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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*.
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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,

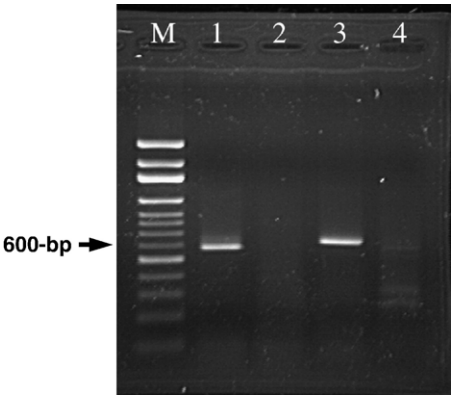

Fig. 1. Screening of T1/T2 doubly transgenic mice. T1/T2 doubly transgenic mice were screened using PCR with 0.2 μg tail DNA as the PCR template. Both T1- and T2-amplified DNA fragments were screened from the mice used in this study. A representative experiment of 3 trials is displayed. Lane M, molecular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

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