EPSAH, an exopolysaccharide from Aphanothece halophytica GR02, improves both cellular and humoral immunity as a novel polysaccharide adjuvant

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[ABSTRACT] EPSAH is an exopolysaccharide from Aphanothece halophytica GR02. The present study was designed to evaluate its toxicity and adjuvant potential in the specific cellular and humoral immune responses in ovalbumin (OVA) in mice. EPSAH did not cause any mortality and side effects when the mice were administered subcutaneously twice at the dose of 50 mg·kg⁻¹. Hemolytic activity in vitro indicated that EPSAH was non-hemolytic. Splenocyte proliferation in vitro was assayed with different concentrations of EPSAH. The mice were immunized subcutaneously with OVA 0.1 mg alone or with OVA 0.1 mg dissolved in saline containing Alum (0.2 mg) or EPSAH (0.2, 0.4, or 0.8 mg) on Day 1 and 15. Two weeks later, splenocyte proliferation, natural killer (NK) cell activity, production of cytokines IL-2 from splenocytes, and serum OVA-specific antibody titers were measured. Phagocytic activity, production of pro-inflammatory cytokines IL-1 and IL-12 in mice peritoneal macrophages were also determined. EPSAH showed a dose-dependent stimulating effect on mitogen-induced proliferation. The Con A-, LPS-, and OVA-induced splenocyte proliferation and the serum OVA-specific IgG, IgG1, and IgG2a antibody titers in the immunized mice were significantly enhanced. EPSAH also significantly promoted the production of Th1 cytokine IL-2. Besides, EPSAH remarkably increased the killing activities of NK cells from splenocytes in the immunized mice. In addition, EPSAH enhanced phagocytic activity and the generation of pro-inflammatory cytokines IL-1 and IL-12 in macrophages. These results indicated that EPSAH had a strong potential to increase both cellular and humoral immune responses, particularly promoting the development of Th1 polarization.

[KEY WORDS] Exopolysaccharide from Aphanothece halophytica GR02 (EPSAH); Adjuvant; Antibody; Th1

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Introduction

Vaccines can stimulate appropriate immune response contributing long-lasting protection against infection. In many cases, the vaccine consisting of antigen alone only stimulates weak immunogenicity, which is not enough to prevent infection. Adjuvants are agents that strengthen the immune response against inoculated antigens [1]. Vaccines usually require additional exogenous adjuvants to improve the immune response to the antigens following immunization [2]. It is one of the most significant challenges in vaccinology to select suitable adjuvants.

Despite many compounds or agents, such as Freund’s complete adjuvant and lipopolysaccharide, possess adjuvant activities, they are unsuitable for human use, due to toxicities and side effects. Particulate aluminum salts (known as alum) have been the only adjuvants approved for human use because of alum’s simplicity, tolerability, safety, and cost-efficiency [3-4].

Recent development of vaccines, especially increasing applications of recombinant subunit and synthetic vaccines, dwarfs alum adjuvants’ immunity stimulating function to antigens, since alum adjuvants can only induce Th2 type immune responses, not highly effective at stimulating cell-mediated immune responses, including the vaccination against pathogens that require Th1-cell-mediated immunity [2, 5-6].

Freund’s complete adjuvant promotes a marked commitment...
to the Th1 pathway, but has a generally unacceptable level of adverse effects. Thus, minimizing toxicity remains as one of the major challenges in adjuvant research. There is an urgent requirement to look for the adjuvants capable of boosting Th1-type responses without unacceptable toxicity.

Carbohydrate structures play critical roles in immune system function and carbohydrates also have the virtue of a strong safety and tolerability record. A number of carbohydrate compounds from plant, bacterial, yeast and synthetic sources have emerged as promising vaccine adjuvant candidates. Carbohydrates are readily biodegradable and therefore unlikely to cause problems of long-term tissue deposits seen with alum adjuvants. Carbohydrate-based compounds have many favorable properties that could place them in a unique position to challenge alum’s monopoly over human vaccine usage. Polysaccharides have attracted many experts to explore their ability of regulating immune system function and inhibitory activity against inflammation and cancer. Such polysaccharides have been used as vaccine adjuvants for the induction of mucosal and systemic immune responses.

