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Polysaccharides from *Antrodia camphorata* mycelia extracts possess immunomodulatory activity and inhibits infection of *Schistosoma mansoni*

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Abstract

Antrodia camphorata (AC) is a commonly used fungus in folk medicine for the treatment of viral hepatitis and cancer. AC polysaccharides (AC-PS) are reported to possess anti-inflammatory, anti-hepatitis B virus, and anticancer activities. In this study, we tested the *in vivo* effect of AC-PS on immune function by evaluating cytokine expression; on immunomodulation, by evaluating spleen cells; and on *Schistosoma mansoni* infection in mice. The induction of high levels of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) mRNA was detected in BALB/c mice after 2, 4, and 6 weeks of oral AC-PS administration. After 6 weeks of oral AC-PS administration to the BALB/c mice, the number of splenic dendritic cells, macrophages, and the surface expression of $CD8\alpha^+$ and major histocompatibility class II I-A/I-E on dendritic cells increased. The $CD4^+/CD8^+$ ratio and number of B cells among splenocytes were also augmented. By using a disease model of parasitic infection, we found that AC-PS treatment inhibited *S. mansoni* infection in BALB/c mice. AC-PS appears to modulate the immune system of mice and has potential for preventing *S. mansoni* infection.

1. Introduction

Antrodia camphorata (AC) is a fungus native to Taiwan, which has been widely used for many years as a medicinal herbal tea to protect against hepatoma and hepatitis virus infection. It is

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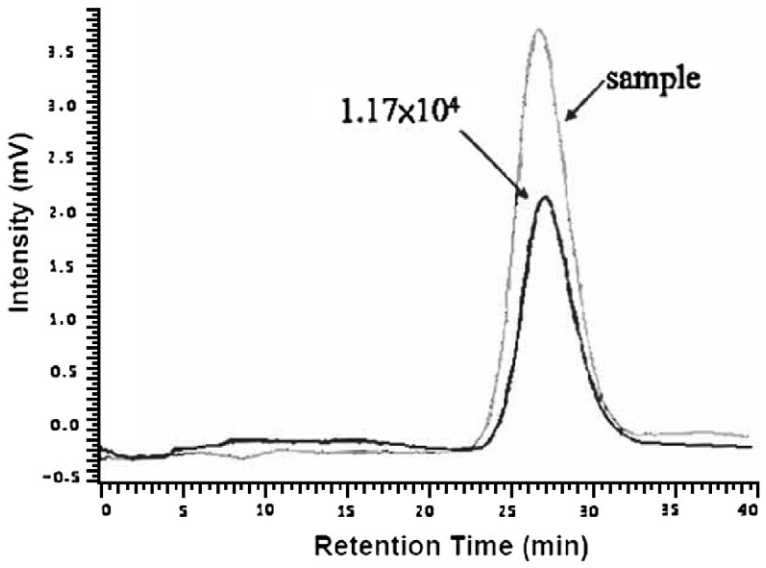

Figure 1 HPLC analysis of polysaccharides extracted from *A. camphorata* mycelia. A lyophilized polysaccharide-containing preparation was dissolved in 10 ml of distilled water and was separated on a PolySep-GFC-P 300×7.8-mm column (Phenomenex) which had been equilibrated with distilled water. The flow rate was 1 ml/min.

reported that several ingredients isolated and derived from AC possess various pharmacological and biological activities. Ethylacetate extract from its fruiting bodies induced apoptosis in hepatoma Hep G2 and PLC/PRF/5 cells via a mitochondrial pathway and NF-κB inhibition [1]. Fermented culture broth of AC induces apoptosis of human leukemic HL-60 cells [2]. Polysaccharides isolated from AC inhibits expression of surface and e antigen of hepatitis B virus [3]. AC also possesses anti-oxidative [4], anti-inflammatory [5] and hepatoprotective properties [6,7]. In an antitumor immunity model, a partially purified polysaccharide from AC was reported to inhibit the proliferation of U937 cells via activation of human mononuclear cells [8].

Dendritic cells (DC) are specialized leukocytes that present antigens to naive Tcells, thus playing a pivotal role in both cell-mediated and humoral immune responses *in vivo* [9]. The exceptional ability of DCs to stimulate T cells both *in vitro* and *in vivo* is attributed, at least in part, to their ability to capture antigens, migrate into lymphoid organs, and express high levels of immunostimulatory molecules such as major histocompatibility complex (MHC) class II, B7.1, B7.2, and interleukin-12 (IL-12) [9,10]. Upon exposure to various microbial and inflammatory products (e.g., lipopolysaccharide, IL-1, and tumor necrosis factor-α (TNF-α)), DCs mature and migrate to lymphoid tissues where they interact with both T and B cells [11–14]. In mouse, 2 subclasses of DCs that differ in CD8α expression have been described, and it has been suggested that $CD8\alpha^+$ DCs could play a role in the regulation of immune responses, while the more common $CD8\alpha^-$ DCs could have a more stimulatory function [15,16].

Schistosomiasis is a chronic parasitic disease that affects more than 200 million people globally [17]. The life cycle of the causative agent, the helminth parasite *Schistosoma spp.*, is complex and initiated by skin penetration of the larvae, followed by rapid transformation into schistosomula [18,19]. In the infected skin lesion, schistosomula closely interact with immunocompetent cells, including DCs, to manipulate the host immune responses [20,21].

