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Full Length Research Paper

Evaluation of immunologic enhancement mediated by a polysaccharide isolated from the fruit of *Physalis alkekengi* L. var. *francheti (Mast.) Makino*

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The study was to investigate the immunologic enhancement mediated by a polysaccharide (designated PPSB) purified from the mature fruits of *Physalis alkekengi* L. var*. francheti (Mast.) Makino*. Male ICR mice were immunized subcutaneously with ovalbumin (OVA) alone, OVA/PPSB, OVA/QuilA, and saline two times at 14-day interval. The mice were sacrificed two weeks after the last immunization to analyze humoral and cellular immunity. Delayed-type hypersensitivity (DTH) response was further detected. Moreover, toxicity of PPSB was evaluated by haemolytic activity and acute toxicity assay. The results showed that PPSB significantly enhanced OVA-specific antibody titers (IgG, IgG1, IgG2b) in serum, the concanavalin (ConA), lipopolysaccharide (LPS), OVA-induced splenocyte proliferation, proportions of $CD4^+$ and $CD8^+$ T lymphocytes in spleen, DTH response when compared with OVA-injected mice ($P < 0.05$ or $P < 0.01$). Toxicity assay indicated PPSB was non-toxic. Therefore, PPSB mediated both humoral immunity and cellular immunity, and it was non-toxic. Thus, PPSB should be considered as a promising adjuvant eliciting both Th1 and Th2 responses to improve the efficacy of vaccine.

Key words: Herbal medicine, polysaccharide, humoral response, cellular response, adjuvant.

INTRODUCTION

New technology provides a new generation of vaccines such as subunit vaccines, synthetic peptide and plasmid DNA. Although they carry no risk of inadvertent infection when compared with live attenuated or whole inactivated organisms, they are less immunogenic and may be costly to produce (McCluskie and Weeratna, 2001; Perrie et al., 2008; Vandepapelière et al., 2008; Yang et al., 2007).

A large number of candidates of novel adjuvant used to augment or replace alum in human vaccine had been evaluated extensively. However, aluminum-based mineral salts (called alum) continue to be the only immunologic adjuvant approved by the US Food and Drug Administration (FDA). Alum adjuvant increase the stability and the effective particle size of an immunogen; they also promote the release of certain cytokines, such as interleukin-1 (IL-1). Unfortunately, alum adjuvant only stimulates humoral immunity; they fail to induce cell-mediated immunity or work with all antigens, and can not be frozen or freeze-dried. It is well known that QuilA, a mixture of partially purified saponins extracted from the bark of *Quillaja saponaria* Molina, can stimulate Th1 immune response and the production of CTLs. Unfortunately, high toxicity and the undesirable haemolytic effect of QuilA limit its use in human vaccines (Sun et al., 2009a). Thus, there is an urgent need for the development of potent adjuvant that enhance both antibody and cell-mediated responses (antibodies, Th1, Th2, and CTLs) to vaccine antigens (Guy and Burdin, 2005; Ebensen and Guzmán, 2008; Reed et al., 2009).

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Abbreviations: OVA, Ovalbumin; **PPSB,** polysaccharide purified from the mature fruits of *Physalis alkekengi* L. var. *francheti (Mast.) Makino*; **IFT,** increase in footpad thickness.

Previous studies have shown that polysaccharides can evoke stronger humoral and cell-mediated immune responses (Ooi and Liu, 2000; Sun et al., 2006; Wang et al., 2005; Wang et al., 2006; Wasser, 2002). More importantly, most polysaccharides from herbal plants are typically less immunogenic, non-toxic and biodegradable; they are an unlimited natural resource and low cost to manufacture (Schepetkin and Quinn, 2006; Sun et al., 2009a; Xie et al., 2007). Hence, the use of herbal immuno-modulators may be helpful in overcoming the limitation of Alum or QuilA, and herbal adjuvants are one of the ideal candidates.

