Effects of Water-Soluble Hemicellulose from Soybean Hull on Serum Antibody Levels and Activation of Macrophages in Rats

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Effects of soybean hull water-soluble hemicellulose (WSHC) on serum immunoglobulin (Ig) concentration and production of NO and IL-1β from peritoneal macrophages were examined and compared with those of *Agaricus blazei* in the rat system. WSHC consisted of arabinose, galactose, xylose, glucose, and rhamnose, and the molecular weight was ~500000. Rats were ip administrated each sample at a dose of 0.67, 13.4, or 26.9 mg/kg/day for 14 days. The administration of WSHC resulted in significantly higher productions of IgM ($p < 0.01$ on day 6, $p < 0.05$ on day 14) and IgG ($p < 0.05$ on day 6) than those in other groups. When peritoneal macrophages were stimulated with various concentrations of sample (0.67, 13.4, or 26.9 mg/mL), WSHC significantly increased both NO and IL-1β productions only at the concentration of 13.4 (mg/mL) compared with those of a saline group. These findings demonstrate that WSHC enhances humoral immunity and activation of macrophages, thereby leading to the augmentation of immune responses in rats.

Keywords: *Rat immunoglobulin; soybean hull; water-soluble hemicellulose; rat peritoneal macrophage*

INTRODUCTION

It has been reported that immune functions are stimulated by polysaccharides from the fruiting body or mycelium of a variety of edible mushrooms, fungi, and bacteria. In particular, polysaccharides including β-glucan are now widely recognized as biological response modifiers (BRM).

Mizuno et al. suggested that polysaccharides including β-(1,6)-D-glucans isolated from a variety of edible mushrooms such as cv. Himematsutake (*Agaricus blazei*) (*1*, *2*), cv. Niohsimeji (*3*, *4*), and cv. Ningyotake (*5*) have a potent antitumor effect in the model of Sarcoma 180 solid tumor implanted in mice. It has been proposed that these functions result from the stimulation of immune responses through the activations of various immunocompetent cells of the host. Sakurai et al. (*6*) demonstrated that a polysaccharide, β-(1,3)-glucan, from fungus stimulated pulmonary immune responses including the activation of alveolar macrophages and inhibited the metastasis of 3LL cells. In addition, it has been reported that other polysaccharides such as arabinoxylan, glucogalactan, and xyloglucan contained in foods or plants, which have β-form in their structures, also exerted antitumor activity (*7*−*9*). These studies postulated that immune pharmacological functions of polysaccharides were attributed to the stimulation of cell-mediated immunity, although the precise mechanism of the antitumor activity is not known well.

Soybean hull is a residual product in the manufacturing process of soy protein and cooking oil and is generally discarded as an industrial waste of food manufacturing. Although it is well-known that feeding of water-soluble hemicellulose (WSHC) exerts physiological effects in the health maintenance of as a dietary fiber (*10*), little is known with regard to the immunological functions of WSHC containing β-form in the structure. On the basis of the structural property, however, it has been anticipated that WSHC may modulate immune functions as was shown with *A. blazei*, which has recently been promoted as a biological functional food in Japan.

To elucidate the immunological influence of the soybean hull WSHC, in this study, we examined serum concentrations of immunoglobulin (Ig) M and G in rats that were ip administered either WSHC polysaccharide or β-glucan from *A. blazei*. We also analyzed the production of nitric oxide (NO) and interleukin (IL)-1β from peritoneal macrophages of nontreated rats upon stimulation with the polysaccharide or β-glucan from *A. blazei*. Our ultimate goal is to identify naturally occurring compounds that can be used pharmacologically to augment immune response.

MATERIALS AND METHODS

Immunomodulators. Fruiting bodies of *A. blazei*, which was kind gift from Okinawa Fermentative Chemicals Co., Ltd. (Okinawa, Japan), were freeze-dried and made into powder form. Soybean hull was supplied by The Nisshin Oil Mills, Ltd. (Tokyo, Japan).

