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In vivo immunomodulatory effects of *Antrodia camphorata* polysaccharides in a T1/T2 doubly transgenic mouse model for inhibiting infection of *Schistosoma mansoni*

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Abstract

Antrodia camphorata (*A. camphorata*) is a fungus commonly used for treatment of viral hepatitis and cancer in Chinese folk medicine. Extract of *A. camphorate* is reported to possess anti-inflammatory, antihepatitis B virus and anticancer activities. In this study, we tested the *in vivo* effects of polysaccharides derived from *A. camphorata* (AC-PS) on immune function by detection of cytokine expression and evaluation of the immune phenotype in a T1/T2 doubly transgenic mouse model. The protective effect of AC-PS in mice was tested by infection with *Schistosoma mansoni*. The induction of large amounts of IFN-γ, IL-2 and TNF-á mRNA were detected after 2 and 4 weeks of oral AC-PS administration in BALB/c and C57BL/6 mice. In transgenic mice, 3 to 6 weeks of oral AC-PS administration increased the proportion of $CD4^+$ T cells and B cells within the spleen. More specifically, there was an increase of Th1 $CD4^+$ T cells and Be1 cells among spleen cells as observed by detection the of Type1/Type2 marker molecules. By using a disease model of parasitic infection, we found that AC-PS treatment inhibited infection with *S. mansoni* in BALB/C and C57BL/6 mice. AC-PS appears to influence the immune system of mice into developing Th1 responses and have potential for preventing infection with *S. mansoni*.

Keywords: *Antrodia camphorata*; Immunomodulation; T1/T2 doubly transgenic mice; *Schistosoma mansoni*

Introduction

Antrodia camphorata, a new basidiomycete which grows on the heartwood of *Cinnamomum kanehirai*, has been widely used as a medicinal herb to protect against hepatoma and hepatitis virus infection in Taiwan. It is reported that several ingredients derived from *A. camphorata* 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 (Hsu et al., 2005). Furthermore, fermented culture broth of *A. camphorata* induced apoptosis of human leukemic HL-60 cells (Hseu et al., 2004). *A. camphorata* also possesses antioxidative (Hseu et al., 2002), antiinflammatory (Shen et al., 2004; Hseu et al., 2005) and hepatoprotective properties (Hsiao et al., 2003; Song and Yen, 2003; Lu et al., 2007). In an antitumor immunity model, partially purified polysaccharides from *A. camphorata* were reported to inhibit the proliferation of U937 cells via activation of human mononuclear cells (Liu et al., 2004). The polysaccharides derived from *A. camphorata* (AC-PS) not only inhibited expression of surface antigen of hepatitis B virus (Lee et al., 2002), but also led to the suppression of angiogenesis (Chen et al., 2005; Cheng et al., 2005).

It has been shown that traditional medicinal mushrooms administered under different conditions may induce different

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types of immune responses (Wasser, 2002). The polysaccharide-enriched fraction from *Ganoderma lucidum* had strong stimulatory effects on both macrophages and T-lymphocytes for various cytokines releasing activity (Wang et al., 1997). Extracts of *G. lucidum* are capable of stimulating both Th1 and Th2 cytokine mRNA expression (Chan et al., 2005). The immunomodulatory effects of *Astragalus mongholicus* polysaccharides result from the promotion of dendritic cell-mediated immunity and the modulation of cytokine production (Shao et al., 2006). However, limited information is available on the active ingredients and mechanisms of action of *A. camphorata* underlying its effect on immune function. It is interesting and worthwhile to investigate the actions of *A. camphorata* in regulating immune responses against pathogens.

Th1 and Th2 cells appear to counter-regulate each other, as shown by the suppressive effect of IFN-γ on Th2 development and of IL-4 on Th1 development. This can lead to the predominance of one Th-type in the environment during immune responses against tumors or pathogens (Abbas et al., 1996; Bonecchi et al., 1998). To directly detect Th1/Th2 cells *in vivo*, T1/T2 doubly transgenic mice have been generated. These mice carry two transgenes that express two distinct cell-surface markers: a human *Thy1* transgene (*hThy1*), designated as T1, under the control of the murine *IFN-γ* promoter and a murine *Thy1.1* transgene (*mThy1.1*), designated as T2, under the control of the murine *IL-4* promoter. These transgenic mice have been used previously to monitor the *in vivo* development of Th1 or Th2 cells during infections caused by *Listeria monocytogenes* or *Schistosoma mansoni* (Hsieh et al., 2000). Thus, these T1/T2 doubly transgenic mice provide a valuable model for tracing Th1 or Th2 cells *in vivo*. In an attempt to assess the potential effects of *A. camphorate* on Th-type development and function, *in vivo* experiments on T1/T2 doubly transgenic mice were conducted with polysaccharides derived from *A. camphorata*.

