no reports had been carried out on whether this herb has similar immune stimulant effects to vaccination against FMDV, or on how APS exerts its effects on both cellular and humoral immune responses of FMDV vaccine in mice.

In this research, we attempted to evaluate the effect of APS on both cellular and humoral immune responses of FMDV vaccine using a series of experiments. The results proved that oral administration of APS can significantly promote cellular and humoral response by enhancing the capacity of phagocytes, lymphocyte maturing and splenic lymphocyte proliferation, as well as the expression of cytokines and the increasing of serum antibodies.

2. Materials and methods

2.1. Mice

Female BALB/c mice at 5–6 weeks of age, weighing 18–22 g, were purchased from Beijing Laboratory Animal Center (Beijing, China). The mice were kept in polypropylene cages in an air-conditioned room at $24 \pm 1\,^{\circ}C$ with a 12 h light cycle and fed pathogen-free food and water. All the procedures were performed in strict accordance with internationally accepted principles and the PR China legislation on the use and care of laboratory animals.

2.2. Materials

RPMI-1640 medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG were purchased from Sigma (St. Louis, MO, USA). Concanavalin A (Con A; Sigma, St. Louis, MO, USA), a T-cell mitogen, was prepared at 10 μg/mL in RPMI-1640. Lipopolysaccharide (LPS; Sigma, St. Louis, MO, USA), a B-cell mitogen, was prepared at 5 μg/mL in RPMI-1640. FMDV O-serotyped inactivated vaccine in oil emulsion was acquired from Lanzhou Veterinary Research Institute (Lanzhou, China). Trizol was obtained from Invitrogen (Carlsbad, CA, USA). Goat anti-mouse monoclonal anti-CD11c-FITC, anti-CD40-PE, anti-CD80-PE, anti-CD86-PE and anti-MHC-II-PE antibodies were purchased from eBioscience (San Diego, CA, USA). An ELISA kit coated with FMDV O-type VP1 gene expression antigen to detect antibody against FMDV was from Wuhan Keqian Animal Biological Products (Wuhan, China).

2.3. Astragalus polysaccharide

Astragalus polysaccharide injectable powder (No. HD006001) was provided by Beijing Medicass Biotechnologies (Beijing, China). The polysaccharide preparation (APS content > 60%) was measured and determined by a standard high-performance liquid chromatography-refractive index detection method employed by the Chinese State Drug Administration (WS-330 (Z-029)-2001). APS powder was purified as previously described (Li et al., 2009) and the resultant polysaccharides were freeze-dried and stored in a vacuum desiccator until used.

2.4. Immunization and oral administration of APS

BALB/c mice were randomly divided into five groups ($n = 21$/group), including a vaccine-only control group (VC, 0.0 mg kg^{-1} APS administrated), a 6.25 mg kg^{-1} APS group ($APS_{6.25}$), a 12.5 mg kg^{-1} APS group ($APS_{12.5}$), a 25 mg kg^{-1} APS group (APS_{25}) and a 50 mg kg^{-1} APS group (APS_{50}). One day before each immunization, the mice were orally administered 1.0 mL saline solution containing 0.0, 0.125, 0.25, 0.5 or 1.0 mg APS (equivalent to 0.0, 6.25, 12.5, 25, 50 mg kg^{-1} body weight, respectively), accordingly. All groups of mice were immunized twice subcutaneously at 14-day intervals with 200 μL FMDV O-serotyped inactivated vaccine.

2.5. Phagocytosis assay

One day 3 after the first immunization, three mice from each group were intraperitoneally injected with 1.0 mL starch solution, after 24 h, 1.0 mL of 1% chicken red blood cells had been injected by the same way. Half an hour later, a drop of peritoneal fluid was stained with Wright's stain and observed under a high-power microscope. The results are expressed as the percentage of phagocytic cells (PP) and the phagocytic index (PI), where PP was defined as the percentage of peritoneal macrophage (PM) that ingested one or more chicken red blood cells, PI was defined as the average number of chicken red blood cells ingested per PM and was calculated by dividing the total number of ingested chicken red blood cells by the total number of PM (200 cells).