Preparation of Soybean Hull WSHC. The extraction and fractionation of soybean hull WSHC were performed according to the method illustrated in Figure 1. Briefly, dried and ground

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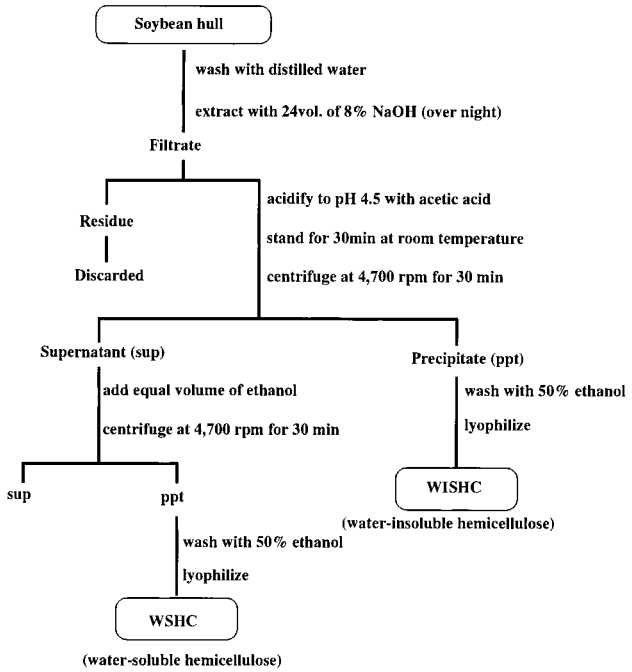

Figure 1. Preparation of water-soluble hemicellulose from soybean hull.

soybean hull was washed with distilled water and extracted with 8% NaOH overnight at room temperature. The filtered extract was acidified to pH 4.5 with acetic acid. The solution was centrifuged at 4500*g* for 30 min. The supernatant was mixed with an equal volume of ethanol. After centrifugation, the precipitate was collected and washed twice with 50% ethanol. The lyophilized sample was used as a WSHC of soybean hull.

Analyses of Sugar Content, Components, and Molecular Weight. The total amount of hexose was determined by using the anthrone reagent with a D-glucose as standard (*11*). The total amount of pentose was determined by using the phloroglucin reagent with a D-arabinose as standard (*12*). The sum of total hexose and total pentose was regarded as total sugar content. Sugar components were analyzed by high-performance liquid chromatography (HPLC; Shimadzu, Japan) equipped with Shim-pack ISA-07, and the molecular weight was determined by HPLC equipped with a Superose 12B column using standard dextran (Amersham Pharmacia) as a marker.

Animals. Male Wistar rats, aged 8 weeks (initially weighing ~150 g), were purchased from Japan SLC (Shizuoka, Japan) and were housed individually in an air-conditioned room with controlled temperature (23−25 °C), artificial lighting (8:30 a.m.−8:30 p.m.). They were divided into seven groups (five rats/group) and maintained on CE-2 pellet diet (Clea, Japan) and water ad libitum. Each polysaccharide was dissolved in 0.9% NaCl on the basis of total sugar content, namely, prepared at the concentrations of 0.67, 13.4 and 26.9 (mg/mL), respectively. Then, aliquots (1 mL/kg of rat body weight) of the sample solution and control vehicle were injected ip to rats once daily for 14 consecutive days. Peripheral blood was collected for quantitation of IgM and IgG at days 0, 3 and 6 after ip administration of the sample. At the end of the experiment, animals were sacrificed under anesthesia with diethyl ether, and blood was collected to quantitate IgM and IgG. This study was approved by the Animal Committee of University of the Ryukyus, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals.