Schistosomiasis is a chronic parasitic disease that affects more than 200 million people world wide (WHO Expert Committee of the Control of Schistosomiasis, 1993). The life cycle of the causative agent, the helminth parasite *Schistosoma* spp., is initiated by skin penetration of the larvae followed by its rapid transformation into schistosomula (MacDonald et al., 2002; Pearce and MacDonald, 2002). In the infected skin lesion, schistosomulum closely interacts with immunocompetent cells to manipulate the host's immune responses (Ramaswamy et al., 2000; Angeli et al., 2001; Jenkins and Mountford, 2005). The immune events elicited in response to the infective larvae of the parasitic helminth *Schistosoma mansoni* remain poorly clarified. Mice infected with *S. mansoni* developed Th2 polarization in which Th1 responses were prevented by IL-10-mediated suppression of IL-12 production (Jenkins and Mountford, 2005). A previous report had presented evidence that worm eggs are crucial for the generation of an optimal Th2 response and for the subsequent liver pathology that develops in infected mice (Faveeuw et al., 2002). Immunization studies, however, suggested that these Th2 responses may not provide protective immunity; instead, the Th1-type immune response appears to be important in the induction of resistance against *S. mansoni* in the murine model (Zhou et al., 2000; Fonseca et al., 2004).

In the present study, we evaluated the immunomodulatory effect of the polysaccharide-enriched fraction from the extracts of wild, air-dried *A. camphorata* mycelia by analysis of cytokine production in healthy mice *in vivo*. We then further analyzed immune phenotypes of B and T cells in the T1/T2 doubly transgenic mouse model to estimate the enhancement of Th development during immunoregulatory activity. Finally, we also examined the protective activity of AC-PS on resistance to infection with *S. mansoni*.

Materials and methods

Preparation of polysaccharide-enriched fraction from A. camphorata extract (AC-PS). The polysaccharides of *A. camphorata* were isolated based on a previous method with little modification (Liu et al., 2004). Briefly, fresh air-dried *A. camphorata* mycelia were obtained from the Biotechnology Center (Grape King Inc., Chungli, Taiwan). Mycelia were filtered through Whatman #1 paper with boiling water three times before being air-dried. For preparation of the aqueous extracts, all air-dried mycelia samples 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. The water-soluble polysaccharide-enriched fraction was then isolated by ethanol precipitation from the concentrated extract. The resulting crude polysaccharides were then passed through a PolySep-GFC-P4000 (Phenomenex, Torrance, CA) gel filtration column. The stock solution was then lyophilized and stored for treatment. The quantitative analysis of *A. camphorata* polysaccharides was conducted as previously described (Chaplin and Kennedy, 1994). The eluted fractions were assayed for hexose by the phenol-sulfuric acid method and the percentage of carbohydrate content in lyophilized extract of *A. camphorata* mycelia was 96.4%. The quantitative percentage of polysaccharides in the extract was 5.24%. Briefly, the polysaccharides 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" (U. S. FDA, 1987). Concentration of endotoxin in the sample is in direct proportion with absorbance and is calculated from a standard curve. The concentration found was significantly below the following limits are approved by the US FDA Devices. So the influence of endotoxin contamination could be ignored.

Experimental animal treatment. Male BALB/c or C57BL/6 mice (6–8 weeks old) were obtained from the National Laboratory Animal Center (Taipei,

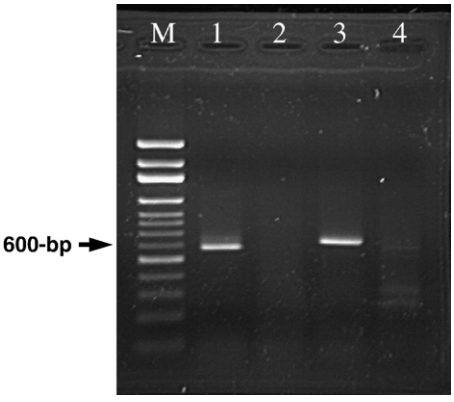

Fig. 1. Screening of T1/T2 doubly transgenic mice. T1/T2 doubly transgenic mice were screened using PCR with 0.2 μg tail DNA as the PCR template. Both T1- and T2-amplified DNA fragments were screened from the mice used in this study. A representative experiment of 3 trials is displayed. Lane M, molecular size marker; lanes 1 and 3, positive results of screening T1/T2 doubly transgenic mice; and lanes 2 and 4, negative results of screening non-T1/T2 transgenic mice.