The immune responses to the infective larvae of the parasitic helminth *Schistosoma mansoni* remain poorly understood. Jenkins et al. report that the early products released by *Schistosoma* larvae stimulate the maturation of DCs and trigger their Th2-polarizing function [22]. Mice infected with *S. mansoni* developed Th2 polarization, and their Th1 responses were inhibited by IL-10-mediated suppression of IL-12 production [23]. Mongolian gerbils infected with *S. mansoni* showed limited recruitment of DCs around attenuated larvae infection, which may partially contribute to the defective induction of protective immunity by the attenuated vaccine [24]. Smith et al. demonstrated that *S. mansoni* induced anergy in T cells via selective up-regulation of programmed death ligand 1, one of the B7 costimulatory molecules within macrophages, via a macrophage-T-cell contact-dependent mechanism [25]. However, immunization studies suggest that the Th1 type of immune response is more important in the induction of resistance against *S. mansoni* in the murine model. [26,27].

In the present study, we evaluated the immunomodulatory effect of extracts from fermented mycelia of *A. camphorata* (AC) by analyzing the immune phenotypes of DCs, macrophages, and B and T cells *in vivo*. The protective activity of the AC mycelia extract against *S. mansoni* infection was also tested by an optimal administration schedule for immunomodulation.

2. Materials and methods

2.1. Preparation of polysaccharide-enriched fraction from *A. camphorata* extracts

The polysaccharide of *A. camphorata* extracts was isolated based on a previously published method [8]. Briefly, fresh air-dried *A. camphorata* mycelia were obtained from the Biotechnology Center (Grape King Inc., Chungli, Taiwan). Mycelia were filtered through Whatman no. 1 paper with boiling water three times before being air-dried. For the preparation of the aqueous extracts, all air-dried mycelia samples

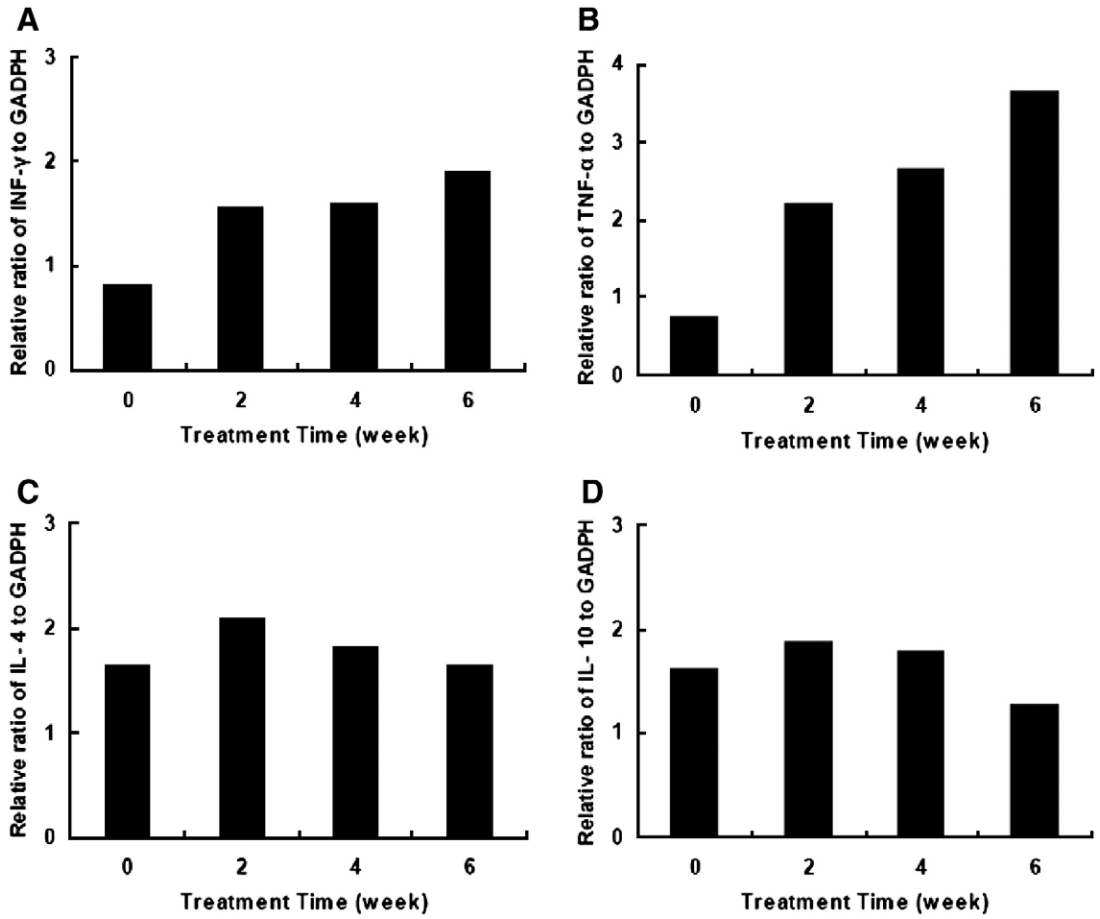

Figure 2 Effects of AC-PS on the mRNA expression of different cytokines in spleen cells. cDNA derived from the splenocytes of mice that were administered 2.5 mg AC-PS orally for 0, 2, 4, and 6 weeks was analyzed for the following by RT-PCR: (A) IFN-γ, (B) TNF-α, (C) IL-4, and (D) IL-10 mRNA. All reactions were normalized to the GADPH levels to ensure that equivalent amounts of template were used. The ratios of cytokine to GADPH expression with the internal control are set at 1.