2.6. Flow cytometry assay

Three mice from each group were sacrificed on day 4 after the first vaccination and flow cytometry was carried out as previously described by Su et al. (2008). In brief, single-cell splenocyte suspensions at a concentration of 2×10^6 cells/200 μL were blocked with 2 μL of Fcγ mAb (0.5 μg/mL) for 30 min at $4\,^{\circ}C$. After rinsing once with phosphate-buffered saline (PBS) solution, cells were stained with isotype control, or double-stained with either anti-CD11c-FITC and anti-CD40-PE, anti-CD11c-FITC and anti-CD80-PE, anti-CD11c-FITC and anti-CD86-PE, or anti-CD11c-FITC and anti-MHC-II-PE. The fluorescence intensities were measured using the FACSCalibur machine and analyzed using FlowJo analysis software (Version5.7.2, Tree Star, Ashland, Oregon, USA).

2.7. Splenic lymphocyte proliferation assay in vitro

Three mice from each group were sacrificed on day 7 after the second immunization. Single spleen lymphocytes suspension was prepared by passing through a fine stell mesh and centrifugation and cultured in 96-well plates at 4×10^6 cells/well in RPMI-1640 media. All the cultures were stimulated with ConA (final concentration 10 μg/mL), LPS (final concentration 5 μg/mL), or unstimulated, respectively, for 48 h at $37.5\,^{\circ}C$ and 5% CO_2. Cells were cultured for 44 h and 50 μL (2 mg/mL) MTT was added to detect T-cell proliferation by colorimetric reaction. After the addition of 100 μL DMSO to each well to stop the color development, plates were read at 570 nm by a microtiter plate reader (Bio-Rad, Hercules, CA, USA). The stimulation index (SI) was calculated according to the following equation: SI = OD_{570} stimulated cells/OD_{570} unstimulated cells.

Table 1
Primer sequences and conditions for polymerase chain reaction (PCR) for the detection of cytokine gene expression.

Target genes	Primers	Parameters of PCR	Product size (bp)
IFN-γ	5′-CATTGAAAGCCTAGAAAGTCTG 5′-TGACTCCTTTTCCGCTTCCTGAG	94 °C for 30 s, 59 °C for 30 s, 72 °C for 40 s	382
IL-10	5′-CCAGTTTTACCTGGTAGAAGTGATG 5′-TGTCTAGGTCCTGGAGTCCAGCAGACTCAA	94 °C for 30 s, 62 °C for 30 s, 72 °C for 40 s	324
IL-4	5′-ATGGGTCTCAACCCCCAGCTAGT 5′-GCTCTTTAGGCTTTCCAGGAAGTC	94 °C for 30 s, 66 °C for 50 s, 72 °C for 30 s	399
β-Actin	5′-TGCTGTCCCTGTATGCCTCT-3′ 5′-TCTTTGATGTCACGCACGAT-3′	94 °C for 30 s, 56 °C for 50 s, 72 °C for 30 s	207

2.8. Semi-quantitative RT-PCR for mRNAs of IFN-γ, IL-4 and IL-10

The pattern of cytokine mRNA expression in splenocytes was determined by standard reverse transcription-polymerase chain reaction (RT-PCR). In brief, total RNA isolated from spleen samples (three from each group) was extracted on day 14 after the second vaccination using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription of RNA to form cDNA was performed using AMV reverse transcriptase (Takara Biotechnology, Dalian, China) and an oligo(dT) 16-mer primer. The resulting cDNA was used to amplify a house-keeping gene (β-actin) used as a normalizing control and IFN-γ, IL-4 and IL-10 targets by PCR with specific primer pairs listed in Table 1. The PCR products were electrophoresed on 1.5% agarose gels and photographed under the UV light. The relative amount of mRNA for the cytokine-specific genes was evaluated by comparing the intensities of amplified PCR products.