Taiwan). The IFN-γ:hThy1, IL-4:mThy1.1 double transgenic mice (T1/T2 doubly transgenic mice) were generated on a BALB/c background as previously described (Hsieh et al., 2000). Transgenic mice were also screened by PCR as reported previously (Hsieh et al., 2000). In brief, primers Tg1 and Tg2 were used to amplify the *mIFN-γ: hThy1* transgene (T1), and primers Tg3 and Tg4 were used to amplify the *mIL-4:mThy1.1* transgene (T2). The DNA sequences of the PCR primers were as follows: Tg1, 5′-GCTGTCTCATCGTCAGAGAGC-3′; Tg2, 5′-TCAAGGACAGGAGATCTTAGGG-3′; Tg3, 5′-CCAAGATATCA-GAGTTTCCAAGG-3′; and Tg4, 5′-AGAGGCTACTTCCCGGGATG-3′. A 600 bp DNA fragment was amplified by primers Tg1/Tg2, and another 600 bp DNA fragment was also amplified by primers Tg3/Tg4 (see Fig. 1).

The transgenic mice were subsequently maintained at the Experimental Animal Facility of the Institute of Tropic Medicine, National Yang-Ming University following the Guidelines of the National Science Council of the Republic of China. 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 with a 12-h light–dark cycle for acclimatization. The dose of AC-PS used for these experiments was decided by referring to previously publication (Liu et al., 2004) and the data of our pilot tests. The BALB/c or C57BL/6 mice were divided into six groups that received either 1.0 or 2.5 mg of lyophilized AC-PS dissolved in 0.2 ml of distilled water once a day i.g. for 2 and 4 weeks plus 2 control groups that received untreated distilled water for the same periods of time. In addition, the T1/T2 doubly transgenic mice were divided into six groups that received 2.5 mg of lyophilized AC-PS dissolved in 0.2 ml of distilled water once a day i.g. for 2, 3 ,4, 5 and 6 weeks, plus a control that received distilled water.