We have previously reported that the exopolysaccharide from Aphanothece halophytica GR02 (EPSAH) is able to induce apoptosis in human cervical cancer HeLa cells. However, there is no published information on EPSAH as a potential adjuvant. Hence, in the present study, the in vivo adjuvant activity of EPSAH was evaluated using ovalbumin (OVA) as a model protein antigen. The aim of the present study was to determine whether EPSAH was able to enhance the cellular immunity in mice subcutaneously immunized with OVA, particularly the Th1-type responses, in addition to the humoral immunity.

Materials and Methods

Materials

Ovalbumin (OVA), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), aconitavlin A (Con A), lipopolysaccharide (LPS), and rabbit anti-mouse IgG peroxidase conjugate were from Sigma Chemical Co., USA Medium RPMI-1640 was purchased from Gibco Invitrogen Co., USA. Goat anti-mouse IgG, IgG1 and IgG2a peroxidase conjugate were from SouthernBiotech Co., Ltd. Cytokine IL-1 and IL-2 detecting ELISA kits were purchased from Nanjing Boqiao Biological Technology Co. Ltd. (Nanjing, China). Cytokine IL-12 detecting ELISA kit was purchased from Beijing 4A Biotech Co. Ltd. (Beijing, China). Human leukemia K562 cell lines were purchased from Institute of Cell Biology, China Academy Sciences (Shanghai, China). All other reagents were of analytical reagent grade.

Animals

Female rabbits (5 months old) were provided by the Experimental Animal Center, Jiangsu Province Academy of Agricultural Sciences. Female ICR mice (5 weeks old) weighing 18–22 g were purchased from the Comparative Medical Center of Yangzhou University (Yangzhou, China). The mice were maintained under controlled conditions with a temperature of 25 ± 1 °C, humidity of 50% ± 10%, and a 12 h/12 h light/dark cycle. They were acclimatized for 1 week prior to experiments.

Culture of Aphanothece halophytica GR02 and isolation and purification of EPSAH

According to the method described previously, after 20 days of culture, the alga cells were removed by centrifugation. The supernatant was filtered, dialyzed, and concentrated. The concentrated sample was purified by anion-exchange chromatography. The major peak were collected, dialyzed, concentrated, and precipitated by ethanol and then lyophilized.

Toxicity assays

Female and male ICR mice were divided into two groups (8/group, half female and half male). The animals were injected subcutaneously on the back with EPSAH at a dose of 50 mg·kg−1 twice on the first day, and monitored daily for 14 days. Saline-treated animals were included as control group. The toxicity was assessed by monitoring lethality, local swelling, and body weight.

Hemolytic activity of EPSAH

Hemolytic activity of EPSAH was determined against rabbit red blood cells. Briefly, three independent triplicates of 250, 500, and 1 000 μg·mL−1 of EPSAH in PBS were mixed with rabbit red blood cells to a final concentration of 4% volume/volume (V/V), incubated at 37 °C for 2 h, and centrifuged at 800 g for 5 min. The release of hemoglobin in the supernatant was monitored by measuring the hemoglobin absorbance at 540 nm. The readings from cell suspension in PBS (without any additives) or ddH2O were used as 0% or 100% hemolysis, respectively. The hemolysis rate (HR) was calculated using the following equation:

\[ HR = \frac{A_{\text{sample}} - A_{\text{negative}}}{A_{\text{positive}} - A_{\text{negative}}} \]

Splenocyte proliferation assay in vitro

Spleens were collected from the mice under aseptic conditions, placed in Hank’s balanced salt solution (HBSS, Sigma), and then minced using a pair of scissors and passed through a fine steel mesh to obtain a homogeneous cell suspension. The erythrocytes were lysed with ammonium chloride (0.8%, W/V). After centrifugation, the pelleted cells were washed with PBS thrice, and re-suspended in complete medium. The cell numbers were counted with a hemocytometer using trypan blue dye exclusion technique (Cell viability exceeded 95%). Splenocytes were seeded into a 96-well flat-bottom microtiter plate at 5 × 10^4 cell/mL in 100
μL of complete RPMI-1640 medium, with or without mitogen (5 μg·mL⁻¹ Con A or 10 μg·mL⁻¹ LPS). EPSAH samples (final concentration 200, 100, 50, 25, and 12.5 μg·mL⁻¹) were added into each well. After a 44-h incubation, 20 μL of MTT solution (5 mg·mL⁻¹) was added to each well and incubated for additional 4 h. The plates were centrifuged (1 400 × g, 5 min) and 150 μL of DMSO was added to each well, and the absorbance at 570 nm was recorded on an ELISA reader. The stimulation index (SI) was calculated based on the following formula: SI = the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

**Immunization**

Six-week-old female ICR mice were divided into six groups (6/group). The animals were immunized subcutaneously with 100 μg of OVA alone or with 100 μg OVA dissolved in saline containing aluminum compounds adjuvant (Alum) (0.2 mg) or EPSAH (0.2, 0.4, or 0.8 mg) on Day 1. Saline-treated animals were included as controls. A boosting injection was given 2 weeks later. Sera and splenocytes were collected 2 weeks after the second immunization for measurement of OVA-specific antibody, proliferation and NK cell activity.