Physalis alkekengi, L. var. *francheti (Mast.) Makino* (Solanaceae) is widely distributed in Europe and Asia including Russia, China, Japan, etc. It is well known that *P. alkekengi*, L. var. *francheti (Mast.) Makino* is an edible and medicinal plant in oriental countries, especially as a traditional Chinese herbal plant (Ge et al., 2009). The broad use of this plant in popular medicine includes anti-inflammatory, anti-cold, anti-cough, and anti-fungal activities (Basey and Woolley, 1973; Vessal et al., 1991; Vessal et al., 1996; Zhou and Wang, 1997). We have previously focused on the biological activities of polysaccharide (designated PPSB below) isolated from fruits of *P. alkekengi* L. var. *francheti (Mast.) Makino*, and reported that PPSB significantly reduces blood glucose levels and holds potential as an anti-diabetic agent (Tong et al., 2008).

In this paper, the immunologic enhancement of the polysaccharide fraction (PPSB) isolated from the fruits of *P. alkekengi* L. var. *francheti (Mast.) Makino* was evaluated. The result shows that PPSB can significantly enhance humoral and cellular responses, and is also considered as an effective adjuvant.

MATERIALS AND METHODS

Experimental animals

The present study was performed according to the National Research Council's guidelines of China. Five week-old male ICR mice (Grade II, body weight 20 ± 2 g) used for experiment were breed at the School of Public Health, Jilin University, China (certificate no. SCXK-(JI) 2007-0003, Changchun, China).

The mice were acclimatized for a period of 2-3 days before using for experiment. Before and during the experiment the mice were fed with standard laboratory diet, given tap water and maintained under a constant 12 h light and dark cycle at 21-23℃.

Materials and chemicals

The mature fruits of *P. alkekengi* L. var. *francheti (Mast.) Makino.* were collected in Shuangyang district, Chanchun city, Jilin province in September, 2007. It was identified by Prof. Hongxing Xiao (School of Life Science, Northeast Normal University, Changchun, China).

Ovalbumin (OVA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ConcanavalinA(ConA), Lipopolysaccharide (LPS), Ureahydrogen peroxide addition compound, Aluminum Hydroxide Gel (Alum) and QuilA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Goat anti-mouse IgG1-HRP and IgG2b-HRP were from Southern Biotechnology Associate Inc (Birmingham, AL, USA); goat anti-mouse IgG-HRP was purchased from Beijing Zhongshan Golden Bridge Biotechnology Co.LTD (China). RPMI-1640 tissue culture medium was from Gibco (USA); Fetal Calf Serum (FCS) was provided by Hangzhou Sijiqing Corp of China. Sepharose CL-6B were purchased from Amersham Pharmacia Co. (Sweden). Fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD4 (L3T4, cloneH129.19) and phycoerythrin (PE)-conjugated rat anti-mouse CD8 (LY-2, clone 53-6.7) monoclonal antibody were from BD Biosciences Pharmingen, CA, USA. All other chemicals were of grade AR.

Extraction, isolation and identification of PPSB

We have previously reported the purification, structure characterization and the hypoglycemic activity assessment of polysaccharide (PPSB) isolated from fruits of *P. alkekengi* L. var. *francheti (Mast.) Makino*. PPSB (Mw=27 kDa) is an acid heteropolysaccharide consisting of Ara, Gal, Glc and GalA in ratio of 2.6:3.6:2:1; it has a backbone composed of (1→5)-linked Ara, (1→6)-linked Gal with 3 branches attached to O-3 of (1→6)-linked Gal and terminated with either Gal or Gal and Glc, and all of Glc and the majority of GalA are distributed in branches (Tong et al., 2008). Isolation and purification of PPSB were conducted as the methods described previously (Tong et al., 2008). Briefly, fresh fruits (1 kg) of *P. alkekengi* L. var. *francheti (Mast.) Makino* were decocted with distilled water at 100℃ for three hours. The Crude Polysaccharide (CP) was extracted from the decoction by precipitating with 85% ethanol. CP dissolved in distilled water was frozen at -20℃, thawed repeatedly to remove insoluble materials by centrifugation. CP was further precipitated with 50% ethanol to discard the residue; and the supernatant was further precipitated with 70% ethanol to obtain precipitate (PPSA). After deproteinated by a combination of proteinase and Sevag method, PPSA was further purified on a Sepharose CL-6B column eluted with 0.15 mol/L NaCl and the main polysaccharide fraction (PPSB) was collected, dialyzed and lyophilized. PPSB was used for activity assessment. The endotoxin level in PPSB solution was less than 0.5EU (endotoxin unit)/ml. The solution of PPSB was sterilized by 0.22 μm millipore filter for all animal experiments.