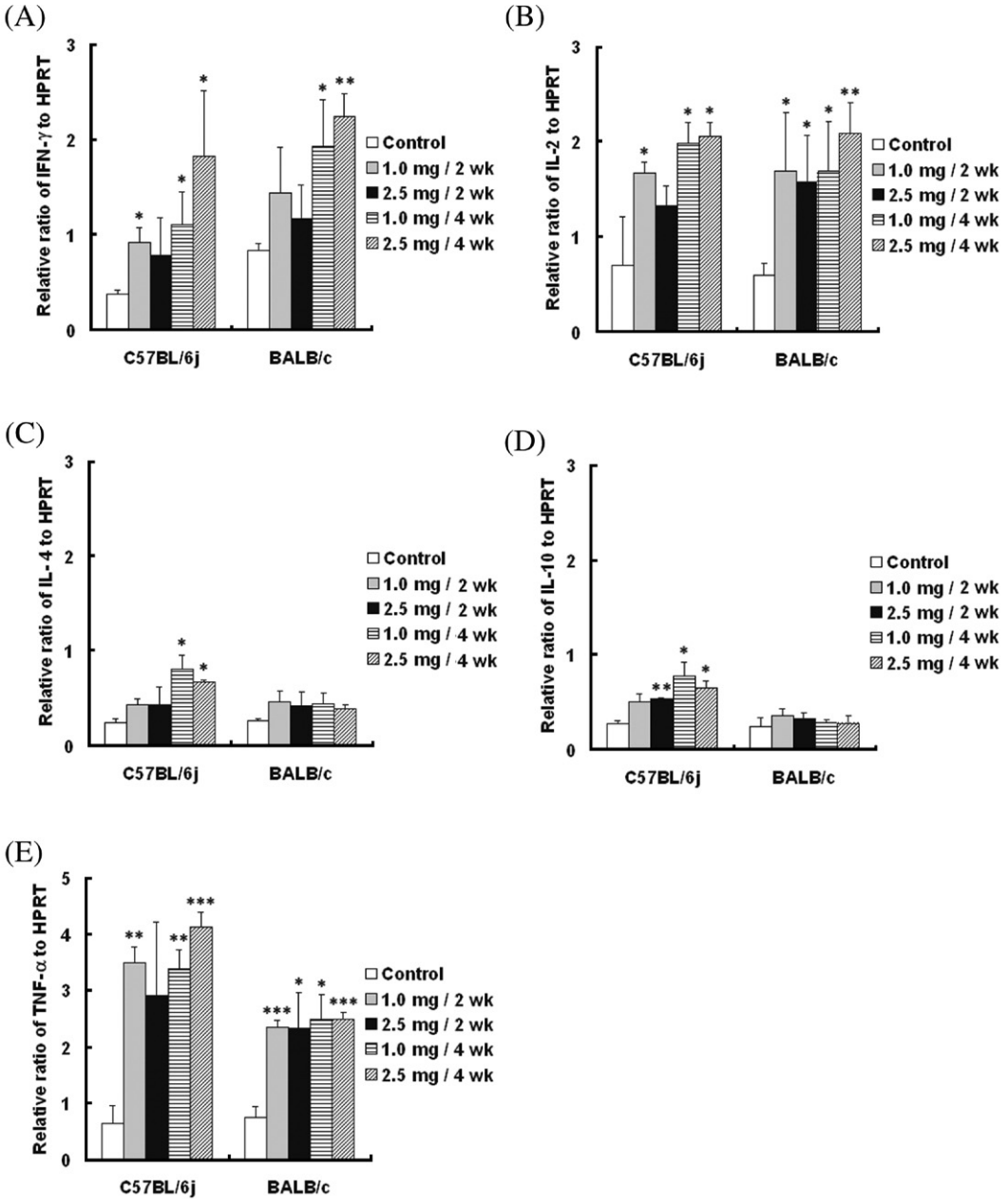

Fig. 2. Effects of AC-PS on different cytokine mRNA expression in spleen cells of C57BL/6j and BALB/c mice. cDNA derived from the splenocytes of mice who received oral administration of 1.0 or 2.5 mg AC-PS for 0, 2, and 4 weeks was analyzed for (A) IFN-γ, (B) IL-2, (C) IL-4, (D) IL-10 and (E) TNF-α mRNA by RT-PCR. All reactions were normalized to HPRT levels to ensure equivalent amounts of template were used. The ratios of cytokines to HPRT expression with the internal control are set at one. The traces represent the mean from three independent experiments, which are expressed as mean ± SD. *$p<0.05$, ** $p<0.01$, and ***$p<0.001$ when compared with control at week 0.

Isolation of splenocytes. Immediately following the final treatment, the mice were sacrificed and the spleen was removed and placed in a Petri dish and then washed to flush out the leucocytes. The leukocytes were dispersed using a PBS-filled syringe equipped with a 23-G needle. Residual red blood cells were hypotonically lysed in PBS containing 150 mM ammonium chloride, 1 mM potassium bicarbonate, and 0.1 mM ethylenediaminetetraacetic acid (EDTA) (all from Sigma-Aldrich, St. Louis, MO). The intact leukocytes were recovered by centrifugation (at 500×*g*, for 3 min, 4 °C) and resuspended for later use. Routinely, 95% of the isolated cells were viable, as determined by the exclusion of Trypan Blue. Animal experiments were conducted in accordance with NIH *Guide for the Care and Use of Laboratory Animals* (DHHS publication No. NIH 85-23, revised 1996) regulation.

Isolation of RNA, cDNA synthesis, and reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was extracted from isolated spleen using TRIzol™ reagent (Life Technologies, Carlsbad, CA) and reverse transcribed using Superscript RT II (Life Technologies) to generate cDNA for use in RT-PCR. Conditions used for all reactions were 30 cycles of 95 °C for 30 s, 60–65 °C for 30 s, and 72 °C for 1 min. Reactions typically contained 2.5 mM $MgCl_2$, 0.2 mM dNTP, 2.0 U Taq polymerase, and 20 pM 5′ and 3′ oligonucleotide primers (Life Technologies). The sequences of the primer pairs used, 5′ and 3′, respectively for each pair, were: INF-γ, AGCGGCTGACTGAACTCAGATTG-TAG and GTCACAGTTTTCAGCTGTATAGGG; IL-2, TGATGGACCTA-CAGGAGCTCCTGAG and GAGTCAAATCCAGAACATGCCGCAGCAG, IL-4, CCAGCTAGTTGTCATCCTGCTCTTCTTTCTCG and CAGT-GATGTGGACTTGGACTCATTCATGGTGC, IL-10, GGACAACATACTGC-TAACCGACTC and AAAATCACTCTTCACCTGCTCCAC, TNF-α, GTTCTATGGCCCAGACCCTCACA and TCCCAGGTATATGGGTTCA-TACC, The hypoxanthine phosphoribosyl transferase gene (HPRT), GTT-GGATACAGGCCAGACTTTGTTG and GAGGGTAGGCTGGCCTATGGCT. PCRs were run on agarose gels and visualized by ethidium bromide staining.

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 type-1, 2 helper T (Th1, Th2) and type-1, 2 effector B (Be1, Be2) cells as follows: anti-CD4-FITC, anti- CD45R/B220-FITC, anti-human CD90-PE, anti-CD8á-Cy, strepavidin-Cy and anti-mouse thy1.1-biotin. Isolated cells were blocked with rat anti-mouse CD16/CD32 antibody (2.4G2, Pharmingen, San Diego, Calif.) in PBS for 30 min on ice to reduce nonspecific antibody binding by Fc receptors and then incubated with 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) and the data were collected and analyzed using CellQuest Software (Becton Dickenson Biosciences).