were ground and then shaken with isotonic phosphate saline buffer (PBS) (154 mM NaCl and 10 mM phosphate buffer at pH 7.4) at a ratio of 1:25 (w/v) at 25 °C for 10 h, then centrifuged at 3000×g for 10 min, followed by passing through a 0.45 μm pore size filter (Pall Life Science, Ann Arbor, MI). The water-soluble polysaccharide-enriched fraction was then isolated by ethanol precipitation from the concentrated extract. The crude polysaccharides obtained were then passed through a PolySep-GFC-P4000 (Phenomenex, Torrance, CA) gel filtration column. The stock solution was lyophilized and stored for treatment. The quantitative analysis of polysaccharide ingredient was performed as previously described [28]. The AC-PS preparations were tested for gram negative bacterial endotoxin contamination by the limulus amebocyte assay QCL-1000 kit (Cambrex, Walkersville, MD) and the protocols were based on the US FDA "Guideline on Validation of the LAL test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices"[29].

2.2. Experimental animals and isolation of splenocytes

Male BALB/c mice (6 to 8-weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animals were provided with water and mouse chow (Labdiet 5001, PMI Nutrition International, LLC, MO) ad libitum and were housed in a rodent facility at 22±1 °C under a 12-h light–dark cycle. The dose of AC polysaccharides (AC-PS) used for these experiments was determined based on a previous publication [8] and the data obtained from our pilot tests. The mice were divided into 4 groups: a control group that received distilled water and those that received AC-PS treatment for 2, 4, or 6 weeks. For treatment, 2.5 mg lyophilized AC-PS dissolved in 0.2 ml distilled water was administered orally daily via intragastric (i.g.) lavage. The mice were sacrificed 72 h after the final administration; the spleens were removed and placed in a petri dish and washed with phosphate-buffered saline (PBS) to flush out the leukocytes. The leukocytes were dispersed in PBS by using a syringe and a 23-G needle to obtain a suspension. Our preliminary work has indicated that the expression of surface markers on DCs and macrophages after the final AC-PS administration was more consistent at 72 h than at 24 h and 120 h. Animal experiments were conducted in accordance with the regulations of the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (DHHS publication No. NIH 85-23, revised 1996).

2.3. Isolation of RNA, cDNA synthesis, and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the isolated spleens by using TRIzol™ reagent (Life Technologies, Carlsbad, CA) and reverse transcribed using Superscript RT II (Life Technologies) to generate cDNA by using reverse transcriptase-polymerase chain reaction (RT-PCR). The protocol used for all the reactions was as follows: 30 cycles at 95 °C for 30 s, 60–65 °C for 30 s, and 72 °C for 1 min. The reaction mixture typically contained 2.5 mM $MgCl_2$, 0.2 mM dNTP, 2.0 U *Taq* polymerase, and 20 pM of 5′ and 3′ oligonucleotide primers (Life Technologies). The following were the sequences of the 5′ and 3′