2.9. Antibody titer analysis

Serum samples, three from each group, were collected on days 0, 7, 14, 21, and 28 after the second vaccination and used for FMDV-specific IgG antibody detection by ELISA, according to the manufacturer's instructions. In brief, mouse sera (100 μL of 1:100 dilution) were added to 96-well microtiter plate coated with FMD O-type VP1 gene expression antigen and incubated for 30 min at 37 °C. Subsequently, 100 μL goat anti-mouse IgG conjugated to HRP was added to each well and incubated for 30 min at 37 °C. The colorimetric detection reaction was developed using tetramethylbenzidine (TMB) reagent (Sigma, St Louis, MO, USA) and stopped by the addition of H_2SO_4. The OD of each well was read at 450 nm in a microtiter plate reader (Bio-Rad, Hercules, CA, USA).

2.10. Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using an independent-sample *t*-test available in the SPSS software (Version12.0, Chicago, USA), where a p-value < 0.05 was considered significant.

3. Results

3.1. Effects of APS on the PM phagocytic function

The effect of APS dose on the phagocytosis by PM is listed in Table 2. Compared with the control vaccine-only VC group, both PP and PI were significantly higher in all APS administered groups (except for the $APS_{6.25}$ group). This effect was especially noteworthy for APS_{25} and APS_{50} groups, which demonstrated the highest PP and PI of all groups. No significant differences of PP and PI were observed between the $APS_{6.25}$, APS_{25} and APS_{50} groups.

3.2. APS enhances the expression of CD40, CD80, CD86 and MHC-II on dendritic cells (DCs)

The effects of APS on CD antigen expression on DCs are shown in Fig. 1. Compared with the VC group, CD80 expression on DCs was slightly decreased for the $APS_{6.25}$ group (Fig. 1B). No significant differences were observed for other APS-treated groups. CD40, CD86 and MHC-II expression was slightly decreased on DCs in the $APS_{6.25}$ group but were not statistically significant (Fig. 1A, C and D). However, CD40, CD86 and MHC-II expression was significantly increased in other groups, with the exception of CD86 in the $APS_{12.5}$ group. Moreover, both the APS_{25} and APS_{50} groups showed a strong upregulation of CD86, CD40 and MHC-II antigen expression, especially for CD40 and MHC-II.

3.3. Splenic lymphocyte proliferation assays

The lymphocyte proliferation response to ConA and LPS treatments are presented in Fig. 2. Seven days after the second vaccination, the stimulation indices of APS administered groups (except the $APS_{6.25}$ group), whether stimulated with ConA and LPS, were significantly higher than that of VC group. Among all groups, the stimulation indexes of APS_{25} and APS_{50} group (highest doses of APS administered groups) were significantly higher than those of $APS_{6.25}$ and $APS_{12.5}$ group.

3.4. IFN-γ, IL-4 and IL-10 mRNA expression in splenocyte

The levels of mRNA expression of the Th1-type cytokine, IFN-γ, and the Th2-type cytokines, IL-4 and IL-10, were determined by RT-PCR (Fig. 3). Compared with the VC group, the expression of IFN-γ in APS administered groups was not significantly different. However, both IL-4 and IL-10 were determined to be significantly upregulated in APS administrated groups, with the exception of IL-4 in the $APS_{6.25}$ group.

Table 2
The effect of astragalus polysaccharide (APS) on the function of peritoneal macrophage (PM).

Groups	PM been observed (number)	PP	PI
VC	200	0.68 ± 0.04	0.82 ± 0.07
$APS_{6.25}$	200	0.67 ± 0.07	0.85 ± 0.11
$APS_{12.5}$	200	0.71 ± 0.09[a]	1.09 ± 0.51[a]
APS_{25}	200	0.77 ± 0.07[a]	1.34 ± 0.16[a]
APS_{50}	200	0.78 ± 0.09[a]	1.31 ± 0.09[a]

Mice were immunized subcutaneously with 200 μg FMDV O-serotyped inactivated vaccine with 14-day intervals and orally administered with 1 mL APS solution (equivalent to 0, 6.25, 12.5, 25 and 50 mg kg^{-1} body weight) one day before each immunization. PP was defined as the percentage of PM that ingested one or more chicken red blood cells, PI was defined as the average number of chicken red blood cells ingested per PM and was calculated by dividing the total number of ingested chicken red blood cells by the total number of PM.

[a] Denotes significant difference (p < 0.05) compared to vaccine-only control group (VC).