Infection with S. mansoni and assessment of infection rate. BALB/c or C57BL/6 mice were divided into control ($n=10$) and AC-PS treatment (1, 2, and 4 weeks) groups ($n=10$). We had previously determined via pilot studies the treatment regime that induced an optimal immune response. For treatment, 2.5 mg lyophilized AC-PS dissolved in 0.2 ml distilled water was administered i.g. daily. At the indicated time points after the AC-PS treatment, each mouse from all the groups was infected with 120 *S. mansoni* cercariae via the tail vein. The cercariae were freshly isolated from their intermediate host *Biomphalaria*

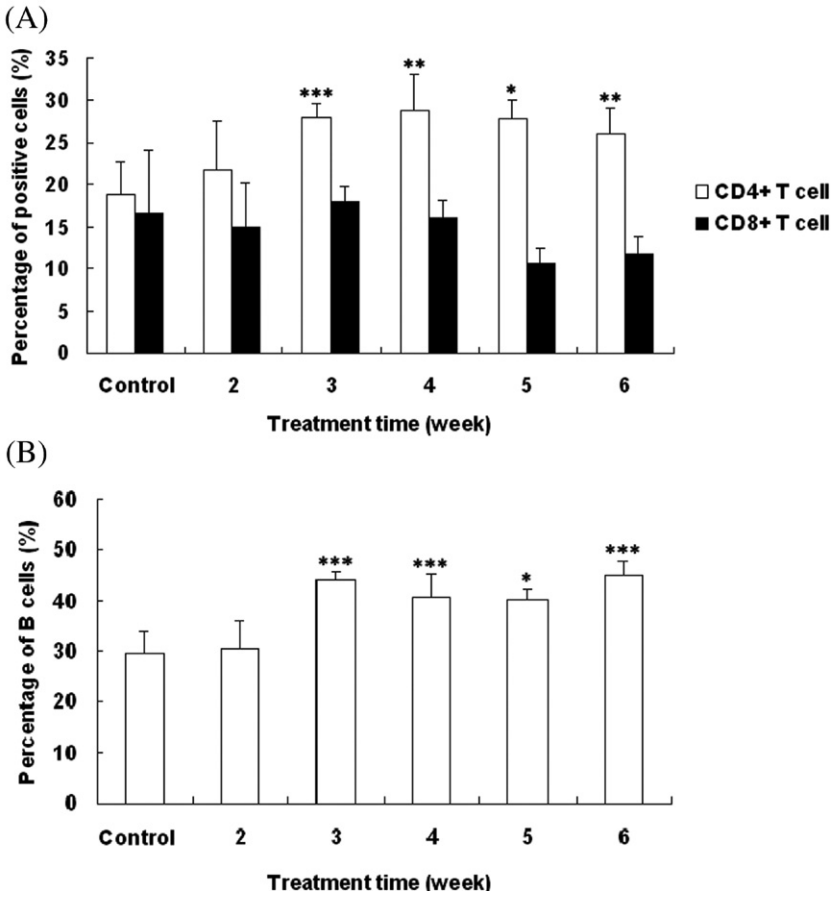

Fig. 3. The percentages of surface $CD4^+$ and $B220^+$ expression on spleen cells. Splenocytes of mice who received oral administration of 2.5 mg AC-PS for 0, 2, 3, 4, 5, and 6 weeks were harvested and further incubated with primary (A) CD4/CD8 or (B) CD45R/B220 monoclonal antibodies followed by FITC-conjugated IgG at 4 °C for 30 min. After washing twice with PBS, cells were applied to a FACS caliber flow cytometer. Data from 3 to 5 separate experiments are expressed as mean ± SD. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ when compared with control at week 0.

glabrata. The mice that did not receive AC-PS treatment were included in the control groups. After 8 weeks, the adult worms were isolated from the portal and mesenteric veins and counted.

Statistics. Data were expressed as mean ± standard deviation (SD). Analysis of variance was used to analyze the differences 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 significantly.

Results

Cytokine mRNA expression of splenocytes induced by polysaccharides of A. camphorata

To investigate the immunomodulating effects of *A. camphorata*, C57BL/6j and BALB/c mice were fed different amounts of AC-PS for 2 and 4 weeks, respectively, and subsequently, the cytokine mRNA expression levels of IFN-γ, IL-2, IL-4, IL-10, and TNF-α in the splenocytes of the mice of the two strains were observed and compared (Fig. 2). HPRT is expressed at a relatively constant level in cells, and the panel shows the relative ratio of each cytokine to HPRT. After the mice were fed *A. camphorata*, induction of large amounts of IFN-γ, IL-2, and TNF-α mRNA was detected at 2 and 4 weeks by comparing these levels with the relevant values of the control groups ($p<0.05$) (Fig. 2A, B, and E). The level of gene expression was relatively greater in the group of mice fed 2.5 mg AC-PS once a day for 4 weeks ($p<0.01$). Our observations revealed that C57BL/6j and BALB/c mice exhibit very similar expression profiles of these genes. However, IFN-γ was induced at a higher level in BALB/c mice than in C57BL/6j mice, whereas the levels of TNF-α were higher in the C57BL/6j mice than in the BALB/c mice.