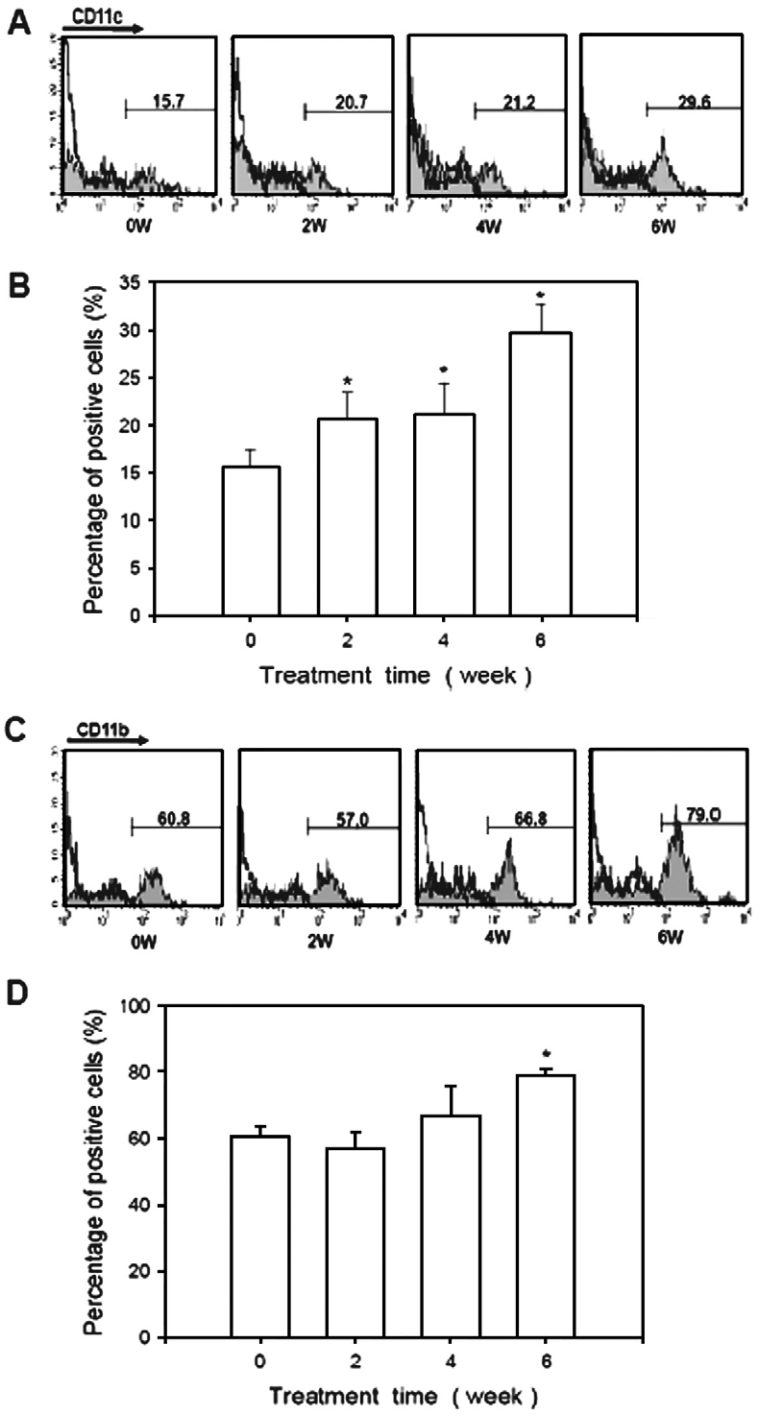

Figure 3 Expression of surface CD11c and CD11b on splenocytes. Splenocytes were identified and analyzed for CD11c or CD11b expression by FACS. For FACS, myeloid cells were gated and the percentages of cells expressing CD11c or CD11b marker were estimated. The histograms and bars depict the percentage of $CD11c^+$ (A and B) and $CD11b^+$ (C and D) cells at different weeks following the AC-PS treatment. The numbers on each histogram represent the mean percentage. Actual percentages of cells were determined for each group. The data shown is representative of a typical result. Cells were stained with mouse IgG1 (gray shade) or CD11c or CD11b mAbs (black line), respectively. Data from 3–5 separate experiments are expressed as mean ± SEM. * $p < 0.05$ when compared with the control at week 0.

primer pairs used for each molecule, respectively: INF-γ, AGCGGCTGACTGAACTCAGATTGTAG and GTCACAGTTTTCAGCTGTATAGGG; IL-4, CCAGCTAGTTGTCATCCTGCTCTTCTTTCTCG and CAGTGATGTGGACTTGGACTCATTCATGGTGC; IL-10, GGACAACATACTGCTAACCGACTC and AAAATCACTCTTCACCTGCTCCAC; TNF-α, GTTCTATGGCCCAGACCCTCACA and TCCCAGGTATATGGGTTCATACC; GADPH, CCACCTTCTTGATGTCATCA and TATTGGGCGCCTGGTCACCA. The PCR products were run on agarose gels and visualized by ethidium bromide staining. Band intensity was analyzed using Quantity One 1-D analysis software (Bio-Rad Laboratories Inc, Hercules, CA).