Quantitation of IgM and IgG. Rat serum IgM and IgG were quantified with a sandwich ELISA. In brief, 96-well ELISA plates (Corning Glass Works, Corning, NY) were coated overnight at 4 °C with 100 μL of either rabbit anti-rat IgM(μ) or IgG (H+L) (Zymed Laboratories). Plates were incubated overnight at 4 °C with 200 μL of 0.1% BSA in PBS. Fifty microliter aliquots of each sample and standard dilutions of IgM or IgG (Zymed Laboratories) were then added to each well. Plates were incubated at 37 °C for 1 h. Either peroxidase-conjugated anti-rat IgM or IgG (Zymed Laboratories) was added to each well and incubated for 30 min. *o*-Phenylenediamine was added as a substrate solution, and the reaction was stopped by the addition of 50 μL of 50% H_2SO_4. Plates were read in an ELISA plate reader at a wavelength of 495 nm (model 450, Bio-Rad Laboratories, Richmond, CA).

Preparation of Peritoneal Macrophages and Cell Culture. Peritoneal macrophages were harvested from the peritoneal fluid 3 days after ip injection of 3% thioglycolate (w/v). The cells were washed three times with Hanks buffer. The pellet was resuspended in RPMI-1640 medium containing 10% FCS, and the cell suspension (5×10^6) was plated on 35-mm plastic Petri dishes (Corning Glass Works). After overnight incubation at 37 °C in a humidified atmosphere of 5% CO_2 in air, unattached cells were washed off. These dishes were replaced with fresh RPMI-1640 medium and incubated with 50 μL of various concentrations of sample (0.67, 13.4, and 26.9 mg/mL, respectively) under similar conditions for another 18 h. Thereafter, supernatants were recovered and used for quantitation of NO and IL-1β.

NO Production. NO production was determined by analyzing nitrite (NO^{2-}, a product of the L-arginine-dependent NO pathway) concentration in culture supernatants of peritoneal macrophages using a colorimetric assay based on the Griess reaction (*13*). Briefly, 100 μL of culture supernatant was combined with 100 μL of Griess−Romijn reagent (Wako, Osaka, Japan) and incubated for 10 min at room temperature in the dark. Absorbance was measured at 540 nm in a microtiter plate reader (model 450, Bio-Rad Laboratories), and NO concentrations were determined using a standard curve generated with sodium nitrite in complete culture medium.

Quantitation of IL-1β. The concentration of IL-1β in culture supernatants of peritoneal macrophages was determined by a commercially available enzyme-linked immunosorbent assay kit (Toyobo).

Statistical Analysis. Values are presented as the mean ± standard error of the mean (SEM). Data were analyzed by factorial ANOVA (Statview 4.02, Abacus Concepts Inc.). Statistical difference between groups was assessed by Duncan's multiple-range test (*14*).

Table 1. Sugar Content, Components, and Molecular Weight of Soybean Hull WSHC and *A. blazei*[a]

	WSHC	*A. blazei*
total sugar content (wt %)	27.4	67.2
sugar component (wt %)		
rhamnose	2.42	0.47
ribose	0.06	0.16
mannose	0.24	0.43
arabinose	32.3	3.30
galactose	47.9	1.29
xylose	11.3	4.64
glucose	5.28	87.1
unknown	0.53	2.66
pentose	43.6	8.1
hexose	55.8	89.2
molecular weight	500000	10000−185000

[a] WSHC, water-soluble soybean hull hemicellulose; *A. blazei* is freeze-dried powder of fruiting bodies.

RESULTS

Structure of WSHC and *A. blazei*. Sugar content, components, and molecular weight of WSHC and *A. blazei* are listed in Table 1. Total sugar content of WSHC was one-third that of *A. blazei*. Soybean hull WSHC was composed of different monosaccharides, which were arabinose, galactose, xylose, glucose, and rhamnose in the ratio of 32.3, 47.9, 11.3, 5.28, and 2.42

