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Effect of Oral Administration of Dried Royal Sun Agaricus, *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae), Fruit Bodies on Anti-β-Glucan Antibody Titers in Humans

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ABSTRACT: *Agaricus brasiliensis* (= *Agaricus blazei* Murrill sensu Heinem.) is a health food that has received recent attention. β-Glucan is one of the major components of *A. brasiliensis*. We have reported that an anti-β-glucan antibody was detected in sera from human volunteers. In this study, we examined the reactivity of the anti-BG antibody to *A. brasiliensis* extracts in human sera and change in the anti-BG antibody titer of healthy volunteers taking *A. brasiliensis* for 6 months (N = 27, average age = 43 ± 11, male = 13, female = 14). Individual differences in the anti-BG antibody titer existed. We evaluated the rate of change in the titer in each individual. The volunteers in the *A. brasiliensis* group showed an increase in the anti-BG antibody titer as compared with those in the placebo group. Individual differences existed in the rate of the increase. We first demonstrated a clinical effect of the oral administration of *A. brasiliensis* on the anti-BG antibody titer. The oral administration of *A. brasiliensis* induced a β-glucan-specific response and there were individual differences in this response. The resulting anti-BG antibody production could be useful as an index of the immune response to β-glucan in humans.

KEY WORDS: *Agaricus brasiliensis,* Royal Sun Agaricus, medicinal mushrooms, oral administration, β-glucan, anti-β-glucan antibody

ABBREVIATIONS

Ab: antibody; **AgCA:** *A. brasiliensis* cold alkaline extract; **AgHWE:** *A. brasiliensis* hot water extract; **AgHA:** *A. brasiliensis* hot alkaline extract; **ASBG:** *Aspergillus niger* solubilized cell wall glucan; **BG:** β-glucan; **BRM:** biological response modifier; **BSA:** bovine serum albumin; **CSBG:** *Candida albicans* solubilized cell wall glucan; **ELISA:** enzyme-linked immunosorbent assay; **GRN:** Grifolan; **IFN-γ:** interferon-γ; **IL-12:** interleukin-12; **LAM:** Laminaran; **NK cells:** natural killer cells; **OVA:** ovalbumin; **SPG:** Sonifilan; **TMB:** tetramethlbenzidine; **TNF-α:** tumor necrosis factor-α; **Y-Man:** yeast mannan.

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I. INTRODUCTION

β-Glucan is a major component of fungal cell walls and is widely distributed in fungi, plants, algae, and microbes. β-Glucan is also an important immunomodulating substance used as a biological response modifier (BRM) for the treatment of cancer and infectious diseases.[1–3] The effects of Lentinan from *Lentinus edodes* and Sonifilan (SPG) from *Schizophyllum commune* in cancer therapy have been proven clinically.[4,5]

Agaricus brasiliensis (= *Agaricus blazei* Murrill sensu Heinem.) is used as a medicinal mushroom, medicine, and food. There have been reports that the extracts from the fruit bodies and mycelium of *A. brasiliensis* showed immunopharmacological effects such as antitumor activity and an influence on cytokine (TNF-α and IL-12) production.[6–8] We performed studies on a murine model and human volunteers to examine the immunoenhancing effects of the naturally outdoor-cultivated fruit body of *A. brasiliensis* KA21. *A. brasiliensis* KA21 is cultivated outdoors in Brazil. Fruit bodies were air dried by a ventilator with a blowing temperature lower than 60°C. *A. brasiliensis* KA21 has a high protein and fiber content. It also has high levels of vitamins B_1, B_2, B_6, D, niacin, pantothenic acid, folic acid, and biotin. It contains many minerals, including large amounts of iron, potassium, phosphorus, magnesium, zinc, and copper, and certain amounts of manganese and selenium. In addition, it has a high vitamin D content because it is cultivated under sunlight. Antitumor, leukocyte-enhancing, hepatopathy-alleviating, and endotoxin shock-alleviating effects were found in mice.[9] In a human study, percentage body fat, percentage visceral fat, the blood cholesterol level, and the blood glucose level were decreased, and NK-cell activity was increased.[9] Taken together, the results strongly suggest that the *A. brasiliensis* fruit body is useful as a health-promoting food. Also, it was suggested that these activities were induced by polysaccharides or a protein-polysaccharide complex. β-Glucan is one of the major components responsible for the immunomodulating activity of *A. brasiliensis*.

We have developed various β-glucans derived from fungi, namely, Grifolan (GRN) from *Grifola frondosa*,[10–12] SSG from *Sclerotinia sclerotiorum*,[13,14] OL-2 from *Omphalia lapidescens*,[15] PVG from *Peziza vesiculosa*,[16] CSBG from *Candida* spp.,[17] ASBG from *Aspergillus* spp.,[18] OX-ZYM from a yeast-cell preparation, zymosan,[19,20] SCG from *Sparassis crispa*,[21] and AgHWE and AgCA from *Agaricus brasiliensis*.[6] In addition, we reported that these glucans show immunopharmacological effects, such as antitumor activity, adjuvant activity, and activity to generate $CD8^+$ T cells, the production of cytokines such as IFN-γ, and the production of nitric oxide.[22,23] β-1,3- and β-1,6-glucans show a diversity of structural and physical properties, such as degree of branching, conformation—the triple helix, single helix, and random coil, molecular weight, and solubility in water. Because of these physical properties, the biological activity of β-glucan seems to vary significantly.

The system recognizing β-glucan that plays a role in the induction of biological activity has been extensively studied. Some of the cell surface molecules, such as Dectin-1, complement receptor 3, and lactosylceramide, were cited as candidates for a β-D-glucan receptor.[24–26] They may be important for phagocytosis and other biological activities.

Specific antibodies are key molecules in acquired immunity and have been shown to promote phagocytosis, enhancing the presentation of antigens and co-stimulatory molecules and modifying the production of cytokines and so on. It is generally accepted that 6-branched β-1,3-D-glucans in mushrooms are not good antigens for inducing a specific response.[27] However, an anti-BG antibody was detected in sera from human volunteers.[28] This antibody was highly reactive and specific to fungal β-1,3- and β-1,6-glucans. Furthermore, most recently, Chiani et al.[29] reported that the anti-BG antibody was detected in sera of the healthy human subjects, and the main class was IgG2. The anti-BG antibody titer fluctuated in a patient with a deep mycosis whose serum was β-1,3-glucan-positive, a patient with autoimmune disease (rheumatoid arthritis, antineutrophil cytoplasmic-antibody-associated vasculitis), and a cancer patient.[30] The sera of DBA/1 and DBA/2 mice, as well as bovine species, also contained higher titers of anti-BG antibody.[31,32] In addition, it was recently suggested that anti-BG antibody formed an antigen-antibody complex and participated in the immunopharmacological activity

of BG.[33] Furthermore, the anti-BG monoclonal antibodies that reacted in intact cells of pathogenic fungi were developed, and it is suggested that these antibody worked as the protective antibody for the infection of *C. albicans* and *C. neoformans*.[34–36] Anti-BG antibody could play a role in the recognition of BG and induce the clearance of pathogenic fungi and biological activity in collaboration with other molecules such as the BG receptor and complement in humans.