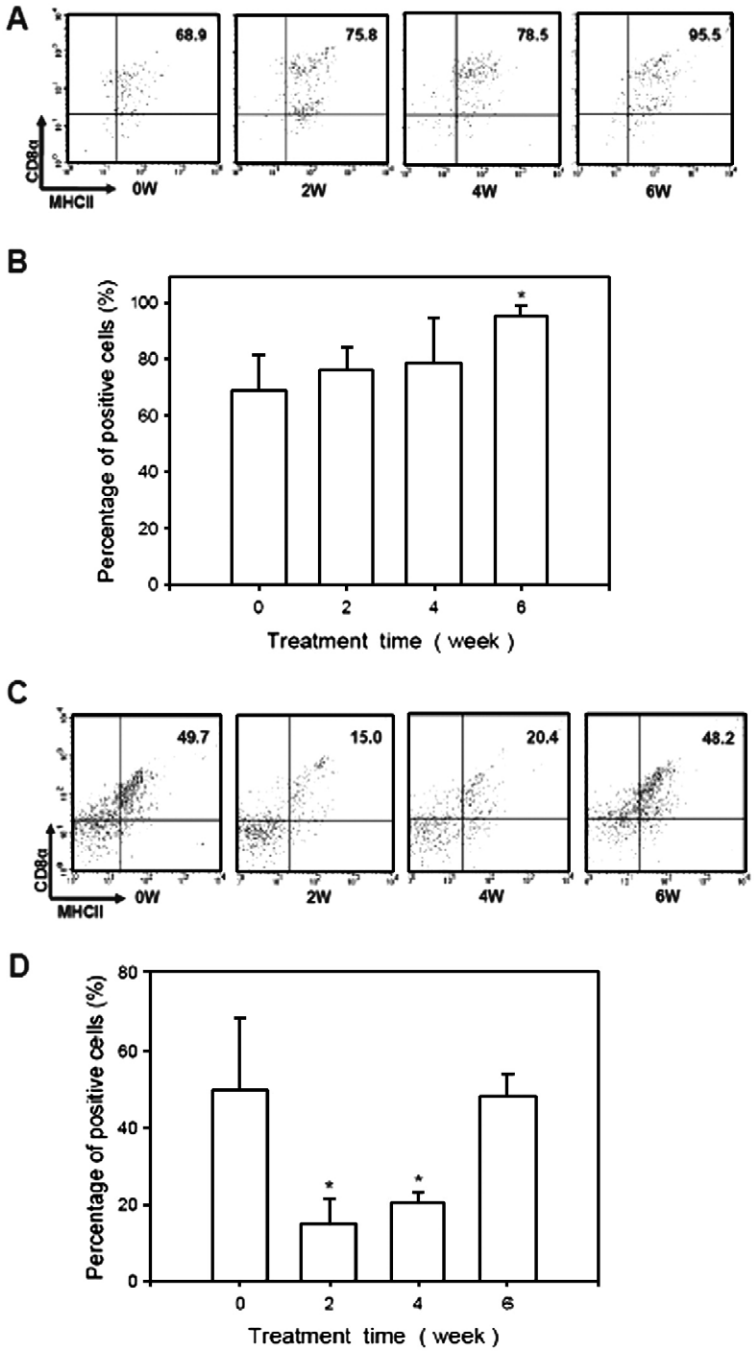

Figure 4 Expression of surface CD8α and the MHC class II molecule I-A/I-E on $CD11c^+$ and $CD11b^+$ splenocytes. Splenocytes were identified and analyzed for MHC class II I-A/I-E and CD8α protein coexpression by flow cytometry. For FACS, $CD11c^+$ or $CD11b^+$ cells were gated and the percentage of cells coexpressing CD8α and I-A/I-E was estimated. Data of I-A/I-E /$CD8\alpha^-$ coexpressing $CD11c^+$ (A) and $CD11b^+$ (C) cells at different weeks following the AC-PS treatment are shown as dot blots from all the analyzed samples with one representative mouse per time point. The quadrants were set according to the unstained controls. Numbers in the right upper quadrant show the cell frequency in each of the 4 quadrants from the total number of gated cells. Bars depict the percentage I-A/I-E /$CD8\alpha^-$ coexpressing $CD11c^+$ (B) and $CD11b^+$ (D) cells. Actual percentages of the cells were determined for each group. The data shown is representative of a typical result. Data from 3–5 separate experiments are expressed as mean ± SEM. * $p<0.05$ when compared with control at week 0.

2.4. Flow cytometric analysis

Three-color immunolabelling was performed using fluorochrome isothiocyanate (FITC)-, phycoerythrin (PE)-, Cy-chrome (Cy)-conjugated monoclonal antibody and appropriate isotype controls were purchased from Serotec (Pharmingen, San Diego, CA) and used for characterization of DC, macrophages and T and B cells as follows: anti-CD11c-FITC, anti-CD11b-FITC, anti-MHC class II (I-A/I-E)-PE, anti-CD8α-Cy, anti-CD4-FITC and anti-CD45R/B220-FITC. Isolated cells were blocked with rat anti-mouse CD16/CD32 antibody (2.4G2, Pharmingen, San Diego, Calif.) in 1×PBS for 30 min on ice to reduce nonspecific antibody binding by Fc receptors and then incubated with

Table 1 Expression of surface CD4 and CD8 on splenocytes

	$CD4^+$	$CD8^+$ (%)	CD4/CD8 ratio
0 week	35.8±3.0	23.6±1.0	1.5
2 week	33.0±2.8	19.0±2.7*	1.7
4 week	36.9±3.1	18.9±1.4*	2.2
6 week	32.0±2.8	14.6±1.1*	2.4

Splenocytes from variously treated mice (administration of AC-PS for 0–6 weeks) were harvested and further incubated with monoclonal antibodies raised against CD4 followed by FITC-conjugated IgG and Cy5-conjugated anti-mouse CD8 at 4 °C for 30 min. After washing twice with PBS, cells were applied to a FACS caliber flow cytometer. For cytometric analysis, lymphocytes were gated and the percentage of cells expressing CD4 or CD8 marker respectively were estimated. Data from 3 to 5 separate experiments are expressed as mean±SEM. * $p<0.05$ when compared with control at week 0.

5 μg/ml concentrations of color-conjugated monoclonal antibody and primary monoclonal antibody, followed by immunoglobulin G (IgG)-FITC at 4 °C for 30 min. After washing twice with PBS, 1×10^6 cells were applied to a FACS flow cytometer (Becton Dickenson Biosciences, San Jose, CA). Data were collected and analyzed using CellQuest Software (Becton Dickenson Biosciences).

2.5. Infection with *S. mansoni* and assessment of infection rate

The BALB/c mice were divided into control ($n=10$) and AC-PS treatment (2, 4, and 6 weeks) groups ($n=10$ for each group). For treatment, 2.5 mg lyophilized AC-PS dissolved in 0.2 ml distilled water was administered orally daily via i.g. lavage. Each mouse was infected with 120 *S. mansoni* cercariae, freshly isolated from their intermediate host *Biomphalaria glabrata*, via the tail vein. After 8 weeks, the adult worms in the portal and mesenteric veins were isolated and counted. Another group of mice ($n=5$) were infected with *S. mansoni* for flow cytometry (FACS) analysis experiments. Splenocytes from the infected mice were obtained 8 weeks later, and the modulation of cell type and coexpression of markers were analyzed by FACS.

2.6. Statistics

Data were expressed as mean±standard error of the mean (SEM). Analysis of variance was used to analyze the dif0ferences in surface marker expression between various treatment groups and controls. Statistical differences between groups were determined using Student's *t* test. A *p* value of, <0.05 was considered significant.