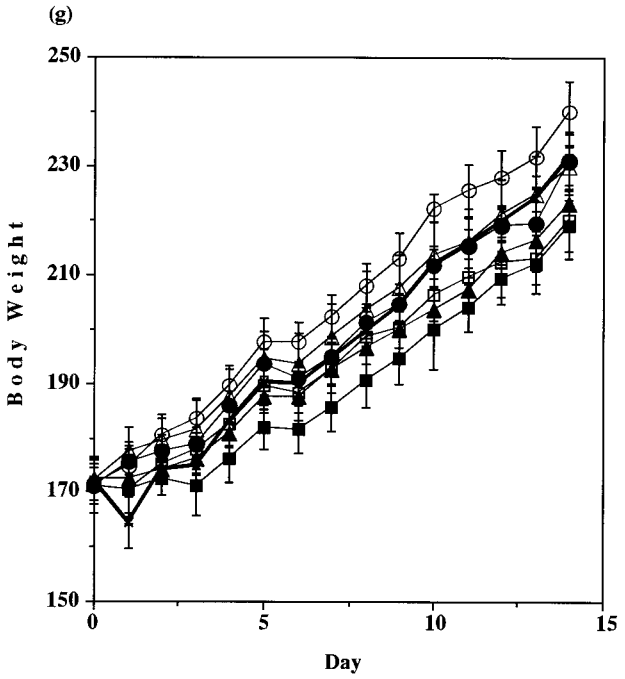

Figure 2. Time course of rat body weight. Rats were given ip each sample at a dose of 0.67, 13.4, or 26.9 mg/kg of body weight per day for 14 days: (×) 0.9% NaCl; (○) 0.67, (△) 13.4, and (□) 26.9 mg/kg of soybean hull WSHC; (●) 0.67, (▲) 13.4, and (■) 26.9 mg/kg of *A. blazei*. Each value represents the mean ± SEM of five rats. No significant differences were observed among groups.

(by wt %), respectively. On the other hand, most of the components of *A. blazei* were glucose (~90 wt %). The molecular weight of WSHC was ~500000, whereas *A. blazei* was made up by the various amounts of polysaccharides and the molecular weights were in the range of ~10000−185000. These observations suggest that the physicochemical properties of soybean hull WSHC are different from those of *A. blazei*, although both polysaccharides contain a common β-form in structure.

Influence of WSHC and *A. blazei* on Body Weight. Rats were administered WSHC, *A. blazei*, or vehicle alone. Both polysaccharides were given ip to rats once a day at dose of 0.67, 13.4, or 26.9 mg/kg/day for 14 days. Changes of the body weight of these rats are illustrated in Figure 2. No significant differences are recognized in the body weight between animals of the experimental and control groups, although there was a trend toward lower weight with increasing amounts of polysaccharide injection for each time point. In this study, however, it is unlikely that the administration of these two kinds of polysaccharides showed harmful influences on the growth of rats.

Serum Concentration of IgM and IgG in Rats Administered WSHC or *A. blazei*. Time courses of IgM and IgG concentrations in rat sera are depicted in Figure 3. The serum IgM concentrations of rats administrated WSHC were significantly higher ($p < 0.01$ on day 6, $p < 0.05$ on day 14) than those of rats administered either saline or 0.67 mg/kg *A. blazei*. The serum IgG concentrations of rats administered WSHC at the concentrations of 0.67 and 26.9 mg/kg were significantly higher ($p < 0.05$) than those of either saline or *A. blazei*-treated rats on day 6. Although administration of soybean hull WSHC resulted in the significant augmentation of both IgM and IgG production, administration of *A. blazei* induced significant augmentation of IgM production but not of IgG production.

NO and IL-1β Productions by Peritoneal Macrophages Stimulated with WSHC or *A. blazei*. We next measured productions of NO and IL-1β from peritoneal macrophages to examine in vitro effects of WSHC and *A. blazei*. As shown in Figures 4 and 5, the stimulation of macrophages by these polysaccharides induced distinctive productions of both NO and IL-1β from these macrophages.