**Measurement of OVA-specific antibody**

OVA-specific IgG, IgG1 and IgG2a antibodies in sera were detected by an indirect ELISA. In brief, microtiter plate wells were coated with 100 μL of OVA solution (50 μg·mL⁻¹) in 10 mmol·L⁻¹ carbonate–bicarbonate buffer, pH 9.6) overnight at 4 °C. The wells were washed thrice with PBS containing 0.05% (V/V) Tween 20 (PBST) and blocked with 5% nonfat milk powder in PBS at 37 °C for 1 h. After three washings with PBST, 100 μL of a series of diluted sera sample or 2.5% nonfat milk/PBS as control were added in triplicate. The plates were then incubated for 2 h at 37 °C, followed by three times of washing. Aliquots of 100 μL of goat anti-mouse IgG, IgG1, or IgG2a horseradish peroxidase conjugate diluted 1 : 50 000 with 2.5% nonfat milk/PBS were added to each well. The plates were further incubated for 2 h at 37 °C. After washing, the peroxidase activity was assayed as follows: 100 μL of TMB substrate solution was added to each well, the plate was incubated for 10 min at 37 °C in dark, and enzyme reaction was terminated by adding 50 μL/well of 2 mol·L⁻¹ of H₂SO₄. The optical density was measured using an ELISA reader at 450 nm. The data were expressed as the mean OD value of the samples/the mean OD value of the control. P/N = ODpositive serum / ODnegative serum

**Proliferation assay of splenocyte from the OVA-immunized mice**

Preparation of Splenocyte from the OVA-immunized mice was conducted as above. Splenocytes were seeded into 6 wells of a 96-well flat-bottom microtiter plates at 5 × 10⁶ cell/mL in 100 μL of complete medium. Con A (final concentration 5 μg·mL⁻¹), LPS (final concentration 10 μg·mL⁻¹), OVA (final concentration 10 μg·mL⁻¹), EPSAH (final concentration 200, 100, 50, 25, and 12.5 μg·mL⁻¹), and control medium were added giving a final volume of 200 μL. The plates were incubated at 37 °C in a humid atmosphere with 5% CO₂. After a 44-h incubation, 20 μL of MTT solution (5 mg·mL⁻¹) was added to each well and incubated for further 4 h. The plates were centrifuged (1 400 × g, 5 min) and 150 μL of DMSO was added to each well, and the absorbance was evaluated in an ELISA reader at 570 nm. The stimulation index (SI) was calculated based on the following formula: SI = the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

**Assay of natural killer (NK) cell activity**

KS62 cells were used as target cells and seeded into 96-well microtiter plates at 2 × 10⁶ cells/well in 100 μL RPMI 1640 complete medium. Splenocytes from the OVA-immunized mice prepared as described above were used as the effector cells and added at 1 × 10⁶ cells/well to yield an effector/target cell ratio of 50 : 1. The plates were then incubated for 20 h at 37 °C in 5% CO₂ atmosphere. 20 μL of MTT solution (5 mg·mL⁻¹) was added to each well and the plate was incubated for another 4 h and subjected to MTT assay. Three kinds of control measurements were performed: target cells control, blank control, and effector cell control. NK cell activity was calculated as following equation: NK activity (%) = ([ODT − (ODb − ODs)]/ODT) × 100, where ODₜ is optical density value of target cells control, ODₚ is optical density value of test samples and ODₑ is optical density value of effector cells control.

**Preparation of peritoneal macrophages**

Peritoneal exudates were obtained from male ICR mice by lavage 4 days after the intraperitoneal injection of 1 mL of sterile 4% thioglycollate broth (Difco Laboratories, Detroit, MI) as reported previously [18]. After being washed with RPMI 1640 medium containing 2% FBS, the peritoneal macrophages were plated in 100-mm tissue culture dishes and incubated for 4 h at 37 °C in a 5% CO₂ humidified atmosphere.