Haemolytic activity

Red blood cell suspension (0.6 ml of 0.5%) from New Zealand rabbit was mixed with 0.6 ml diluents of PPSB or QuilA in saline solution (0.89% w/v NaCl, pyrogen free) with the various concentrations. Mixtures were incubated at 37℃ for 30 min and centrifugated at 70 g for 10 min. Free haemogloblin in the supernates was measured at 412 nm (Xie et al., 2008a). Saline and distilled water were included as minimal and maximal haemolytic controls. The concentration inducing 50% of the maximum haemolysis was considered the HD50. Each experiment included triplicates at each concentration. Four independent experiments were performed for the analysis of the HD50.

Toxicity assays

Six-week-old female ICR mice were divided into five groups, each consisting of five mice. Animals were injected twice subcutaneously on the back with PPSB at a single dose of 50, 100, 200, 400 mg/kg /mouse in saline solution (0.3 ml) at weekly intervals, and monitored daily for 14 days. Saline-treated animals were included as control

group and the toxicity was assessed by lethality, local swelling and loss of hair at the site of injection.

For further histological evaluation, ICR mice were sacrificed to excise kidneys, livers, and spleens two weeks later. Kidneys, livers, and spleens were immediately fixed in 10% (v/v) formalin. Sections (3-4 μm) of buffered paraffin-embedded tissues were stained with periodic acid-schiff (PAS) reagent, hematoxylin and eosin (H and E).

Immunization, collection of blood samples and spleen lymphocytes

Preliminary experiments showed that mice immunized with OVA containing PPSB at dose of 5 mg/kg (body weight)/mouse had a higher level of antibody titers against OVA as compared to other groups immunized with different doses (1.25, 2.5, 10, 20, 40, 80 mg/kg /mouse) (data not shown). Thus, the dose of PPSB 5 mg/kg /mouse was used for all experiments.

In order to detect the immunologic enhancement of PPSB on the mice, the male ICR mice (5 mice per group) were immunized subcutaneously with OVA (5 mg/kg) alone, or with OVA (5 mg/kg) containing QuilA (0.5 mg/kg) (OVA/QuilA) or PPSB (5 mg/kg) (OVA/PPSB) twice at 14-day interval. Mice were treated with saline as a control group. Before the first immunization, the mice from groups OVA/QuilA, OVA/PPSB were immunized with QuilA (0.5 mg/kg), and PPSB (5 mg/kg) alone respectively for two successive days. Other groups were injected saline. OVA was dissolved in saline for injection. The mice were sacrificed two weeks after the second immunization. The mice serum and the spleen lymphocytes were collect to detect humoral and cellular immune responses.

Splenocyte proliferation assay

Splenocytes were aseptically prepared from immunized ICR mice in Hank's balanced salt solution by mincing with a pair of scissors and filtering through a fourply gauze. After the erythrocytes were lysed with red blood cytolysate (Tris-NH_4Cl), splenocytes were collected by centrifugation (206 × g at 4°C for 2 min). The splenocytes were later washed twice in Hank's balanced salt solution and re-suspended in complete medium RPMI1640, which was supplemented with 100 IU/ml penicillin, 100 mg/L streptomycin and 10% FCS. The splenocytes were seeded into 3 wells of a 96-well flat-bottom microtiter plate (Nunc) at 2×10^6 cells/ml in 100 μl complete medium. Thereafter ConA, LPS, OVA (final concentration 5, 10, 10 mg/L respectively), or medium were added to a final volume of 200 μl. After 68 h incubation at 37°C in 5% CO_2, the splenocytes were incubated with 10 μl of MTT solution (5 g/L) for 4 h. The supernatant was removed carefully. 200 μl of a DMSO working solution (192 μl with 8 μl 1 mol/L HCl) was added to each well, and the absorbance was evaluated in an ELISA reader at 570 nm with a 630 nm reference after 15 min. The stimulation index (SI) was calculated by the following formula: SI = the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures (Sun, 2006; Yang et al., 2008).