In this study, we examined the reactivity of anti-BG antibody to *A. brasiliensis* extracts in human sera and the anti-BG antibody titer in volunteers taking *A. brasiliensis*.

II. MATERIALS AND METHODS

A. Materials

All strains of *Aspergillus* spp. and *Candida albicans*, purchased from NITE Biological Resource Center (Chiba, Japan), were maintained on Sabouraud agar (Difco, Detroit, MI, USA) at 25°C and transferred once every 3 months. Sodium hypochlorite solution and sodium hydroxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Distilled water was from Otsuka Co., Ltd. (Tokyo, Japan). *Candida albicans* and *Aspergillus niger* solubilized cell-wall glucans (CSBG and ASBG) were prepared by the NaClO-oxidation method according to a procedure used previously.[17,18] Grifolan (GRN) was prepared by the fermentation of the mycelium of *Grifola frondosa*, as described in a previous report.[11] Polysaccharide fractions (AgHWE, AgCA-1, AgCA-2, AgHA) from *Agaricus brasiliensis* were also prepared as described previously.[6] Sonifilan (SPG) was purchased from the Kaken Pharmaceutical Co. (Tokyo, Japan). Dextran was obtained from the Seikagaku Corp. (Tokyo, Japan). Laminaran (LAM) and yeast mannan were from Nakarai Tesque, Ltd. (Kyoto, Japan).

B. Volunteers and Study Design

The study was conducted in accordance with the Declaration of Helsinki of 1964 (revised version of Edinburgh 2000). The study protocol was approved by the ethics committee of the Feel Fine Clinic (http://www.ffclinic.or.jp), and all volunteers provided written informed consent before study entry.

Group 1: Fifty-two healthy volunteers were randomly divided into 2 groups. One group was given *A. brasiliensis* tablets (Toei Pharmaceutical Co., Ltd., Tokyo, Japan) 3 g/day for 6 months (N = 27, average age = 43 ± 11, males = 13, females = 14). The tablets were made of 100% powder of the fruit body of *A. brasiliensis* cultivated outdoors in Brazil, by direct tableting. The other group was given a placebo for 6 months (N = 25, average age = 45 ± 9, males = 12, females = 13). Blood was collected from each volunteer at 0, 3, and 6 months.

Group 2: Twenty-five healthy volunteers were randomly divided into 2 groups. One group was given *A. brasiliensis* tablets (Toei Pharmaceutical Co., Ltd., Tokyo, Japan) 3 g/day for 3 months (N = 14, average age = 49 ± 9, males = 9, females = 5). The other group was given a placebo for 3 months (N = 11, average age = 46 ± 11, males = 6, females = 5). Blood was collected from each volunteer before and after these samples were taken.

After centrifugation, samples were stored at –20°C before the carrying out of an ELISA with anti-BG antibody.

C. ELISA for Anti-BG Antibody

A 96-well Nunc plate was coated with a glucan preparation in 0.1 M carbonate buffer (pH 9.6) by incubation at 4°C overnight. The plate was washed with PBS containing 0.05% Tween 20 (Wako Pure Chemical Industries Ltd.) (PBST) and blocked with 1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO, USA) (BPBST) at 37°C for 60 minutes. After being washed, the plate was incubated with serum at 37°C for 60 minutes. The plate was washed with PBST and then treated with peroxidase-conjugated anti-human IgG+M+A antibody (Sigma) in BPBST and developed with a TMB substrate system (KPL Inc., MD, USA). Color development was stopped with 1 M phosphoric acid, and the optical density was measured at 450 nm. An immune plate (Nunc 442404, F96 Maxisorp) was used for all ELISA experiments in this study.

D. Statistical Analysis

The paired *t* test was used to evaluate statistical significance. $P < 0.05$ was considered significant in all analyses.

III. RESULTS

A. The Titer and Reactivity of Anti-β-Glucan Antibody in Human Sera

First, the titer and reactivity of the anti-BG antibody in healthy volunteers (N = 20) were examined by ELISA with various BG preparations (Fig. 1a, Table 1). The structural characteristics of the polysaccharides used in this experiment are summarized in Table 1. The sera of healthy volunteers showed the highest titer to CSBG, a β-1,3-glucan containing a slightly branched long β-1,6-glucan segment. A significant anti-BG antibody titer to AgHWE, mainly composed of β-1,6-glucan, or ASBG, mainly composed of β-1,3-glucan, was also detected. On the other hand, there was only a weak response to GRN, a 6-branched β-1,3-glucan from *Grifola frondosa* and yeast mannan. The level of the anti-BG antibody titer varied among individuals. About a fivefold difference existed between the highest and lowest levels (Fig. 2).

Further, we examined the reactivity of human sera to each fraction of *A. brasiliensis*. A significant titer to all fractions tested was detected, and, again, individual differences existed (Fig. 1b).

Next, the specificity of the anti-BG antibody was examined by adding soluble BG, ASBG, or AgHWE as a competitor to CSBG-coated plates (Fig. 3a). The binding of the antibody to CSBG-coated plates was inhibited by ASBG or AgHWE. Moreover, when both antigens were added at the same time, the binding was inhibited further. These results suggested that the anti-BG antibody in humans was broadly specific, with reactivity to β-1,3- and β-1,6-glucan chains.

Moreover, we performed a competitive ELISA to examine the reactivity of each *A. brasiliensis* fraction to CSBG-coated plates. The binding of anti-BG antibody to CSBG was strongly inhibited by all fractions, and the inhibition became saturated with a low concentration of *A. brasiliensis* antigens (Fig. 3b). Anti-BG antibody showed cross-reactivity to CSBG and β-glucan, each containing *A. brasiliensis* extract. Furthermore, the cross-reactivity between each *A. brasiliensis* fraction and CSBG was examined by competitive ELISA using plates coated with each fraction (Fig. 4). The binding of anti-BG antibody was inhibited by each *A. brasiliensis* fraction in each plate. Anti-BG antibody showed cross-reactivity among these fractions. The inhibition by CSBG did not correspond to that by the *A. brasiliensis* fractions. These differences probably resulted from the difference in the content ratio or structure of the polysaccharide and the protein in each fraction. Hence, CSBG, as a standard antigen, was used in the following examination of the binding of the antibody to BG.