Furthermore, the data showed no significant change in IL-4 and IL-10 expression after the BALB/c mice were treated with *A. camphorata* (Fig. 2C and D). Similar trends for these 2 cytokines were also observed in C57BL/6j mice, although the levels of gene expression were slightly higher in the group of mice treated for 4 weeks. These results suggest that a Th1 response (IFN-γ and IL-2) was induced in the splenocytes after the mice were treated with *A. camphorata*, and this response was found to be significantly up-regulated in BALB/c mice as compared to C57BL/6j mice. By these experiments, the mice in following treatments were treated with 2.5 mg *A. camphorata*.

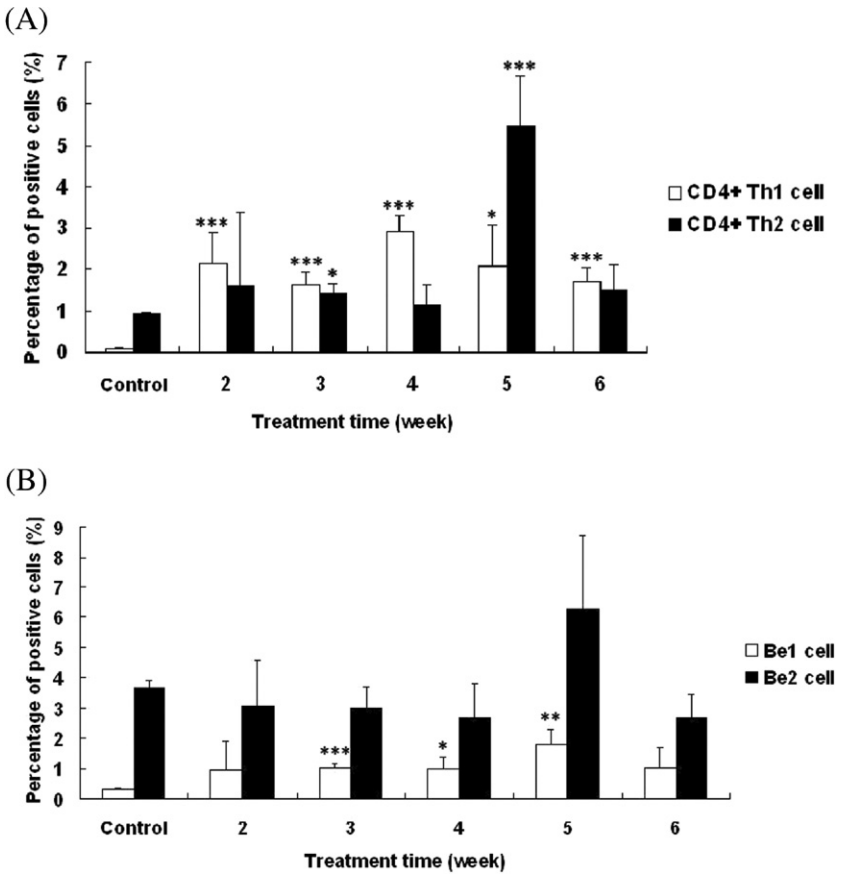

Fig. 4. The percentages of surface Type1 and Type2 marker molecule expression on $CD4^+$ and $B220^+$ splenocytes. Splenocytes from mice receiving oral administration of 2.5 mg AC-PS for 0, 2, 3, 4, 5, and 6 weeks were harvested and further incubated in primary Type1 (hThy1) and Type2 (mThy1.1) marker monoclonal antibodies followed by FITC-conjugated IgG, Biotin-conjugated anti-mouse Thy1.1 and PE-conjugated anti-human CD90 at 4 °C for 30 min. After washing twice with PBS, cells incubated with Cy5-conjugated streptavidin at 4 °C for 30 min. For cytometric analysis, (A) $CD4^+$ and (B) $B220^+$ cells were gated and the percentage of cells expressing Type1 (hThy1) or Type2 (mThy1.1) marker respectively were estimated. Data from 3 to 5 separate experiments are expressed as mean ± SD. $*p<0.05$, $**p<0.01$, and $***p<0.001$ when compared with control at week 0.