Measurement of OVA-specific antibody

OVA-specific antibodies (IgG, IgG1 and IgG2b) in serum of the immunized mice were evaluated by an indirect ELISA according to the methods of Yang et al. (2005) and Xie et al. (2008b). Briefly, microtiter plates (Nunc) were coated with 100 μl OVA (50 mg/L) in 0.05 mol/L carbonate-bicarbonate buffer pH 9.6 for 24 h at 4°C. The wells were washed three times with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20, and blocked with PBS containing 5% skim milk powder at 37°C for 1 h. After washing with PBS containing 0.05% (v/v) Tween 20 three times, 100 μl of a series of diluted serum from immunized mice or PBS containing 5% skim milk powder as control were added to the triplicate wells. The plates were then incubated for 1 h at 37°C, followed by washing with PBS containing 0.05% (v/v) Tween 20. Aliquots of 100 μl of goat anti-mouse IgG-HRP, IgG1-HRP and IgG2b-HRP (diluted 1:5000 with PBS containing 5% skim milk powder respectively) were added to each plate. The plates were further incubated for 1 h at 37°C. Substrate 3,3',5,5'-tetramethylbenzidine (TMB) was added to each well after washing with PBST, and the plate was incubated for 15 min at room temperature. Reaction was terminated by adding 50 μl of 2 mol/L H_2SO_4 to each well, and Optical Density (OD) was detected at 450/630 nm with ELISA reader (Model 680, Bio-Rad, USA). Antibody titer was expressed by Log_2 value of the highest dilution of serum.

Flow cytometric assays

The method for flow cytomeric assay was conducted following procedures by Zaharoff et al. (2007). Lymphoid cells (1×10^6) prepared from the spleen of immunized mice were incubated with either FITC- conjugated rat anti-mouse CD4 mAbs or PE-conjugated rat anti-mouse CD8 mAbs for 1 h at 4°C, and collected by centrifugation at 380 × g for 15 min. Lymphoid cells were washed with PBS and centrifuged at 380 × g for 5 min, re-suspended with 200 μl PBS for immediate flow cytometric analysis. The percentage of positively stained cells, determined over 10,000 events, was analyzed by a FACScan cytofluorimeter (EPICS XL, Beckman Coulter, USA).

Delayed-type hypersensitivity (DTH) assay

The male ICR mice (5 mice per group) were immunized subcutaneously with OVA (5 mg/kg) alone, or with OVA (5 mg/kg) containing QuilA (0.5 mg/kg) (OVA/QuilA) or PPSB (5 mg/kg) (OVA/PPSB). One week after the immunization, the mice were injected on one footpad with 20μ l of OVA (1 g/L), and 20 μl of saline was injected on the contralateral footpad. Increase in footpad thickness (IFT) was measured by a dial caliper 48 h later (Ajdary et al., 2007; Fang et al., 2005; Wang et al., 2006).

Statistical analysis

All results were expressed as mean ± SD. Data were analyzed by standard *t*-test. P values less than 0.05 were considered statistically significant.

RESULTS

Haemolytic activities and toxicity assays

To compare the haemolytic activities between PPSB and QuilA, the HD50 was investigated. Results indicated the HD50 value of QuilA was 17.11 ± 0.30 μg/ml. However, no haemolytic activity of PPSB at concentrations of 10-1000 μg/ml was found.