B. Anti-β-Glucan Antibody Titer in Subjects Taking *Agaricus brasiliensis*

We next examined the change in the anti-BG antibody titer of healthy volunteers taking *A. brasiliensis* for 6 months (group 1; N = 27, average age = 43 ± 11, males = 13, females = 14). The average titer before the taking of *A. brasiliensis* was 3198.4 ± 3337.7 units, and 7 volunteers had a high titer (above 4000 units). The average titer after 3 and 6 months of taking *A. brasiliensis* was 3378.0 ± 3391.7 and 3481.2 ± 2922.7 units, respectively (Fig. 5). In all these cases, the change was not significant (0 vs. 3 months, 0 vs. 6 months, $p > 0.05$ paired *t* test). Among the volunteers with high anti-BG antibody titers, one showed a decrease in the titer after taking *A. brasiliensis*. However, the volunteers with an average (about 2500 units) or lower titer before taking *A. brasiliensis* tended to show an increase in the anti-BG antibody titer. Because the individual difference in the anti-BG antibody titer was considerable, we evaluated the change in the titer in each individual (Fig. 6). In the *A. brasiliensis* group, the titer increased significantly (0 vs. 6 months $p = 0.026 < 0.05$ paired *t* test). The average increase in the *A. brasiliensis* group was 21.8% at 3 months and 33.8 % at 6 months. The average increase in the placebo group was –5%

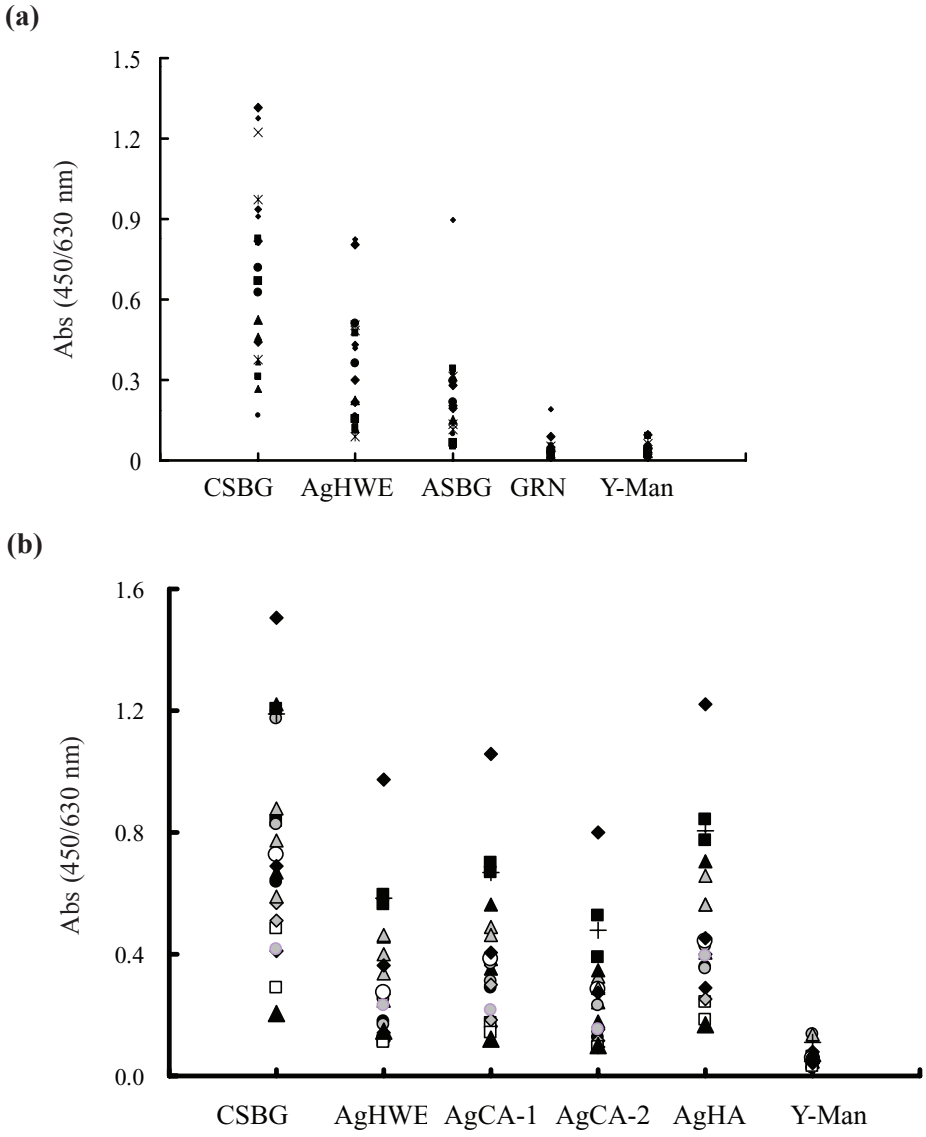


FIGURE 1. Comparison of reactivity of human sera to various polysaccharide-coated plates. An ELISA plate was coated with (**A**) each polysaccharide and (**B**) *Agaricus brasiliensis* extract (AgHWE, AgCA-1, AgCA-2, AgHA; 25 μg/mL in carbonate buffer) and blocked by BSA before use. Sera were diluted and the amount of plate-bound Ig was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate. Data from 20 healthy volunteers are shown.

at 3 months and 10.3% at 6 months. Individual differences existed in the rate of increase. One volunteer showed an increase of about fourfold. But some individuals exhibited no change. Fourteen volunteers had an increased titer at 3 months and 17 volunteers at 6 months. Thirteen volunteers had a decreased titer at 3 months and 10 volunteers at 6 months. On the other hand, in the placebo group, no significant change was observed. Ten volunteers had an increased titer at 3 months and 14 volunteers at 6 months, whereas 15 volunteers had a decreased titer at 3 months and 11 volunteers at 6 months. To examine whether the increase of anti-BG antibody was specific or not, we measured the antibody titer to ovalbumin (OVA), a general antigen in the *A. brasiliensis* group (Table 2). The average increase

TABLE 1
List of Polysaccharides and Their Characteristics

Source	Abbreviations	Main components	References/remarks
Agaricus brasiliensis	AgHWE	β-1,6-glucan with β-1,3-glucan segment	Ohno et al.[6]
	AgCA-1	β-1,6-glucan with β-1,3-glucan segment	Ohno et al.[6]
	AgCA-2	β-1,6-glucan with β-1,3-glucan segment	Ohno et al.[6]
	AgHA	β-1,6-glucan with β-1,3-glucan segment	Ohno et al.[6]
Grifola frondosa	GRN	β-1,6-branching β-1,3-glucan	Ohno et al.[11,12]
Candida albicans	CSBG	β-1,3-linked β-1,6-glucan	Ohno et al.[17]
Aspergillus niger	ASBG	β-1,6, β-1,3-glucan	Ishibashi et al.[18]
Saccharomyces cerevisiae	Y-Man	Mannan	Yeast mannan prepared from *Saccharomyces cerevisiae* purchased from Nakalai Tesque, Ltd.

was 13.8% at 3 months and 7.2% at 6 months. The anti-OVA antibody titer did not change significantly after the taking of *A. brasiliensis*.