**Peritoneal macrophages phagocytosis**

Peritoneal macrophages were used to examine the influence of EPSAH on phagocytic activity by the neutral red phagocytosis assay system [19]. Briefly, peritoneal macrophages cells (1 × 10⁶ cells/well) were incubated in 96-well plates with various concentrations (6.25, 12.5, 50, 100, and 200 μg·mL⁻¹) of EPSAH or LPS (20 μg·mL⁻¹) for 24 h. After incubation, the supernatant was removed. The stimulated cells were washed twice with PBS, 100 μL⁻¹ of 0.1% neutral red were added to each well, and the cells were then co-incubated with macrophages for 4 h at 37 °C. After the removal of unphagocytized neutral red by PBS, 100 μL of cell lysate (the volume ratio of acetic acid to ethanol was 1 : 1) was added into the wells and kept for 8 h at 4 °C for sufficient schizolysis of cells and release of phagotrophic neutral red. The optical density was determined at 570 nm. The phagocytosis index (%) was calculated as follows: (ODₑ –
ODs/ODc × 100, in which ODs represented the OD value of stimulated well while ODc stood for that of control well.

**Cytokine determination by ELISA**

The levels of IL-1, IL-2 and IL-12 in the supernatants were determined by the ELISA kits that were specific against murine cytokines. Assays were performed according to the manufacturer's instructions.

**Statistical analysis**

The data were expressed as mean ± SD and examined for their statistical significance of difference with Student's t-test. P-values of less than 0.05 were considered statistically significant.

### Results

#### Toxicity profile

When the animals were immunized subcutaneously on the back, there is no lethality and local swelling detected at a dose of 50 mg·kg⁻¹ of EPSAH. As shown in Table 1, there was no significant difference in the body weight of the mice between the two groups (P > 0.05). From the above results, the safety dose for EPSAH as adjuvant with OVA to vaccinate the mice could be decided.

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#### Statistical analysis

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### Table 1 Effects of EPSAH on the body weight of the mice (means ± SD, n = 8)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Increase rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>18.9 ± 1.7</td>
<td>23.4 ± 1.3</td>
<td>27.1 ± 1.8</td>
<td>27.5</td>
</tr>
<tr>
<td>EPSAH</td>
<td>50</td>
<td>18.1 ± 1.6</td>
<td>21.2 ± 2.9</td>
<td>23.9 ± 1.0</td>
<td>27.7</td>
</tr>
</tbody>
</table>

### Hemolysis

All the hemolysis rates of the three concentrations of EPSAH were less than 0.07%, much lower than the 5% threshold, and, therefore, all the samples were considered non-hemolytic [15].

#### Effects on splenocyte proliferation in vitro

The effects of EPSAH on mitogen-induced splenocyte proliferation were investigated in a dose range of 6.25–200 μg·mL⁻¹, as shown in Fig. 1. EPSAH exhibited significant stimulation on ConA-induced or LPS-induced proliferation, compared with the ConA or LPS control, showing a dose-dependent stimulating effect on mitogen-induced proliferation.

#### Effects of EPSAH on the OVA-specific serum antibody response

The OVA-specific IgG, IgG1, and IgG2a antibody levels in the sera were measured 2 weeks after the last immunization using ELISA and the results are shown in Fig. 2. OVA alone induced low levels of OVA-specific IgG antibody. However, addition of Alum and EPSAH to OVA resulted in an increase in total IgG antibody titers. With the serum dilution 1 : 1 000, the mice immunized with OVA/Alum and OVA/EPSAH had a dramatic increase in total IgG antibody titers, compared with OVA alone group (Fig. 2A). So the serum OVA-specific antibody IgG1 and IgG2a production in immunized mice was measured using the serum dilution 1 : 1 000. With regard to OVA-specific serum IgG1 and IgG2a titers, the mice immunized with OVA/EPSH had a dramatic increased IgG2a production, compared with OVA alone group. The findings indicated that EPSAH significantly enhanced serum OVA-specific total IgG and IgG2a production in immunized mice (Fig. 2B).