To detect the toxicity of PPSB, ICR mice were administered subcutaneously twice ranging from 50 to 400 mg/kg at weekly intervals. The results showed that no lethality, local swelling or loss of hair was observed in ICR mice at the tested doses. Furthermore,

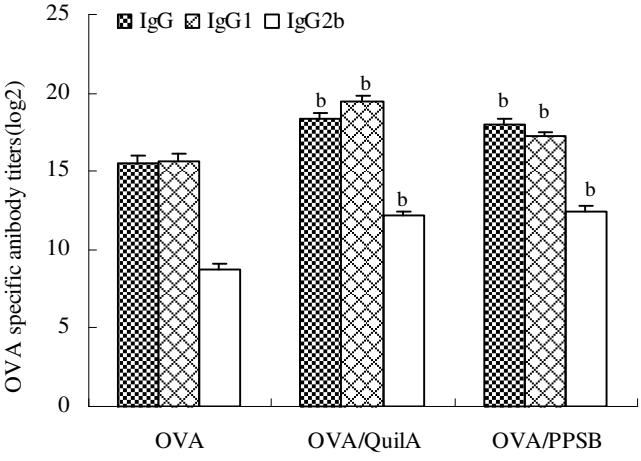

Figure 1. Effect of PPSB on OVA-specific IgG, IgG1, IgG2b antibody titers in OVA-immunized mice.The values are present as mean ± SD. [b] P < 0.01 *vs* group OVA.

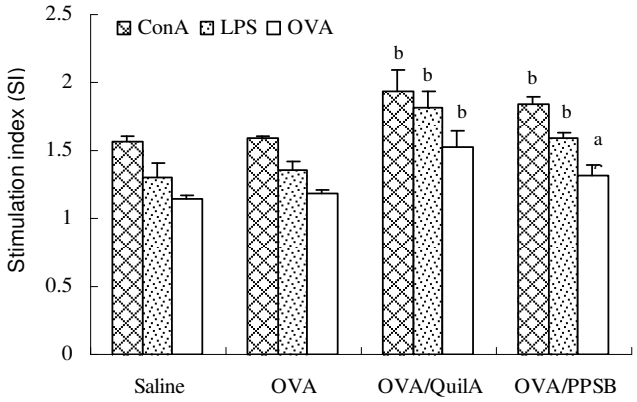

Figure 2. Effect of PPSB on splenocyte proliferation assay.The values were present as mean ± SD. [a] *P* <0.05, [b] P<0.01 *vs* group OVA.

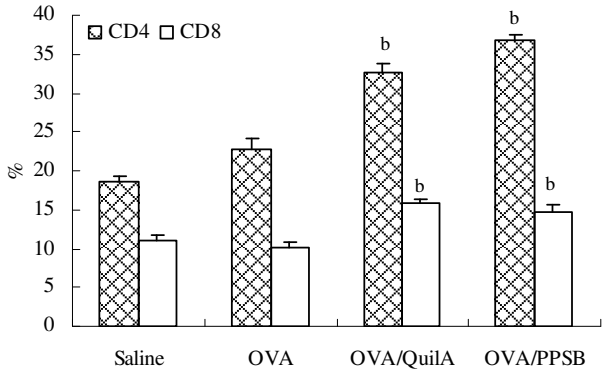

Figure 3. Effect of PPSB on $CD4^+$ and $CD8^+$ T cell populations of spleen in OVA-immunized mice. The values are present as mean ± SD. [b] P < 0.01 *vs* group OVA.

Histopathological examination (figures not shown) indicated no difference in kidneys, livers, and spleens between PPSB-injected mice and saline-treated mice.

OVA- specific antibody response mediated by PPSB

Anti-sera of the immunized mice was collected two weeks after the last immunization to analyze OVA-specific antibody titers (IgG, IgG1 and IgG2b) by indirect ELISA. Data shown in (Figure 1) demonstrated that IgG antibody titers in OVA-immunized mice were enhanced significantly by PPSB and QuilA (P < 0.01). Moreover, considerable enhancements of IgG1 and IgG2b antibody titers in groups OVA/PPSB and OVA/QuilA were detected when compared with group OVA (P < 0.01). The data indicated clearly that PPSB mediated a stronger antibody response in OVA-immunized mice.