Similar results were obtained in another independent set of experiments (group 2; healthy volunteers taking *A. brasiliensis* for 3 months; N = 14, average age = 49 ± 9, male = 9, female = 5).

Figure 7 shows some examples of the kinetics of the anti-BG antibody titer in the *A. brasiliensis* group. Individual differences in the rate and timing of the increase were found. There was an increase from a low titer (500 units) to an average titer (about 2500 units) in one subject, an increase at 3 months in another subject, and an increase at

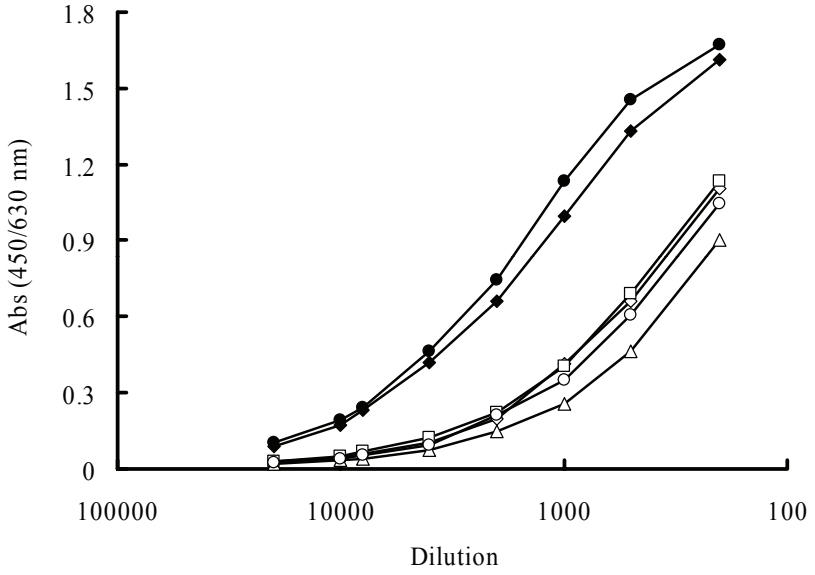


FIGURE 2. Comparison of anti-BG antibody titer of individuals with high and low titers. An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer) and blocked by BSA before use. Sera were diluted (×200, 500, 1000, 2000, 4000, 8000, 10000, and 20000), and the amount of plate-bound Ig was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate. Data from the sera of the individuals with low (n = 4; open symbol) and high (n = 2; close symbol) titers in Figure 1a are shown. Open symbols (○ △ □ ◇): low-titer volunteers; closed symbols (● ◆): high-titer volunteers.