#### Effects of EPSAH on splenocyte proliferation in OVA-immunized mice

The effects of EPSAH on mitogen- and OVA-stimulated splenocyte proliferation in the immunized mice are shown in Fig. 3. EPSAH significantly increased Con A-, LPS-, and OVA-stimulated splenocyte proliferation in the immunized mice (P < 0.05 or P < 0.01). There were no differences (P > 0.05) between the OVA/EPSA and OVA alone group.
Fig. 2 Effects of EPSAH on OVA-specific IgG, IgG1 and IgG2a antibody. A. Effect of EPSAH on OVA-specific IgG antibody. B. Effect of EPSAH on OVA-specific IgG1 and IgG2a antibody. Groups of six female ICR mice were immunized subcutaneously with OVA 100 μg alone or with OVA 100 μg dissolved in saline containing aluminum compounds adjuvant (Alum) (0.2 mg) or EPSAH (0.2, 0.4, and 0.8 mg) on day 1 and 15. Sera were collected 2 weeks after the last immunization. OVA-specific IgG, IgG1 and IgG2a antibodies in the sera were measured by an indirect ELISA. P/N: OD_{positive} serum / OD_{negative} serum. The values are presented as mean ± SE (n = 6). *P < 0.05 and **P < 0.01 vs OVA groups.

0.05) observed between the OVA and OVA + Alum groups.

Effects of EPSAH on NK cell activity in OVA-immunized mice

The effects of EPSAH (0.4 mg) on NK cell activity in OVA-immunized mice are shown in Fig. 4. EPSAH significantly enhanced the killing activity of NK cell in the OVA-immunized mice. The findings indicated that EPSAH could promote lytic activity of NK cells in mice immunized with OVA.

Effects of EPSAH on cytokine IL-2 levels in splenocytes from OVA-immunized mice

Pro-inflammatory cytokines from splenocytes have an important effect on the development of T cell-mediated immune responses. Compared with the levels of cytokines IL-2 in the culture supernatant of splenocytes from OVA-immunized mice, it was observed that the OVA/EPSAH vaccine had differential cytokine expression profiles. The culture supernatant of splenocytes from the OVA/EPSAH-immunized mice showed higher levels of IL-2 (Fig. 5) in comparison with the OVA/Alum, OVA alone or saline group.

Effects of EPSAH on phagocytic activity

One of the most distinguished features of macrophage activation is an increase in phagocytic activity. Phagocytic activity of EPSAH-activated macrophages was examined by the uptake of neutral red, using LPS as a positive control. A dose-dependent enhancement of phagocytic activity was observed in macrophages treated with 6.25–200 μg·mL⁻¹ doses of EPSAH (Fig. 6).

Effects of EPSAH on IL-1 and IL-12 secretion from peritoneal macrophages

In order to determine whether EPSAH was coupled with the production of pro-inflammatory cytokines IL-1 and IL-12 by stimulating macrophages, EPSAH was applied to peritoneal macrophages (6.25 to 200 μg·mL⁻¹). As shown from Fig. 7, the production of IL-1 and IL-12 was significantly increased in EPSAH-treated cells in a dose-independent manner, compared with the control, which might have an implication in the development of T cell-mediated immune responses.

Fig. 3 Effects of EPSAH on mitogen- or OVA-stimulated splenocyte proliferation in the mice immunized with OVA. Splenocyte proliferation was measured by the MTT method. SI: stimulation index. Values were expressed as means ± standard deviation (n = 6). *P < 0.05 and **P < 0.01 vs OVA alone group.
Fig. 4  Effect of EPSAH on NK cell activity in mice immunized with OVA. K562 cells were used as target cells and splenocytes from the OVA-immunized mice were used as the effector cells. \(^* P < 0.05\) vs OVA alone group

**Discussion**

The majority of vaccines require association with adjuvants to increase the potency and stimulate an appropriate immune response. Cell-mediated immunity, mediated by T lymphocytes, plays an important role to combat

Fig. 5  Effects of EPSAH on cytokine IL-2 production in splenocytes from the OVA immunized mice. Splenocytes from the immunized mice were incubated with Con A (final concentration 5 μg·mL\(^{-1}\)) in 96-well culture plates at 37 °C in 5% CO\(_2\). After 24 h, culture supernatants were collected for the detection of IL-2 levels using commercial ELISA kits. \(^* P < 0.01\) vs Saline group