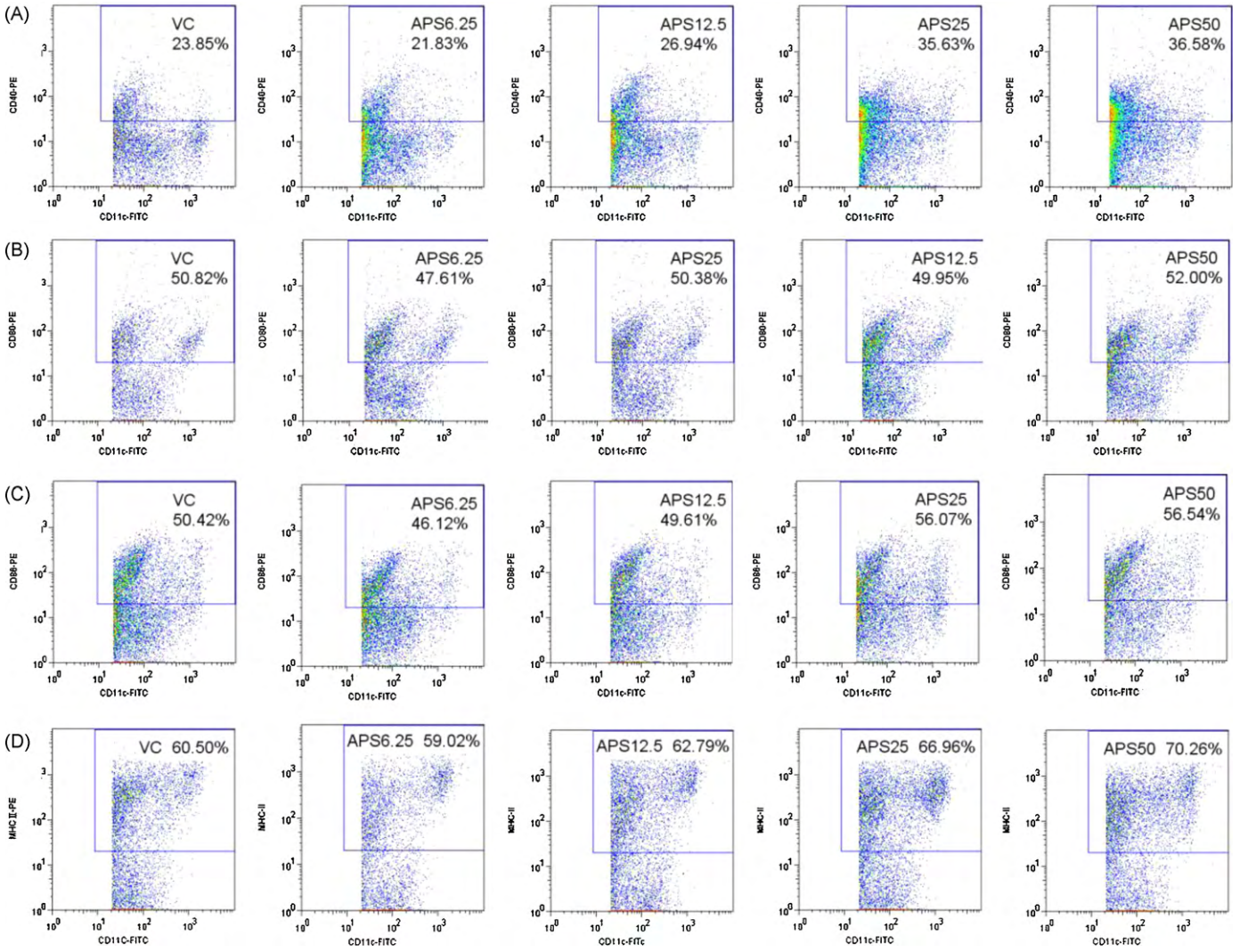

Fig. 1. Flow cytometric analysis of mouse dendritic cells (DCs) for the expression of CD40, CD80, CD86 and MHC-II. Cells were gated on $CD11c^+$ DCs. Similar results were obtained in three independent experiments.