Cellular immune response mediated by PPSB

Effect of PPSB on splenocyte proliferation

The effects of PPSB on ConA, LPS, and OVA-stimulated splenocyte proliferation in immunized mice are shown in Figure 2. ConA, LPS, and OVA -stimulated splenocyte proliferation in the mice immunized with OVA/PPSB or OVA/QuilA were notably higher than these in group OVA-injected alone (P < 0.05 or P < 0.01). The results showed that PPSB greatly promoted the ConA, LPS, and OVA-stimulated splenocyte proliferation.

Effect of PPSB on $CD4^+$ and $CD8^+$ spleen T lymphocytes subsets

As seen in Figure 3, the percentage of $CD4^+$ T lymphocytes in OVA-immunized mice were increased remarkably by PPSB and QuilA (P < 0.01) as compared with OVA control group. More importantly, PPSB and QuilA significantly augmented the proportion of $CD8^+$ T lymphocytes in OVA-immunized mice when compared to group OVA (P < 0.01). These results suggested that PPSB increased the proportions of both $CD4^+$ and $CD8^+$ T lymphocytes, that secret cytokines to mediate immunologic enhancement.

Effect of PPSB on DTH response

The results are presented in Table 1. DTH response in OVA-immunized mice was greatly augmented by PPSB and QuilA compared with group OVA (P < 0.01). These results confirmed that PPSB mediated stronger DTH response.

Table 1. Effect of PPSB on DTH response in OVA-immunized mice.The values were present as mean ± SD. IFT stands for Increase in Footpad Thickness. [a]P < 0.01 *vs* group OVA. *n*=5.

Groups	IFT (mm)
OVA	0.26 ± 0.04
OVA/QuilA	0.46 ± 0.03[a]
OVA/PPSB	0.64 ± 0.07 [a]

DISCUSSION

T cells are divided into two categories based on their functions as well as cell surface phenotypes. These include: helper T cells (Th), that promote cell-mediated (termed Th1) and antibody responses (termed Th2), CTLs, that kill antigen-bearing target cells. Adjuvants can be classified according to their capacity to stimulate Th1 and Th2 responses. Th1 response is required for protective immunity against intracellular infection such as viruses, certain bacteria or protozoa and presumably against cancer cells. Th1 immune response is a requisite for CTLs, which are necessary for subunit vaccines and vaccines directed against intracellular pathogens as well as therapeutic cancer vaccines (Yang et al., 2005). Th2 response is effective for protection against most bacteria as well as some viral infections (Cox and Coulter, 1997). In general, production of immunoglobulin isotypes is associated with Th response. Th1 cells produce IFN-γand IL-2, promoting the production of IgG2a, IgG2b, and IgG3. Th2 cells produce the production of IL-4, IL-5, and IL-10, promoting the production of IgG1 and IgE (Guy, 2007; Marciani, 2003; Chiarella et al., 2007). It has been broadly reported that current available adjuvants such as alum, water/oil emulsions evoke only Th2 immunity, and are unable to stimulate Th1 immunity. Therefore, development of novel adjuvant, which are capable of strongly eliciting both humoral and cellular immune responses (both Th1 and Th2 responses), are necessary to maximize the efficacy of new or available vaccines (Fang et al., 2005; Petrovsky, 2006; Silva et al., 2004).

PPSB was isolated with hot water from the mature fruits of *P. alkekengi* L. var*. francheti (Mast.) Makino*, fractionated with ethanol and purified by Sepharose CL-6B gel filtration chromatography. High-performance liquid chromatography (HPLC) demonstrated that PPSB had a single and symmetric sharp peak revealing its homogeneousness. Male ICR mice were immunized subcutaneously with OVA alone or with either OVA/PPSB or OVA/QuilA, and the OVA-specific antibody response as well as cellular response mediated by PPSB have been discussed in this paper. It was found that the adjuvant potential of PPSB in OVA-immunized mice was as effective as that of QuilA.

Serum antibody titer is the indicator of humoral immunity. Analysis of antibody showed clearly that PPSB not only substantially enhanced OVA-specific IgG level in OVA-immunized mice ($P < 0.01$), but also increased OVA-specific IgG1 and IgG2b antibody titers ($P < 0.01$) (Figure 1). The finding demonstrated PPSB was effective on Th1 and Th2 cells as associated sensitively with an enhancement of IgG2b and IgG1 levels, respectively.