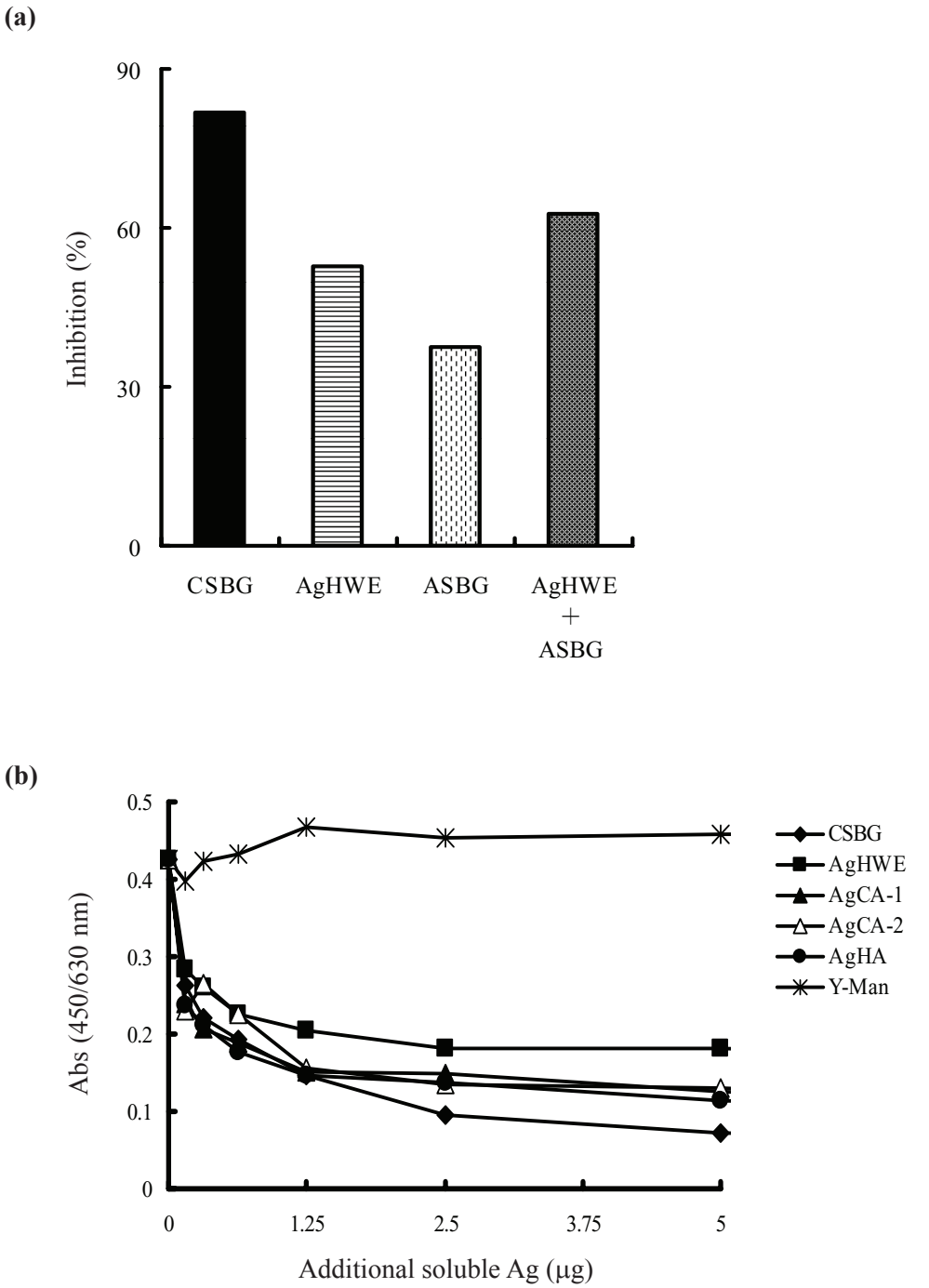


FIGURE 3. Specificity of human sera against plate-bound glucan. (**a**) An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer). Sera were mixed with serially diluted polysaccharides (CSBG or AgHWE, ASBG, ASBG+AgHWE) and then applied to the ELISA plate. The amount of plate-bound Ab was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate. (**b**) An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer). Sera were mixed with serially diluted polysaccharides (CSBG or *Agaricus brasiliensis* extracts [AgHWE, AgCA-1, AgCA-2, AgHA], Y-Man) and then applied to the ELISA plate. The amount of plate-bound Ab was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate.

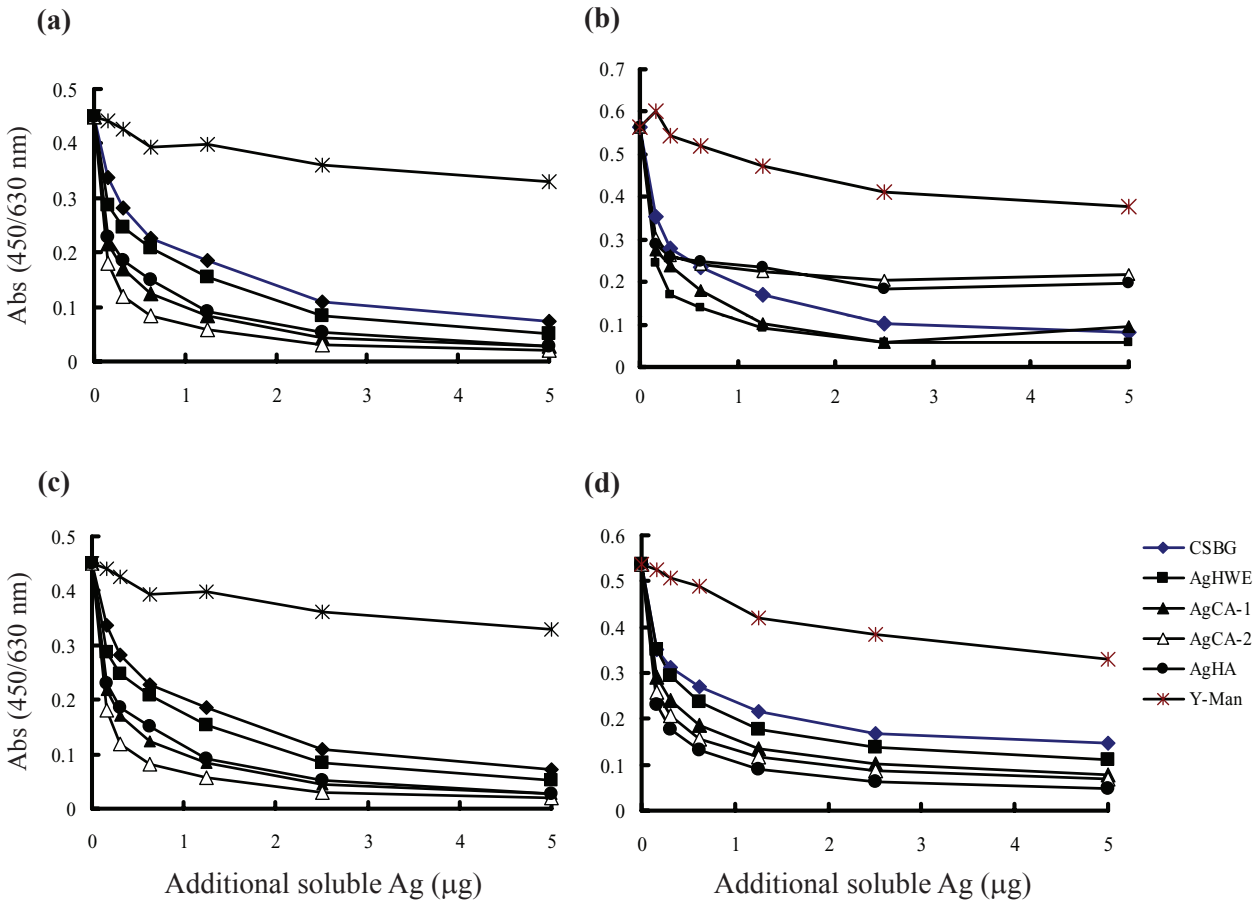
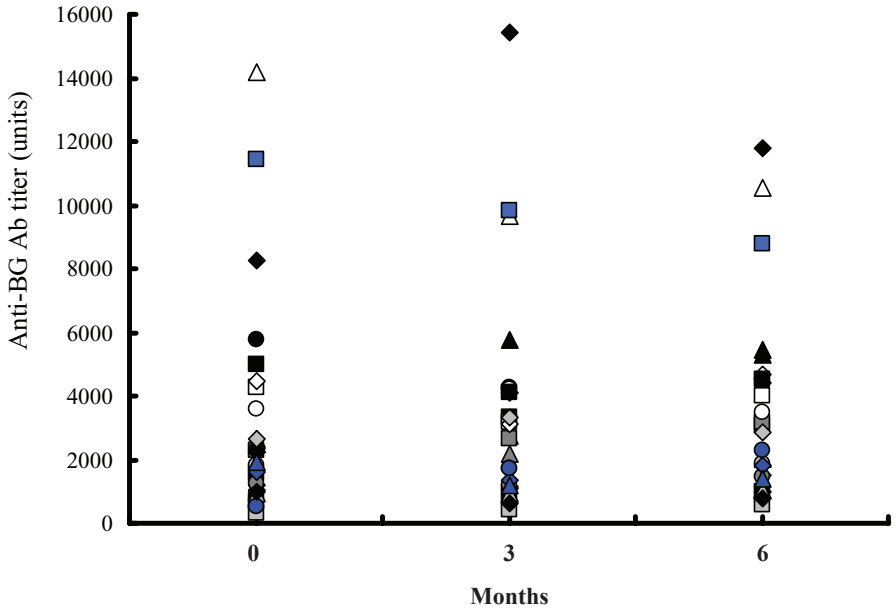


FIGURE 4. Cross-reactivity of human sera against *Agaricus brasiliensis* extracts. An ELISA plate was coated with *A. brasiliensis* extracts [(a) AgHWE, (b) AgCA-1, (c) AgCA-2, (d) AgHA; 25 μg/mL in carbonate buffer]. Sera were mixed with polysaccharides (CSBG or *A. brasiliensis* extracts [AgHWE, AgCA-1, AgCA-2, AgHA], Y-Man; 25 μg/mL) and then applied to the ELISA plate. The amount of plate-bound Ab was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate.