3. Result

3.1. Quantitative and endotoxin analysis of polysaccharides of *A. camphorata*

The results showed that the lyophilized AC-PS have a molecular weight of 1.17×10^4 Daltons (Fig. 1). In addition, the quantitative percentage of polysaccharide in the lyophilized extract of AC mycelia was 5.24%. The eluted fractions were assayed for hexose by using the phenol-sulfuric acid method. The carbohydrate content of the lyophilized extract of AC mycelia was found to be 96.4%. To ascertain that the immunomodulatory effects of AC-PS were not due to endotoxin contamination, the lyophilized AC-PS were tested by the limulus amebocyte lysate (LAL) assay; the

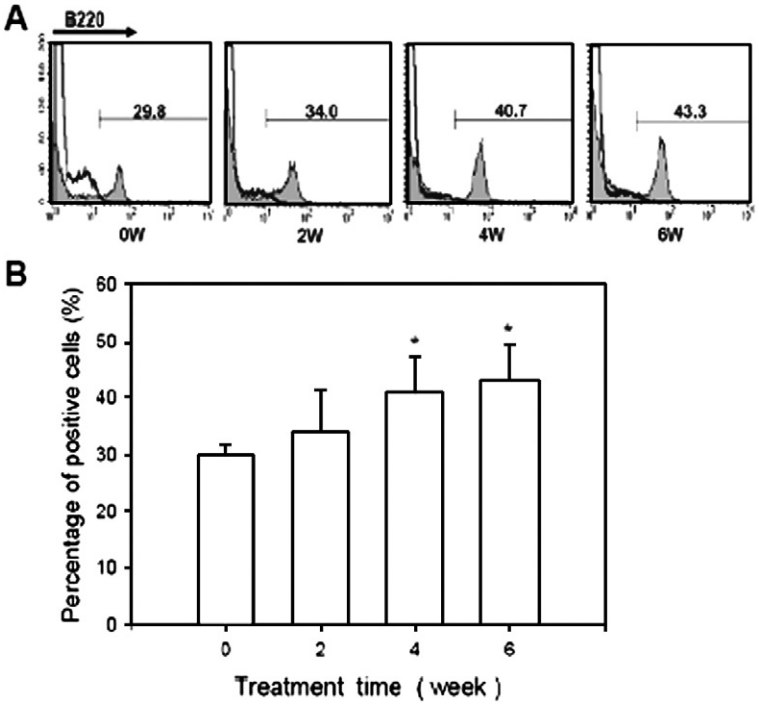

Figure 5 Expression of surface CD45R/B220 on splenocytes. Splenocytes were identified and analyzed for CD45R/B220 expression by FACS. For FACS, lymphocytes were gated and the percentages of cells expressing CD45R/B220 marker were estimated. The histograms and bars depict the percentage of CD45R/B220 marker (A and B) cells at different weeks following the AC-PS treatment. The numbers on each histogram represent mean percentage. Actual percentages of cells were determined for each group. The data shown is representative of a typical result. The cells are stained with mouse IgG1 (gray shade) or CD45R/B220 mAbs (black line), respectively. Data from 3–5 separate experiments are expressed as mean±SEM. * $p<0.05$ when compared with control at week 0.

Table 2 Effect of AC-PS on *Schistosoma mansoni* infection

Oral administration	Worm burden
0 week	61.3±5.7
2 week	45.0±4.8*
4 week	32.1±3.3*
6 week	15.6±3.5*

BALB/C mice after administration of Ac-PS for 0–6 weeks were infected with 120 *S. mansoni* cercariae. Eight weeks after challenge, all mice were sacrificed concurrently and the adult worm was counted. Data from 3 separate experiments are expressed as mean±SEM. *$p<0.05$ when compared with control at week 0.

protocols were based on the U.S. Food and Drug Association (FDA) "Guideline on validation of the LAL test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices" [U. S. FDA, [29]]. The concentration of endotoxin in a sample is directly proportional to the absorbance and is calculated from a standard curve. The endotoxin concentration in our sample was significantly below the limits approved by the U.S. FDA Devices. Thus, the influence of endotoxin contamination on immunomodulation could be disregarded.

3.2. Cytokine mRNA expression in splenocytes induced by AC-PS

To investigate the immunomodulating effects of AC-PS, BALB/c mice were fed on AC-PS for 2, 4, and 6 weeks, and then the cytokine mRNA expression levels for interferon-γ (IFN-γ), TNF-α, IL-4, and IL-10 in the splenocytes were observed (Fig. 2). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is expressed at relatively constant levels in cells, and the panel shows the relative ratio of each cytokine to GADPH. After the mice were fed with AC-PS, large amounts of IFN-γ and TNF-α mRNA were induced at 2, 4, and 6 weeks (Fig. 2A and B). The level of gene expression was relatively stronger in the group of mice fed with AC-PS once a day for 6 weeks. However, the data showed no significant change in IL-4 and IL-10 expression after the mice were treated with AC-PS (Fig. 2C and D). These results suggest that splenocytes were induced toward a Th1 response (IFN-γ and TNF-α) after the mice were treated with AC-PS.