In WSHC-stimulated macrophages, significantly high NO production was observed at the concentration of 13.4 mg/mL compared with those of saline and other WSHC-treated groups (Figure 4). A similar result was also obtained in IL-1β production (Figure 5). In the case of *A. blazei*-stimulated macrophages, NO production was increased in accordance with the concentration of *A. blazei* (Figure 4). IL-1β production in the presence of *A. blazei* showed a pattern similar to that of NO production (Figure 5). We have observed a similar

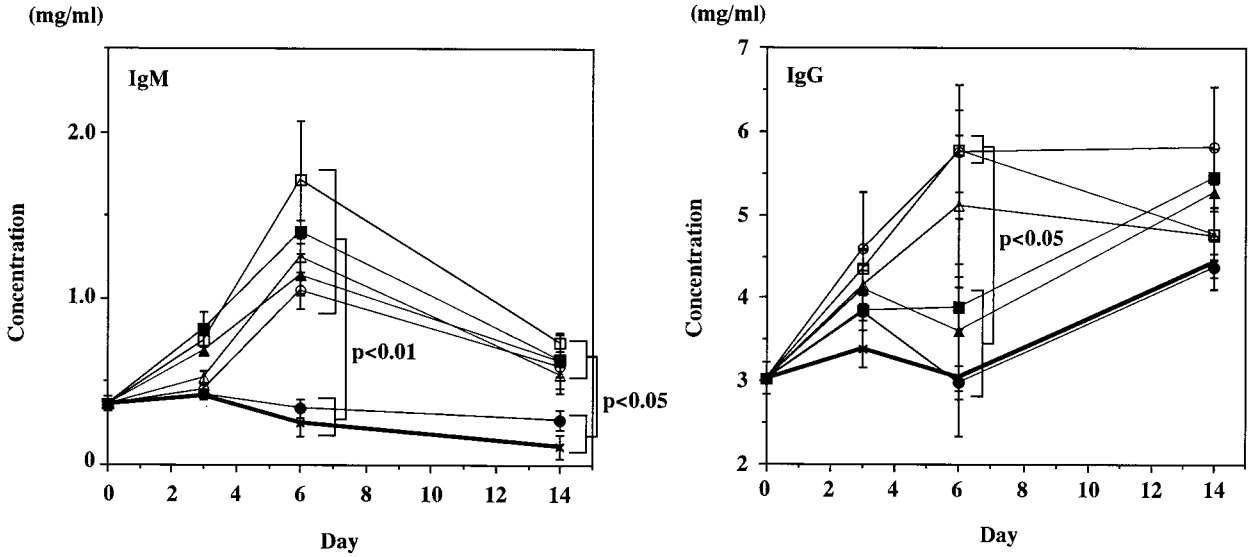

Figure 3. Time course of rat serum IgM and IgG concentrations: (x) 0.9% NaCl; (○) 0.67, (△) 13.4, and (□) 26.9 mg/kg of soybean hull WSHC; (●) 0.67, (▲) 13.4, and (■) 26.9 mg/kg of *A. blazei*. Each value represents the mean ± SEM of five rats. Data were analyzed by ANOVA. Significant differences were shown at $p < 0.01$ or $p < 0.05$.

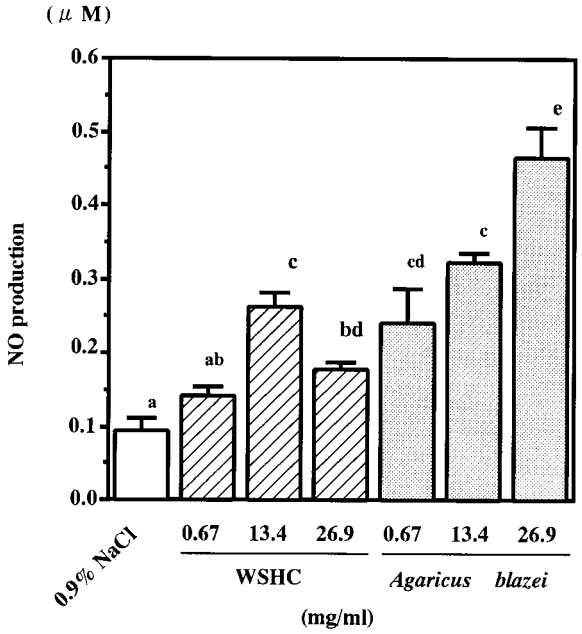

Figure 4. NO production from rat peritoneal macrophage: (white bars) 0.9% NaCl; (slashed bars) WSHC; (dotted bars) *A. blazei*. Each value represents the mean ± SEM of five dishes. Values not sharing a common letter are significantly different at $p < 0.05$.