6 months in yet another subject. The kinetics of the anti-OVA antibody titer was examined in the individuals above. The anti-OVA antibody response was different from the anti-BG response. In addition, this titer was minimally changed by the taking of *A. brasiliensis*. These results suggested that *A. brasiliensis* specifically caused the change in the BG antibody titer.

The antibody was characterized by structure and function. We examined the anti-BG antibody titer according to each class (IgG, IgM, IgA) in human sera (Fig. 8). The IgG class antibody showed the highest titer. IgM and IgA class antibodies were also detected, but showed low titer. Individual differences existed in each class. The number of individuals with twice or more the average absorbance was three for IgG, one for IgM, and two for IgA. Next, we evaluated the effect of *A. brasiliensis* on the titer of each class of anti-BG antibody in each individual (Fig. 9). Individual differences existed in the increase in each class, and the class that increased was different in each individual. After 6 months of taking *A. brasiliensis,* the IgG class antibody showed the highest titer. However, the IgA class showed the greatest average increase (IgG: 30.4%; IgM: 49.5%; IgA: 90.3%). Moreover, the IgA class increased in the greatest number of individuals.

(a) *A. brasiliensis*



(b) Placebo

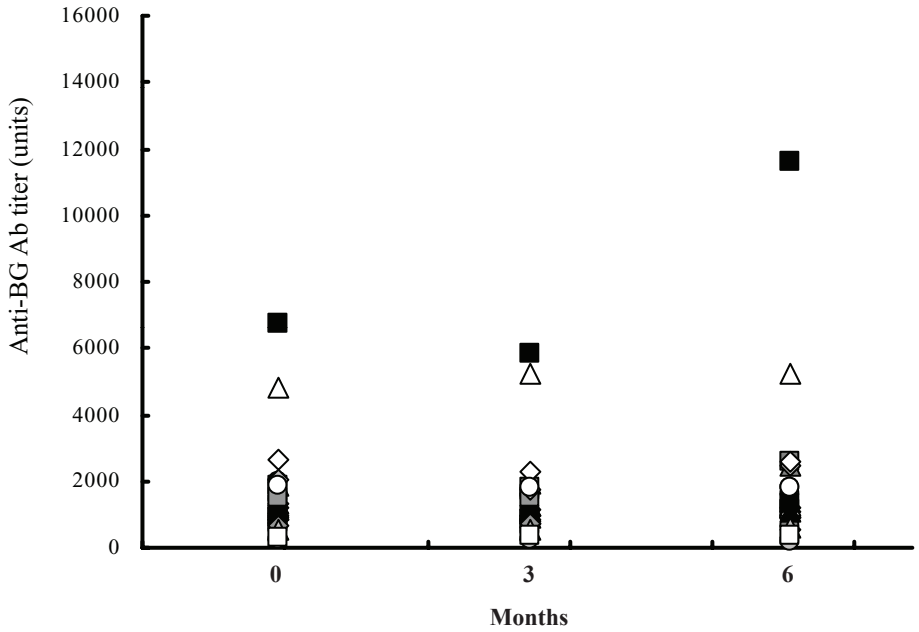


FIGURE 5. Effect of the oral administration of the *Agaricus brasiliensis* preparation on the anti-BG antibody titer [(**a**) *A. brasiliensis* or (**b**) placebo group]. An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer) and blocked by BSA before use. Sera were diluted and the amount of plate-bound Ig was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate.