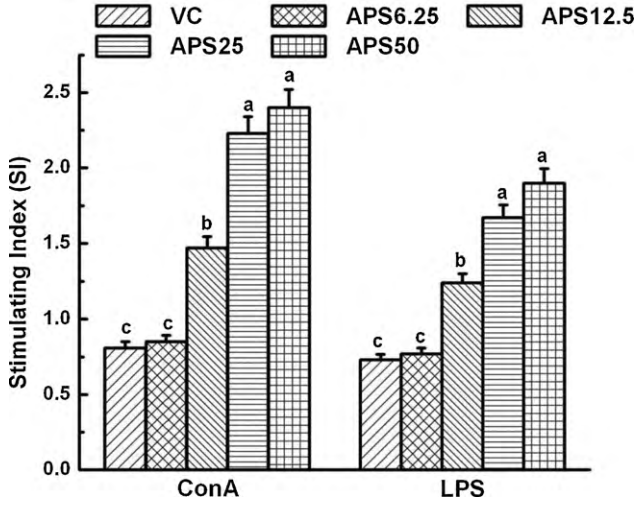

Fig. 2. *In vitro* splenic lymphocyte proliferation assays from mice treated with APS 1 day before the second vaccination of FMDV. Letters (a–c) denote significant differences ($p < 0.05$).

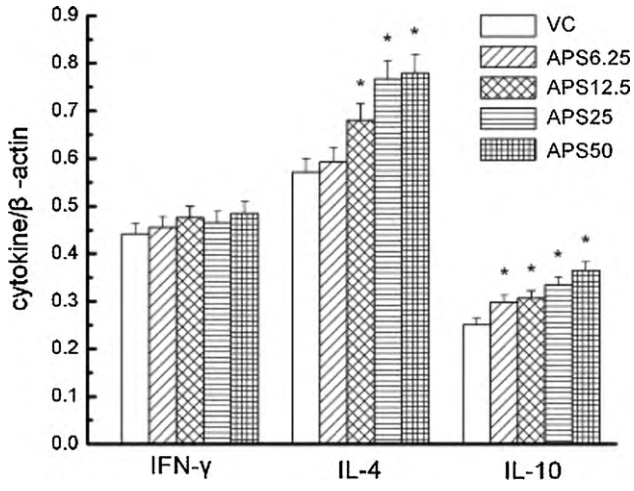

Fig. 3. Cytokine mRNA expression in mouse splenocyte treated with APS one day before the second immunization with FMDV vaccine. *Data differed significantly ($p < 0.05$) when compared to VC group.

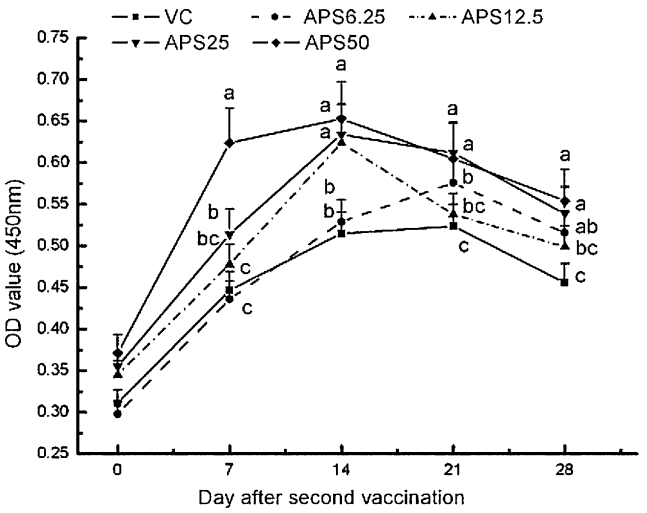

Fig. 4. Serum antibody response to FMDV vaccination in mice pre-treated with APS. Data at the same time point with different letters (a–c) differed significantly ($p < 0.05$).

3.5. Antibody titer analysis

The dynamic changes of serum antibody response to FMDV vaccination in different groups of mice after the second vaccination are shown in Fig. 4. The antibody response to FMDV tended to increase with higher dose of APS administered in a dose-dependent manner. On days 7, 14, 21 and 28 post-vaccination, the titer of $APS_{12.5}$, APS_{25} and APS_{50} groups were significantly higher than those of the VC group. The antibody response in the $APS_{6.25}$ group was not significantly different from the VC group on days 7 and 14, but significantly increased on days 21 and 28. The serum antibody responses of mice in the APS_{25} and APS_{50} groups were consistently higher compared with other groups at all the time points.