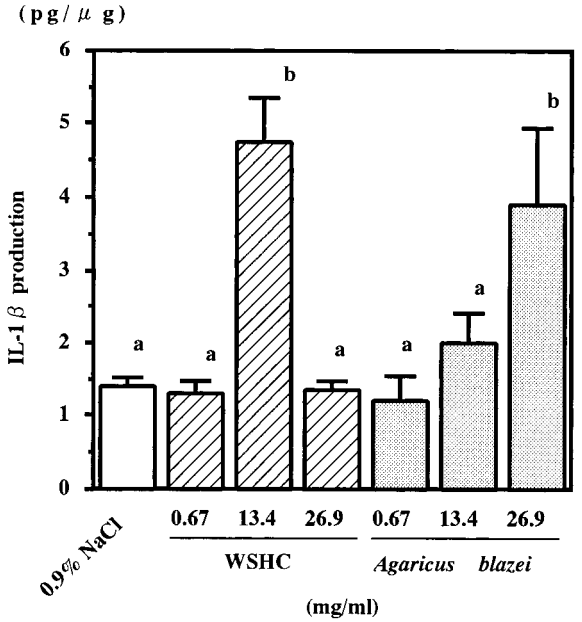

Figure 5. IL-1 β production from rat peritoneal macrophage: (white bars) 0.9% NaCl; (slashed bars) WSHC; (dotted bars) *A. blazei*. Each value represents the mean ± SEM of five dishes. Values not sharing a common letter are significantly different at $p < 0.05$.

tendency relating to NO and IL-1β productions in WSHC-stimulated macrophages with good reproducibility (data not shown).

When maximum production of NO or IL-1β was compared between the WSHC-treated group and the *A. blazei*-treated group, NO production in the 13.4 mg/mL WSHC group was significantly lower ($p < 0.05$) than that of the 26.9 mg/mL *A. blazei* group (Figure 4). On the other hand, no significant difference was noted in IL-1β production between the 13.4 mg/mL WSHC group and the 26.9 mg/mL *A. blazei* group (Figure 5).

It was notable that the maximum productions of NO and IL-1β from WSHC-stimulated macrophages were detected at the narrow range of the WSHC concentration. It appeared that the stimulation of macrophages by *A. blazei* was dose-dependent.

DISCUSSION

In the present study, we examined the influences of soybean hull WSHC compared to *A. blazei* on the antibody production in rats and the activation of macrophages from rats. It was shown that the ip administration of soybean hull WSHC significantly increased production of both IgM and IgG and remarkably activated peritoneal macrophages only at the concentration of 13.4 mg/mL.

As previously reported, polysaccharide from *A. blazei* contains many kinds of water-soluble fractions such as β-(1,6)-D-glucan (*15*), β-(1,3)-D-glucan with β-(1,6)-glycosyl branching (*2*), and polysaccharide−protein complexes (*16*), which have molecular weights from about 10000 to 2000000. It is well-known that β-D-glucan is a key structure for expression of the potent antitumor activity and immune modulating functions (*17*). In fact, we observed that polysaccharides extracted with hot water from *A. blazei* were composed of four distinct types of polysaccharides with molecular weights of ∼10000−185000, and the constitutive sugar was chiefly glucose (Table 1).