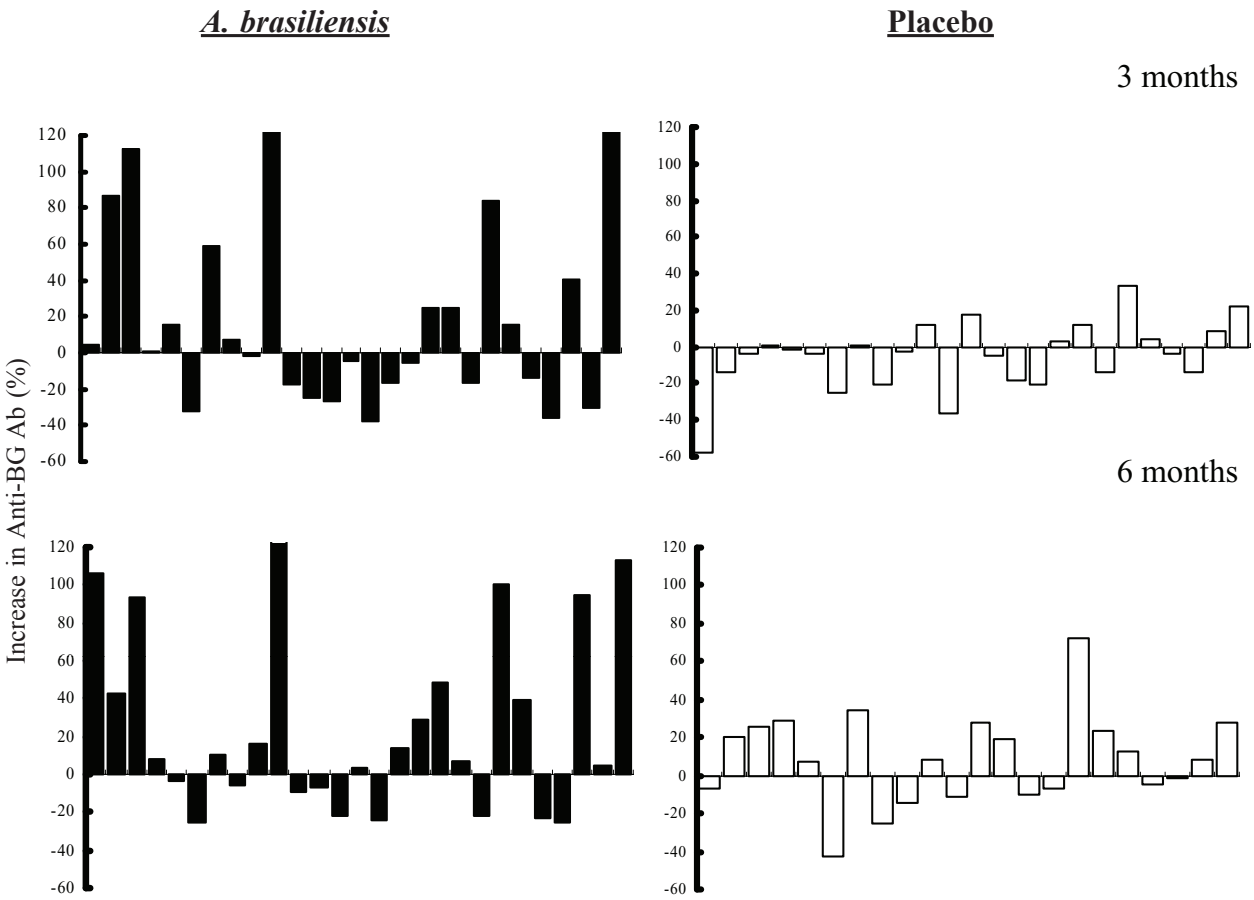


FIGURE 6. Rate of increase in the anti-BG antibody titer in the *Agaricus brasiliensis* or placebo group. The anti-BG antibody titer was measured by ELISA using CSBG-coated plates. The rate of increase in the titer was calculated with the following formula: Increase in anti-BG antibody titer (%) = (anti-BG antibody units after taking—anti-BG antibody units before taking)/anti-BG antibody units before taking the preparation.

IV. DISCUSSION

We recently found an anti-BG antibody in human sera and discovered that this antibody formed an antigen-antibody complex and participated in the immune response as a molecule-recognizing β-glucan. In this study, we examined the effect of the oral administration of *Agaricus brasiliensis* on the anti-BG antibody titer in human volunteers.

First, we examined the titer in healthy volunteers using ELISA plates precoated with various polysaccharides. It was shown that the anti-BG antibody recognized a β-1,6-glucan chain and/or β-1,3-glucan chain. The reactivity of each anti-BG antibody in sera depended on the individual. β-Glucan is one of the main components of the fungal cell wall. Also, fungi are distributed in the surrounding environment. It is known that fungi

TABLE 2
Increase in Titers of Anti-β-Glucan and Anti-OVA Antibody in the *Agaricus brasiliensis* Group

		Average titer increase (%)
3 months	Anti-β-glucan	21.8 ± 63.6
	Anti-OVA	13.8 ± 50.5
6 months	Anti-β-glucan	33.8 ± 77.0
	Anti-OVA	7.17 ± 42.2

such as *Candida* spp. colonize the intestinal tract. It was suggested that production of the anti-BG antibody in normal human sera was induced by fungi that colonized the mucosal surface. We previously examined the anti-BG antibody in bovine sera.[31] The bovine anti-BG antibody was detected in calf and bovine sera but not fetal-calf sera. The bovine sera showed higher anti-BG antibody titers than the calf sera. Also, the titer rose with time in the calves. It is thought that production of the anti-BG antibody was therefore induced by an environmental factor. β-Glucan is widely distributed in nature, including foods. The anti-BG antibody was reactive to AgHWE mainly composed of β-1,6-glucan, the extract of the fruit body of *A. brasiliensis*. On the other hand, it showed only a weak response to GRN, a 6-branched β-1,3-glucan from medium cultivated with the mycelial form of *G. frondosa*. Such differences in cultivation and growth conditions may influence the structure and architecture of β-glucan, which stimulate the mucosal immune system and induce antibody production. Environmental factors

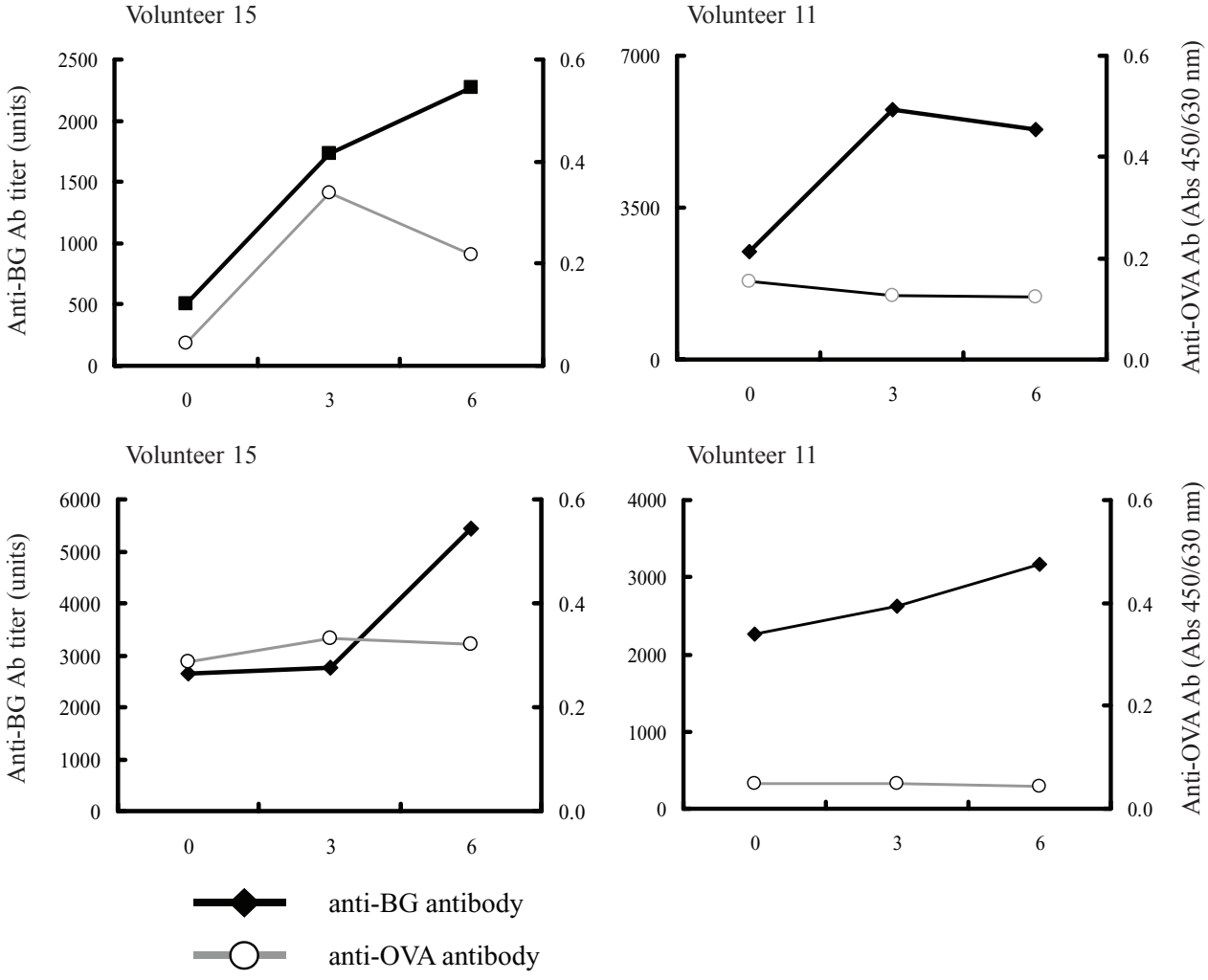


FIGURE 7. Kinetics of anti-BG and anti-OVA antibody titers in volunteers taking the *Agaricus brasiliensis* preparation. An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer) and blocked by BSA before use. The sera of human volunteers at 0, 3, and 6 months were added to the plate, and the amount of plate-bound Ig was determined with peroxidase-conjugated anti-human IgG+M+A antibody. Enzyme activity was measured by the addition of TMB substrate.