Cellular immunity plays an important role in intracellular infections. It is well known that the stimulation of lymphocyte proliferation response can be used to assess the capacity of eliciting cell immunity. The proliferation assay indicated that PPSB could significantly promote the ConA, LPS, and OVA-induced splenocyte proliferation when compared with OVA group ($P < 0.05$ or $P < 0.01$) (Figure 2). The results indicated that PPSB could significantly increase the activation potential of T and B cells.

T cells could differentiate into two different subsets according to their specific membrane molecule, that is $CD4^+$ and $CD8^+$ T lymphocytes, which play different roles in immunomodulation. It is well known that Th cells and Cytotoxic T lymphocytes (CTLs) responses are associated with the enhancement of $CD4^+$ and $CD8^+$ T lymphocytes respectively. It is well known that Th can promote proliferation, maturation, and immunologic function of other cell types, and specific lymphokines secreted by Th cells are very important for the activities of B cells, macrophages, and CTLs (Seder and Paul, 1994; Zhang et al., 2009b). In contrast, CTLs are extremely important in the defense against viral infections. To further elucidate the mechanism of PPSB as an immunomodulator, the effects of PPSB on both $CD4^+$ and $CD8^+$ spleen T lymphocytes populations in OVA-immunized ICR mice were analyzed by flow cytometric assay. The results of present investigation (Figure 3) revealed that the percentage of $CD4^+$ T lymphocytes in OVA-immunized mice was greatly augmented by PPSB and QuilA ($P < 0.01$). More importantly, the percentage of $CD8^+$ T lymphocytes in groups OVA/PPSB and OVA/QuilA were higher than that in group OVA ($P < 0.01$).The enhanced percentages of both $CD4^+$ and $CD8^+$ spleen T lymphocytes indicated that both Th and CTLs were activated greatly by PPSB.

$CD4^+$ T cells can be subdivided into two functional categories - Th1 and Th2. IFN-γ and IL-2 produced by Th1 are the primary mediators of host defenses associated with DTH response and activation of phagocytes (Seder and Paul, 1994). DTH is a typical method used to measure *in vivo* T cell mediated immunity. In the present study, treatment of PPSB in OVA-immunized mice strongly enhanced DTH response when compared to group OVA ($P<0.01$) (Table 1). Results further revealed that Th1 immunity was evoked remarkably by PPSB.

General alum adjuvant only greatly induced Th2 immune response. Indeed, it was poor at eliciting Th1 immune response, and very poorly activated $CD8^+$ T lymphocytes (Yang et al., 2007; Sun et al., 2009; Giudice et al., 2002). Although it has been extensively proved that QuilA can stimulate Th1 and Th2 immune responses, its

high toxicity and haemolytic activity are the main restriction fro human use (Sun et al., 2009a). Fortunately, the data from haemolytic activities and toxicity assays indicated clearly no haemolytic activity of PPSB and safety of PPSB in ICR mice at the tested doses. Generally, the majority of polysaccharides derived from higher plants enhances or activates the host defensive immune responses. On the basis of this work, the mechanism of immunologic enhancement mediated by PPSB was thought to occur through the induction of humoral and cellular immunity (antibodies, Th1, Th2, and TCLs) in ICR mice immunized with OVA. To be specific, PPSB had effects on B and T lymphocyte proliferation, especially on the proliferation of $CD4^+$ and $CD8^+$ T lymphocytes revealing that Th and CTLs were activated by PPSB. Data from antibody isotype (IgG1, IgG2b) and DTH suggested PPSB stimulated the stronger Th1 and Th2 responses. In case of extracellular infection, a good humoral immune response mediated by Th2 cells is required; however, the cell-mediated response, mainly cytotoxic T lymphocytels (CTLs) and T helper 1 (Th1) cells, is the most important for intracellular infection. Thus, PPSB may be a promising adjuvant eliciting Th1 and Th2 responses used in vaccines against both pathogens and cancer.

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