Effect of A. camphorata on the percentage of CD4+ T cell and B cells in splenocytes

As shown in Fig. 3A, the percentage of $CD4^+$ and $B220^+$ splenocytes increased in the AC-PS-treated mice in a time-dependent manner. The percentage of $CD4^+$ cells in the control group was 18.72%±3.99% and increased significantly to 27.94%±1.70%, 28.87%±4.29%, 27.80%±2.23%, and 26.11%±3.02% after 3, 4, 5, and 6 weeks of AC-PS administration, respectively ($p<0.05$). Whereas the percentage of $CD8^+$ cells decreased from 16.50±7.45% without AC-PS treatment to 11.85±1.91% with 6 weeks of treatment (Fig. 3A). In splenocytes from *A. camphorata* treated mice, the percentage of B cells was increased from 29.80±1.90% in the control group to 43.90±1.19% after 3-week treatment in a time-dependent manner and the increment was extended to 6-week ($p<0.05$) (Fig. 3B).

Regulation of the effects of A. camphorata on Th1/Th2 surface markers of Th and B cells

To detect the development of Th1/Th2 cells induced by AC-PS, we used an established T1/T2 doubly transgenic mouse model carrying the different markers before IFN-γ and IL-4 promoters, respectively. The percentage of $CD4^+$ cells that expressed the Th1 surface marker ($CD4^+$ Th1) increased from 0.07%±0.02% of the control at the baseline to 2.14%±0.30%, 1.64%±0.18%, 2.92%±0.19%, 2.07%±0.58%, and 1.71%±0.15%, with 2, 3, 4, 5, and 6 weeks of AC-PS treatment, respectively ($p<0.05$) (Fig. 4A). Furthermore, the percentage of $CD4^+$ cells that expressed the Th2 surface marker ($CD4^+$Th2) increased slightly at 3 weeks and greatly at 5 weeks of AC-PS treatment. As with T lymphocytes, B cells may differentiate into two subsets of "effector" B cells (Be1 and Be2) that produce distinct patterns of cytokines depending on the cytokine environment (Harris et al., 2000). After AC-PS treatment, the expression of Type-1 surface marker on effector B cells (Be1) was increased from 0.31±0.02% of control to 1.81±0.29% at 5 weeks ($p<0.05$) and decreased to 1.04±0.30% at 6 weeks. However, the expression of Type-2 surface marker on effector B cells (Be2) was not significantly different from control group after AC-PS treatments (Fig. 4B). These results suggest that the splenocytes were influenced towards developing a Th1 response following treatment with *A. camphorata* polysaccharides.

Table 1
Effect of AC-PS on *Schistosoma mansoni* infection

Oral administration times	Worm burden	
	C57BL/6	BALB/c
Control	75.8±7.4	61.3±5.7
1 week	68.3±5.8	54.4±3.9
2 weeks	60.6±3.4	45.0±4.8*
4 weeks	30.3±4.7*	32.1±3.3*

C57BL/6 and BALB/C mice after administration of AC-PS for 1–4 weeks were infected with 120 *S. mansoni* cercariae. The mice that received no AC-PS treatment were used for control groups. Eight weeks after challenge, all mice were sacrificed concurrently and the adult worm burden was counted. Data from 5 separate experiments are expressed as mean±SD. *$p<0.05$ when compared with control at week 0.

Effect of AC-PS on the Infection of Schistosoma mansoni

After 1-, 2- or 4-week pretreatment of AC-PS respectively, the infection of *S. mansoni* was inhibited in a time-dependent manner with repeated treatments. As shown in Table 1, the worm burden of C57BL/6 mice was 75.8±7.4 worms for controls versus 30.3±4.7 worms at 4 weeks in the AC-PS-treated group ($p<0.05$). Similarly, the worm burdens of BALB/c mice in 2 to 4 weeks AC-PS-treated groups (45.0±4.8 and 32.1±3.3 worms) were also less than the control group (61.3±5.7 worms) and such differences were statistically significant ($p<0.05$).

Discussion

A. camphorate, a medicinal fungus with a known inhibitory effect on the hepatitis B virus and cancer, was identified in 1990 as a traditional Chinese medicinal treatment in Taiwan (Lee et al., 2002; Liu et al., 2004). The present study revealed that polysaccharides derived from *A. camphorate* modulate the expression of Th1 cytokines in splenocytes as well as the type1 differentiation of T and B lymphocytes. Treatment of mice with AC-PS also reduced the infection rate of *S. mansoni*.

The polysaccharides from mushrooms have potential anti-tumor and immunomodulatory effects mediated through the enhancement of cytokine secretion by T cells (Wang et al., 1997; Oh et al., 2006). Previous reports have indicated that most polysaccharides induce a Th1-dominant state via IL-2 or IFN-γ induction (Kidd, 2000; Wasser, 2002). The present study clearly indicates that AC-PS is a potent inducer of Th1-type cytokines such as IL-2 and IFN-γ, in a time and dose-dependent manner, but not of Th2 cytokines. The cytokine expression profiles of stimulated T lymphocytes differed between the mouse strains. Although C57Bl/6j and BALB/c mice are archetypal Th1 and Th2 strains, respectively (Mills et al., 2000; Murata et al., 2002), the BALB/c mice were observed to be significantly susceptible to the effects of AC-PS as compared to C57BL/6j mice. It is not surprising that the C57BL/6j strain was less susceptible to the effects of AC-PS since Th1 already predominates in this strain. The efficiency of AC-PS was not obviously reflected in the dominant immune responses of the two strains; this response may also result in the slight elevation of Th2 cytokines in the C57BL/6j mice treated for 4 weeks but not in the BALB/c mice.