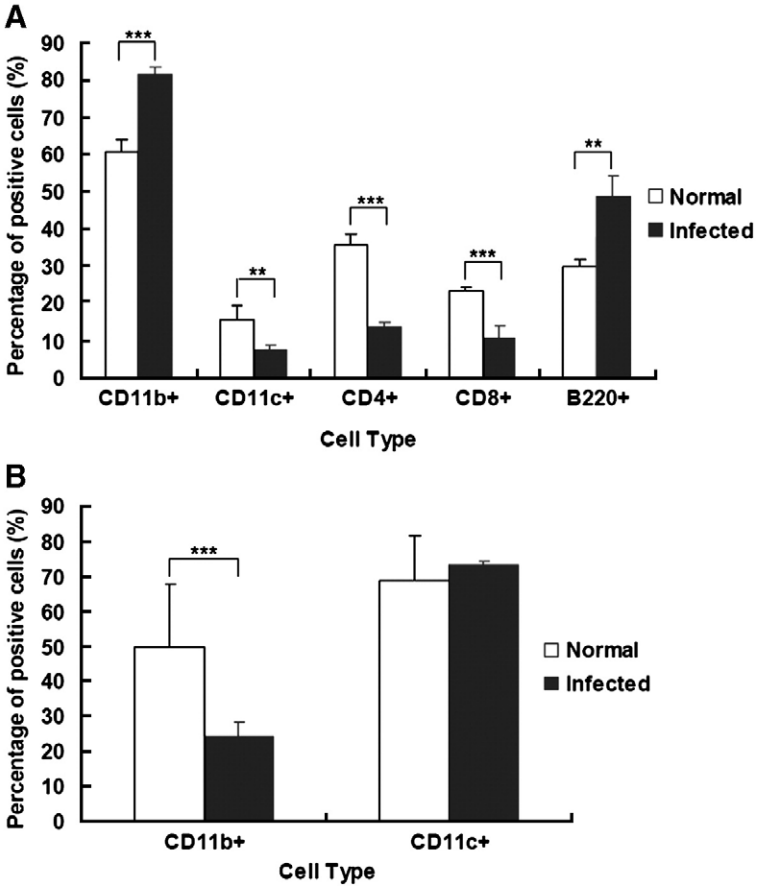

Figure 6 Expression of different surface molecules on splenocytes 8 weeks after schistosoma infection. Splenocytes were identified and analyzed for protein and coexpression by FACS. For FACS, (A) myeloid cells or lymphocytes were gated and the percentages of cells expressing positive markers were estimated. Bars depict the percentage of $CD11c^+$, $CD11b^+$, $CD4^+$, $CD8^+$, or $B220^+$ cells from mice of schistosoma infection. (B) $CD11c^+$ or $CD11b^+$ cells were gated and the percentage of cells coexpressing CD8α and I-A/I-E was estimated. Bars depict the percentage I-A/I-E /CD8α-coexpressing $CD11c^+$ and $CD11b^+$ cells from schistosoma infected mice. Actual percentages of cells were determined for each group. Data from 3–5 separate experiments are expressed as mean±SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared with control sample respectively.

3.3. Expression of surface CD11c and CD11b on splenocytes

The expression of surface CD11c and CD11b increased on splenocytes from AC-PS-treated mice in a time-dependent manner. The number of $CD11c^+$ cells in control group was 15.7±1.8% and significantly increased to 20.7±2.8%, 21.2±3.3% and 29.6±3.1% with 2, 4, and 6 weeks of AC-PS administration, respectively ($p<0.05$) (Fig. 3A and B). Whereas the number of $CD11b^+$ cells increased from 60.8±3.4% without AC-PS treatment to 79.0±2.0% with 6 weeks of treatment (Fig. 3C and D).

3.4. Modulation by AC-PS of surface marker expression on DCs and macrophages

In $CD11c^+$ splenocytes, the putative DCs, identified by coexpression of CD8α and MHC class II molecule I-A/I-E was augmented by *in vivo* treatment of AC-PS (Fig. 4A and B). In contrast, in the putative $CD11b^+$ macrophages the coexpression of these two molecules decreased after 2 and 4 weeks of AC-PS treatment and recovered to the control level after 6 weeks of treatment (Fig. 4C and D).

3.5. Effect of AC-PS on the $CD4^+/CD8^+$ ratio of splenocytes and the number of B cells

The $CD4^+/CD8^+$ ratio of splenocytes in control group was 1.5. This ratio increased to 2.19 after a 6 week treatment of AC-PS in a time-dependent manner (Table 1). In splenocytes from AC-PS-treated mice, the number of $CD45R/B220^+$ B cells also increased from 29.8±1.9% to 43.3±6.0% in a time-dependent manner ($p<0.05$) (Fig. 5A and B).

3.6. Effect of AC-PS on the Infection of *S. mansoni*

After 2, 4, and 6 weeks treatment of AC-PS, the infection of *S. mansoni* was significantly inhibited in a time-dependent manner with repeated treatments. As shown in Table 2, the worm burden of BALB/c mice in 2, 4, to 6 weeks AC-PS-treated groups (45.0±4.8, 32.1±3.3 and 15.6±3.5 worms) were all less than the control group (61.3±5.7 worms) and such differences were statistically significant ($p<0.05$).

3.7. Influence of *S. mansoni* infection on cell populations and surface marker expression

To understand the influence of *S. mansoni* infection on splenic DCs, macrophages, and T and B cells at 8 weeks after the infection, we analyzed the percentage of cell types and coexpression of CD8α and MHC class II molecule I-A/I-E by FACS. The expression of surface CD11b and B220 increased, while that of CD11c, CD4, and CD8 were decreased in splenocytes at 8 weeks after *S. mansoni* infection (Fig. 6A). The $CD4^+/CD8^+$ ratio of splenocytes had also decreased from 1.5 to 1.2. In $CD11c^+$ splenocytes, the coexpression of CD8α and MHC class II molecule I-A/I-E was not statistically different after *S. mansoni* infection. In contrast, in the $CD11b^+$ macrophages, the coexpression of these 2 molecules was significantly inhibited after 8 weeks of *S. mansoni* infection (Fig. 6B).