Fig. 6  Effects of EPSAH on phagocytosis in Peritoneal macrophages. Peritoneal macrophages were treated with EPSAH (6.25, 12.5, 50, 100, 200 μg·mL\(^{-1}\)) or LPS (20 μg·mL\(^{-1}\)) for 24 h and the phagocytic activity was measured using neutral red phagocytosis assay system. The results are shown as means ± SD (n = 5). \(^* P < 0.05, \ ^{*}* P < 0.01\) vs control group
intraplacental microbe infections. Among the T-lymphocytes, helper T cells induce B-lymphocytes to secrete antibodies, and cytotoxic T-lymphocytes help phagocytes destroy ingested microbes and kill intracellular microbes. Humoral immunity, however, mediated by antibodies, which are produced by B-lymphocytes, functions by neutralizing and eliminating extracellular microbes and microbial toxins. The capacity to elicit an effective T cell immunity can be shown by the stimulation of lymphocyte proliferation response [20-21]. OVA is commonly used as a model for immunogen-specific T and B cell mediated immune function [22]. It is generally known that Con A stimulates T cells and LPS stimulates B cell proliferation. The proliferation assay in the present study showed that EPSAH could not only significantly promote the Con A- and LPS-stimulated splenocyte proliferation in the mice, but also significantly enhanced the mitogen- and OVA-stimulated splenocyte proliferation in OVA-immunized mice as comparison with OVA control group (Figs. 1 and 3). The findings indicated that EPSAH could significantly induce cell-mediated immune response.

NK cells are one of major populations of cytotoxic lymphocytes [23-24], and are important in the defense against tumors and viruses [25-26]. In addition to killer activity, NK cells play a role in forming the adaptive immune response through immune modulation [24]. NK cells are able to deliver a response immediately after recognizing specific signals, including stress signals, ‘danger’ signals and signals from molecules of foreign origin [27]. NK cells can react against and destroy target cell without prior sensitization to it. The target cells can be a cancer cells cultured in vitro or from another tissue. NK cell activity assay is a routine method for analysis of a patient’s cellular immune response in vitro [28]. In this investigation, EPSAH significantly enhanced the lytic activity of NK cells in OVA immunized mice, suggesting that EPSAH could enhance cellular immune response.

Adjuvant activity of B cells via helper T cells can be evaluated by measuring total IgG levels as the secondary immune response [21]. Adjuvants are often desirable to induce specific types of immunity, which may direct the immune system towards either Th1 or Th2 type responses [29-30]. Th1 cells producing IFN-γ and IL-12 promote cellular immune responses, including macrophage activation, delayed type hypersensitivity (DTH), and cytotoxicity. Th1 response positively contributes to the humoral (antibody) response to a limited extent by supporting the production of the IgG2a antibody subclass, but inhibits production of several other subclasses like IgG1. In mice, production of IgG1 versus IgG2a is widely interpreted as a reflection of differential Th2–Th1 reactivity. The IgG1/IgG2a ratio can be regarded as an indirect reflection of immune function in vivo [31-33]. The OVA-specific serum IgG1, IgG2a and IgG2b antibody levels in the OVA-immunized mice are shown in Fig. 2, indicating that EPSAH is effective on Th1 response, associated with an enhancement of IgG2a and a descent of IgG1 levels.

Accumulating evidence supports a notion that EPSAH is a potential adjuvant. Cytokines have a central role during Th1 immune adjustment. TNF-α and IL-12 are two major macrophage-derived mediators of immune responses. Specifically, IL-12 is a cytokine that induces the generation of Th1-derived cytokines (e.g., IL-1β, IL-6, and IL-2), as well as differentiation of Th1 cells [34-35]. IL-12 production plays a critical role in driving Th1-type immune responses, as illustrated by Trinchieri et al. [36]. Our results from the present study showed that EPSAH markedly enhanced phagocytic activity and the generation of proinflammatory cytokines, such as IL-1 and IL-12 in macrophages (Figs. 6 and 7). Furthermore, the splenocytes isolated from OVA/EPSAH immunized mice significantly increased Th1-type proinflammatory cytokine IL-2 generation (Fig. 5), suggesting that EPSAH-induced actions may influence the promotion of Th1 polarization.

In addition to promoting an appropriate immune response, an ideal adjuvant should also offer excellent safety. In this investigation, EPSAH was shown to have no toxicity and be non-hemolytic through toxicity assays. Generally polysaccharides show little side effect in many situations in human or animals. So EPSAH is likely to be a safe substance as adjuvant formulations for vaccine.

In conclusion, the present study evaluated the adjuvant activity of the polysaccharide EPSAH. The data indicated that
it was a potential immunity-stimulating adjuvant, particularly promoting the development of Th1 polarization. Future studies should aim at elucidating the mechanism of action for EPSAH as an adjuvant.

References