On the other hand, as shown in Table 1, WSHC isolated from soybean hull was a polysaccharide with a molecular weight of 500000, which consisted of several kinds of monosaccharides. Although detailed analysis of this structure has not been performed in the present study, it seems likely that soybean hull WSHC may have a polymer backbone of xylose with a branched form similar to that of other WSHC (*18*, *19*). Thus, the physicochemical features such as sugar component and molecular weight of soybean hull WSHC are quite different from those of *A. blazei* except that both of these polysaccharides possess β-formed structure. Therefore, it is of interest to determine if soybean hull WSHC exerts unique or similar influences on the immunological functions compared to β-glucan from *A. blazei*.

It is generally considered that polysaccharides such as lipopolysaccharide are thymus-independent antigens and induce no Ig isotype class switch (*20*). In addition, the antitumor effects of polysaccharides from edible mushrooms, fungi, and bacteria are largely attributed to potentiation of the host defense through cellular immunity (*17*, *21*−*23*).

Both humoral and cell-mediated immunities play important roles in the defense against invasion of foreign substances. These substances are processed in antigen presenting cells such as macrophages, and the processed peptide antigens are presented to T lymphocytes. In response to the antigenic stimulation, helper T cells secrete various cytokines (*24*), and some of these cytokines are necessary to induce class switch from IgM to IgG. In addition, it is the general view that the dominant class of secreted antibody in a primary response is usually IgM, and then, in a secondary response, other Ig isotypes such as IgG are increased.

In the present study, we found the significant increase of IgG in addition to IgM production was induced in the rats serum by ip administration of soybean hull WSHC. No significant increase of IgG was observed by ip administration of *A. blazei* (Figure 3). Maeda et al. (*25*)

reported that the soybean hull WSHC composed of arabinoxylan stimulated the production of IgM but not those of IgG and IgE in human−human hybridoma cells and human lymphocytes. These differences may be due, at least in part, to the physiochemical features of WSHC such as the components and molecular weight. In addition, it was reported that murine immune cells and human immune cells responded to polysaccharides in different manners (*26*). It seems to us that WSHC used in the present study stimulated antigen presenting cells, which result in helper T cell activation. Thus, maturation of B cells and class switch of Ig may be induced in rats administrated WSHC. Indeed, significant productions of NO and IL-1β, activation markers of antigen presenting cells, were induced in peritoneal macrophages upon stimulation with WSHC (13.4 mg/mL) in vitro. The optimal concentration of WSHC appears to be of a very narrow range, because less or more concentration of WSHC induced no considerable productions of NO and IL-1β by these macrophages.

On the other hand, *A. blazei* induced NO production in a dose-dependent manner and IL-1β production only at the highest concentration (26.9 mg/mL). Administration of *A. blazei* to rats augmented IgM production but not that of IgG in sera. These results suggest that *A. blazei* activates macrophages and B cells but not helper T cells. It should be investigated in future studies how these WSHC and *A. blazei* modify differently the host immune functions.

In recent years, a number of studies have demonstrated that some polysaccharides such as β-glucan, arabinoxylan, glucogalactan, and xyloglucan generated the antitumor activity (*7*−*9*). As described above, Mizuno et al. reported that many kinds of polysaccharides isolated from a variety of edible mushrooms in addition to *A. blazei* showed potent antitumor activity. Hamuro et al. (*23*) also reported that administration of β-(1,3)-glucan resulted in the augmentation of in vivo cytotoxic T lymphocyte (CTL) responses in mice. Moreover, it has been proposed that galactosylceramide (GalCel) induces activation of antigen presenting cells such as dendritic cells (DCs), which leads to the enhanced antitumor activity (*27*, *28*). Thus, the effect of WSHC on cell-mediated immunity, especially T cell activation, should be further investigated.

In the present study, we demonstrated possible effects of soybean hull WSHC on both humoral and cell-mediated immune responses in rats. However, it remains to be solved how the molecular weight, degree of branching, solubility, and method of administration are related to the WSHC activity. These detailed approaches will serve to further elucidate the effects of soybean hull WSHC on the immune function.

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