4. Discussion

The present study revealed that AC, a medicinal fungus with a known inhibitory effect on hepatitis B virus and cancers, modulates the expression of CD8α and MHC class II I-A/I-E on splenic DCs and macrophages as well as the number of T and B lymphocytes. Furthermore, treatment of BALB/c mice with AC-PS was successful in reducing the infection rate of *S. mansoni*.

Polysaccharides from mushrooms have potential antitumor and immunomodulatory effects that are mediated through enhanced cytokine secretion by T cells [30,31]. Previous reports have indicated that most polysaccharides induce a Th1-dominant state via IL-2 or IFN-γ induction [32,33]. The present study clearly indicates that AC-PS are potent inducers of Th1-type cytokines such as IFN-γ and TNF-α in a time-dependent manner but not of Th2 cytokines. These results suggest that the splenocytes were induced toward a Th1 response following treatment with AC. Previous reports indicate that AC-PS inhibit inflammation by modulating the pro-inflammatory cytokines [34] and elicit an antitumor effect by promoting the Th1-dominant state and natural killer cell activities [8]. The level of TNF-α is also related to inflammatory and immunologic responses during schistosoma infection; this constitutes direct proof of causality [35].

It has been reported that mice splenic DCs that express high levels of CD8α and $CD8\alpha^+$ cells are the dominant population of splenic DCs expressing MHC class II I-A/I-E molecules [36]. Previous studies suggest that the Th1/Th2 balance *in vivo* is regulated by the DCs involved in the primary immune response. $CD8\alpha^+$ DCs trigger the development of Th1-type cells, while the $CD8\alpha^-$ DCs induce the Th2-type response [37–39]. We found that AC-PS treatment *in vivo* augmented the number of both $CD8\alpha^+$ and $I\text{-}A/I\text{-}E^+$ cells, implying that AC-PS may enhance the antigen presenting capability of splenic DCs.

$CD4^+$ Th cells are important regulators of immune system [40]. These cells secrete a series of cytokines to initiate antibody production from B cells and enhance the production of $CD8^+$ cytotoxic T cells [41]. The $CD4^+/CD8^+$ ratio is one of major indicators for assessing the function of cell-mediated immunity. AC-PS significantly promoted the $CD4^+/CD8^+$ ratio and B cell numbers. These results indicate that AC-PS have marked induction effects on increasing the $CD4^+$ T and B cells involved in adaptive immunity.

Murine *S. mansoni* infection demonstrates the development of Th2 polarization in which the Th1 responses are suppressed [23]. It has been suggested that the ability to induce an ineffective Th2-like response may have evolved in schistosomes as an intelligent defense mechanism to ensure that the Th2 cells produced sufficient levels of IL-10 to inhibit protective immunity mediated by the Th1-like subset of the cells involved in $CD4^+$ T responses [18,42]. Our data also indicated a sharp drop in the percentage of splenic $CD4^+$, $CD8^+$, and $CD11c^+$ cells after schistosomal infection, while an increase in B and $CD11b^+$ cells was noted. Relative to the total splenocyte population, both the $CD4^+$ and $CD8^+$ T cell subsets were reduced during schistosoma infection, while B cell expansion was evident [43] On the other hand, the coexpression of CD8α and the MHC class II molecule I-A/I-E on putative $CD11b^+$ macrophages was significantly inhibited after 8 weeks of *S. mansoni* infection, while coexpression on the $CD11c^+$ DCs was not. Although DCs pulsed with soluble egg antigen (SEA) are capable of strongly inducing Th2-polarized SEA-specific responses, $CD8\alpha^+$ DCs are more potent IL-12 producers in response to toll-like receptor (TLR) ligands and Th1 cells in infected animals [44].

AC-PS modulated the immunity and inhibited infection with *S. mansoni*. These activities may correlate with each other; however, their possible mechanisms remain unknown. *S. mansoni* induces anergy of T cells via a macrophage-T cell contact-dependent mechanism [25]. Because AC-PS augmented the expression of MHC class II molecule I-A/I-E and CD8α^+ on macrophages and DCs and concurrently increased the CD4$^+$/CD8$^+$ ratio, AC-PS may inhibit the development of schistosomiasis via immunomodulation of CD8α^+ DCs by promoting a Th1-dominant state. Predominant Th1 responses are important for killer activities against the cercariae and schistosomules stages, which are more susceptible to immune attack [42]. Since the present study revealed modulation of splenic DCs and T cells by AC-PS in uninfected mice, the effect of AC-PS on the immune responses of mice infected with *S. mansoni*, including DC maturation and Th1/Th2 predominance, are currently under investigation.

Our data suggest that AC modulates the expression of CD8α and the MHC class II molecule I-A/I-E in splenic DCs and macrophages; it also modulates the CD4$^+$/CD8$^+$ ratio of T cells and the number of B lymphocytes. Treatment of BALB/c mice with AC-PS reduced the infection rate of *S. mansoni in vivo*. This raises an interesting possibility of AC having therapeutic potential in the treatment or prevention of disorders involving immune responses, such as parasitic infections.

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