4. Discussion

The cellular immune response is critical to the host defense system against infection by accelerating the clearance of pathogens and secreting many cytokines for the regulation of the immune response. A lower level of cellular immune response usually results in a higher rate of infection with FMDV, and also might be one of the reasons for persistent infections in animals after such vaccinations (McVicar & Sutmoller, 1969). Although viruses can be controlled by a vaccine that induces antibodies, the current FMDV vaccine has little effect on cellular immune response. An ideal FMDV vaccine should contain an adjuvant that can induce cellular immunity without damaging, or perhaps even improving, the humoral immune response.

DCs and T cells are the most important cellular components of the cellular immune response. As professional antigen-presenting cells, DCs plays an important role in initiating T-cell responses against microbial pathogens. Matured DCs are characterized by the decreased ability to uptake and process antigen, high expression of surface MHC class II, and have a high capacity to prime T cells (Shao et al., 2006). The maturation or activation of DCs is one of the earliest and crucial events of immune regulation and sets up adaptive immunity (Su et al., 2008). The expression of co-stimulatory factors, CD80/CD86 and CD40, on DCs is correlated with T-lymphocyte differentiation and the upregulation of CD80, CD86 and CD40 typically results in the reinforcement of T-cell activity (Cacere et al., 2008; Munroe, 2009). To investigate the differentiation status of DCs, $CD11c^+$ that highly expressed in DCs was used to distinguish DCs from splenic lymphocytes by flow cytometry as previously described (Su et al., 2008) on day 4 after the first FMDV vaccination. The results showed (Fig. 1) that a high dose of APS (such as APS_{25} and APS_{50} groups) can significantly enhance the expression of MHC class II, CD40, slightly enhance the expression of CD86, and has no significant effect on the expression of CD80 on DCs. However, low doses of APS ($APS_{6.25}$ group) exerted no significant effect on the expression of these cell surface antigens. These results suggest that APS has a dose-dependent regulatory effect on the maturation of DCs in FMDV vaccinated mice.

An appropriate dose of APS is likely to promote the maturation of DCs via the increased expression of MHC II, CD40 and CD86, and amplify the capability of FMDV antigen-presentation during the early stages of the immune response. CD40 expression is typically upregulated on DCs after encountering microbial products (e.g. toll-like receptor ligands) (Clark & Craxton, 2003) and pathogens (e.g. FMDV). Hence, the high expression of CD40 can bridge innate and adaptive immune signals since CD40 expression on DCs plays a direct role in regulating lymphocyte proliferation, and also in the regulation of T-lymphocyte differentiation (Daphne & Edward, 2009). The current study demonstrated that this bridge function was strengthened by the administration of APS via an increased level of CD40 expression on PM (Table 2), T-cell activation (Fig. 2), increased expression of Th2 cytokines (Fig. 3) and serum antibody response (Fig. 4).

Macrophages occupy a unique niche in the immune system where they not only initiate innate immune responses but can also be effector cells that contribute to the resolution of these responses (Song et al., 2002) by inhibiting the growth of a wide variety of microorganisms. The enhancement of phagocytic function is expected to be appropriate for resisting microbial infections. Lymphocyte proliferation is another indicator reflecting the state of cellular immunity since it is generally known that ConA stimulates T cells and LPS stimulates B-cell proliferation (Sun, Wang, Xu, & Ni, 2009). The capacity to elicit an effective T- and B-lymphocyte immunity can be shown by the stimulation of a lymphocyte proliferation response (Xie & Li, 2009). We show that except in the $APS_{6.25}$ group, the PI of peritoneal macrophage and the proliferation index of splenic lymphocytes from FMDV vaccinated mice were significantly increased by prior treatment with APS, especially in APS_{25} and APS_{50} groups. Furthermore, the proliferation indexes of splenic lymphocytes stimulated with ConA or LPS were significantly higher when pre-treated with APS, suggesting that the T cells and B cells of FMDV vaccinated mice were highly activated after APS administration. From these results, we suggest that the immune regulation function caused by treatment with APS prior to FMDV vaccination may be extensive and universal that is not restricted to DCs, but also includes T cells, B cells and macrophages.