These results suggested that the splenocytes were induced towards a Th1 response following treatment with *A. camphorata*. Previous reports indicated that the polysaccharides of *A. camphorata* inhibit inflammation by modulation of the respective cytokines (Wu et al., 2007) and elicit anti-tumor effect by promoting a Th1-dominant state and natural killer cell activities (Liu et al., 2004). We have also observed a rapid increase in TNF-α expression, especially in C57BL/6j strain. The level of TNF-α is related to inflammatory and immunologic responses during schistosoma infection that constitutes direct proof of causality (Adewusi et al., 1996).

Specific immune responses that can be differentiated into Th1 and Th2 responses have distinct roles in the immune system (Mosmann and Coffman, 1989). However, detection of cytokine expression is not revealed entirely by the production and differentiation of lymphocytes *in vivo*. Here we applied a special T1/T2 doubly transgenic mice model to trace the development of Th1 and Th2-type cells *in vivo* by flow cytometry with surface immunofluorescent staining (Hsieh et al., 2000). $CD4^+$ T helper cells are important regulators of immune system (Bennett et al., 1997; Shiku, 2003). The cells secrete a series of cytokines such as IFN-γ, IL-2 and TNF-α to initiate antibody production of B cells and enhance the production of $CD8^+$ cytotoxic T cells (Bennett et al., 1997). In the present study, AC-PS significantly increased the $CD4^+$ and B cell numbers 3–6 weeks after administration. We also examined the potentiality of AC-PS on the Th1/Th2 differentiation of CD4 and B cells and these results showed treated mice displayed a significantly higher frequency of Th1 and Be1 cells when compared to untreated mice. Both $CD4^+$ T cells and humoral antibodies are crucial for the development of an effective response against pathogen invasion (Vignali et al., 1989). The polysaccharides derived from *Ganoderma lucidum* could possess the potential capacity in regulating immune responses by inducing a Th1 response (Lin et al., 2006). These results indicate that AC-PS have marked induction effects on increasing of $CD4^+$ T and B cells and might be useful as an adjuvant to induce Th1 immunity.

AC-PS modulated immune function and inhibited infection with *S. mansoni*. These two activities may correlate to each other and the possible mechanisms remains to be clarified. *S. mansoni* induces anergy of T cells via a macrophage-T cell contact-dependent mechanism (Smith et al., 2004). In mice, *S. mansoni* infection induces the development of Th2 polarization wherein Th1 responses are suppressed (McKee and Pearce, 2004). It has been suggested that the ability to induce ineffective Th2-like responses may have evolved in schistosomes as a clever defense mechanism to ensure that Th2 cells produced sufficient levels of IL-10 to inhibit the protective immunity mediated by the Th1-like subset in the $CD4^+$ T responses (Kuby et al., 2000; Pearce and MacDonald, 2002). AC-PS increased the percentage of $CD4^+$ T cells and augmented the Th1 responses; this suggests that AC-PS may inhibit the development of schistosomiasis via immunomodulation by promoting a Th1-dominant state. Predominant Th1 responses are important for the killer activities against the cercariae and schistosomules; these stages are more susceptible to immune attack (Kuby et al., 2000). This inference is also supported by the result that stronger Th1 cytokine responses were induced in the BALB/c mice that were pretreated with AC-PS and, they acquired higher levels of protection than the C57BL/6j mice. Since the present study revealed modulation of splenic T and B cells by AC-PS in uninfected mice, the effect of AC-PS on mice infected with *S. mansoni*, including Th1/Th2 polarization, are currently under investigation.

Our data suggest that polysaccharides of *A. camphorata* modulate the expression of Type1 cytokines on splenocytes as well as the percentages of $CD4^+$ T cells and B lymphocytes. It was also demonstrated that *A. camphorata* induces these immune cells towards Type 1 differentiation. Furthermore, treatment of mice with AC-PS reduced the infection rate of *S. mansoni in vivo*. This study may provide information for raising the interesting possibility that *A. camphorata* may eventually have therapeutic potential in the treatment or prevention of disorders involving parasite infection. In addition, it is hoped that the special transgenic mice model will be suitable for use in the development of an *in vivo* predictive assay of Chinese herbs.

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