Although many cell types participate in combating infection, T-helper (Th) lymphocytes critically determine the outcome of infection (Frossi, Carli, Piemonte, & Pucillo, 2008) and can be subdivided into two cell subsets, termed as Th1 and Th2, according to differences in their corresponding cytokine expression profiles. Modulation of Th1/Th2 immunity is an important parameter to assess the therapeutic efficacy of immunomodulators. In general, Th1 cells stimulate cellular immunity but only weak and transient antibody responses, whereas Th2 cells evoke humoral immune responses but relatively weak cellular immune activity (Odbileg, Lee, Ohashi, & Onuma, 2005). The regulating functions of Th cells mainly depend on the production and release of cytokines. To determine the effect of APS on the type of Th response, the gene expression of Th1-type cytokines (IFN-γ) and Th2-type cytokines (IL-4 and IL-10) were measured by PCR. Compared to vaccine-only treated mice, the expression of IFN-γ (Th1) in mouse splenocytes from FMDV vaccinated mice in APS administrated groups was not significantly different. However, the expression levels of both IL-4 and IL-10 were significantly increased with prior treatment with

APS, thus indicating that the upregulation caused by APS mainly affects the Th2 response. Since both IL-4 and IL-10 are important cytokines in inducing the proliferation of B cells and the production of antibodies, the high levels of expression of IL-4 and IL-10 suggest that APS is likely to play a role in mediating the humoral immunity through the upregulation of Th2 cytokines in FMDV vaccinated mice. Furthermore, IL-10 is also an immunomodulatory molecule which could inhibit the functions of antigen-presenting cells by downregulating the expression of MHC class II and co-stimulatory molecules on DCs (Rezaei et al., 2009). However, in the current study, APS administered groups (except $APS_{6.25}$) showed a higher expression of MHC II and CD86, and the expression of IL-10 was enhanced simultaneously in splenic lymphocytes. This data suggests a two-pronged regulatory effect of APS on MHC II and CD86 expression. For example, a low dose of APS ($APS_{6.25}$) may promote the expression of CD86 but can also significantly induce the production of IL-10, the inhibitory effect of IL-10 on CD86 expression was greater than the promoting effect of $APS_{6.25}$ which results in a lower expression of CD86 (Fig. 1D).

It is generally believed that an effective means of protection is a vaccine that activates both the cell-mediated and humoral arms of the immune system (Liniger et al., 2008). Accordingly, an efficient FMDV adjuvant should induce both humoral and cellular immune response to a viral antigen. To confirm that APS is responsible for a stimulating effect on the humoral immune response to FMDV vaccine, the dynamic changes of the serum antibody response in different groups of mice after the second vaccination were examined. Higher titers of antibody were found in APS administered groups (except for $APS_{6.25}$) on days 7, 14, 21 and 28 after the second vaccination. This was the case especially for the APS_{25} and APS_{50} groups that reached peak antibody titers 1 week sooner than other groups and maintained a significantly higher titer until day 28. These results provided evidence that APS enhances the humoral response to FMDV vaccination.

In conclusion, these experiments demonstrated that an appropriate dose of APS used as adjuvant for FMDV vaccine is able to upregulate both the cellular and humoral immune response to the vaccine. Compared with the immune response induced by FMDV alone, pre-administration with APS induces increased phagocytic capacity of peritoneal macrophage, DC maturation, T-lymphocyte proliferation, expression of cytokines and antibody production. The successful demonstration of the utility of APS as adjuvant for enhancing FMDV vaccination suggests that it may also enhance the immune response to other vaccines. Considering the many advantages for using APS, including extensive availability, low cost and low side effects, APS could be used as an effective adjuvant for FMDV vaccination with promising use in the field.

Acknowledgments

This work was supported by grants from National Eleven-five Technological Supported Plan of China (Nos. 2008BADB4B01; 2008BADB4B07; 2006BAD31B05-2) and Scientific Research Foundation of Graduate School of China Agriculture University. We are also thankful for the help from the members of CAU-BUA TCVM teaching & research team.

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