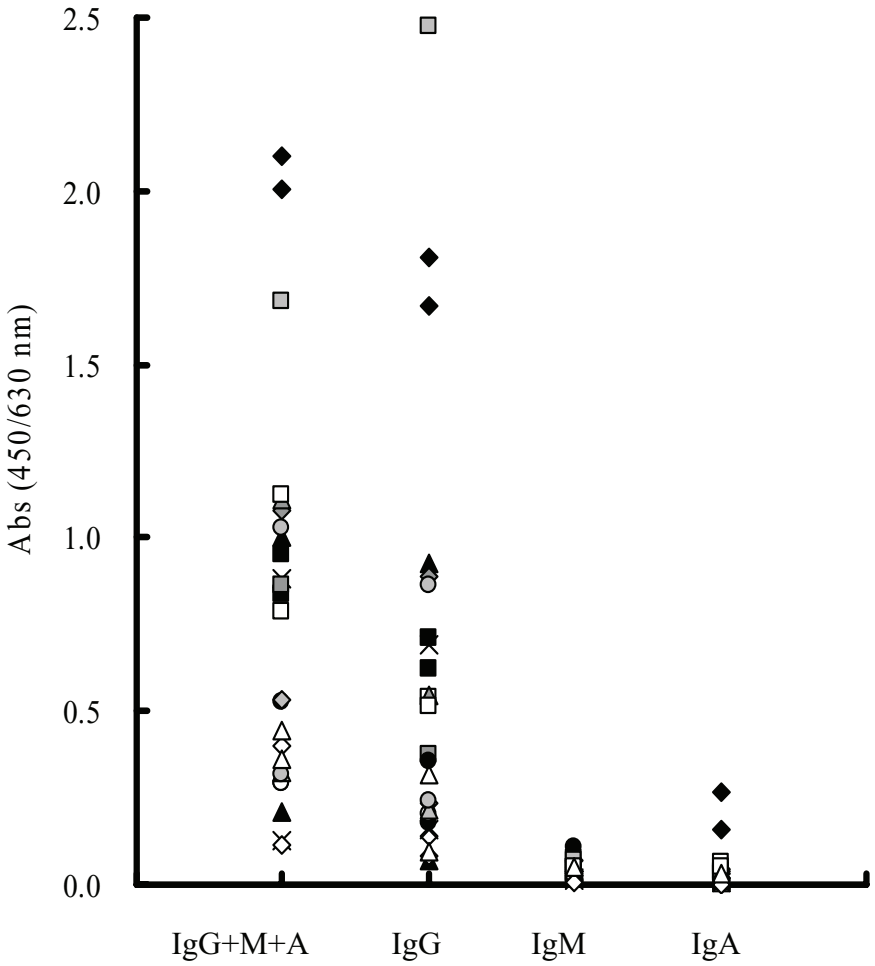


FIGURE 8. Comparison of anti-BG IgG, IgM, and IgA antibody titers in human sera. An ELISA plate was coated with CSBG (25 μg/mL in carbonate buffer) and blocked by BSA before use. Sera were diluted and the amount of plate-bound Ig was determined with peroxidase-conjugated anti-human IgG, IgM, or IgA antibody. Enzyme activity was measured by the addition of TMB substrate. Data from 20 healthy volunteers are shown.

might cause the difference in the titer and reactivity of the anti-BG antibody.

Next, we examined the anti-BG antibody titer in healthy volunteers taking *A. brasiliensis*, one of the main components of which is β-glucan. The volunteers in the *A. brasiliensis* group showed an increasing trend in the anti-BG antibody titer as compared with those in the placebo group. Furthermore, the titer of antibody to OVA was hardly changed by the taking of *A. brasiliensis*. These results suggested that an antigen-specific response to BG was induced by oral administration. Some volunteers exhibited a mild increase in the anti-BG antibody titer in the placebo group. Because there were no environmental restrictions such as limited food intake in this trial, these factors might have induced a mild increase in the titer in the placebo group.

Suzuki et al.[37–39] reported that the oral administration of BG inhibited syngeneic tumor growth and enhanced phagocytic activity, candidacidal activity, and production of interleukin-1 in mouse peritoneal macrophages. In addition, it was reported that β-glucan administered orally was bound and internalized by intestinal epithelial cells and gut-associated lymphoid tissue cells, and increased systemic levels of IL-12 and survival following a challenge with *Candida albicans* or *Staphylococcus aureus* in mice.[40] However, there have been few

reports on the effect of oral BG administration in humans. The present study demonstrated that oral administration of *A. brasiliensis* increased the anti-BG antibody titer and suggested that it induced a β-glucan-specific response via the mucosal immune system in humans. In the *A. brasiliensis* group, the rate at which and process by which the anti-BG antibody titer increased varied in each individual. These results suggest individual differences in the responsiveness to BG in addition to environmental factors. It was reported that there was individual diversity in the level of production of cytokine by human peripheral blood monocytes (PBMCs) stimulated with BG *in vivo* and *vitro*.[41] It is possible that the production of the anti-BG antibody is an index of responsiveness to BG.

The anti-BG antibody cross-reacted with *Candida albicans* solubilized cell-wall glucan, CSBG, and *Agaricus brasiliensis* extracts. An antibody to *A. brasiliensis* extracts (AgHWE, AgCA-1, 2, AgHA) that contains β-1,6-glucan as the main component was detected. These antibodies showed specific reactivity to BG in a competitive ELISA using *A. brasiliensis* extract-coated plates. CSBG is composed of a slightly branched long β-1,6- and β-1,3-glucan chain. *A. brasiliensis* extracts were mainly composed of β-1,6-glucan. We previously reported that anti-BG antibody contributed to host defense against pathogenic fungi such as *Candida albicans* and *Aspergillus* spp.[33] In addition, it was recently shown that anti-BG antibody mediated protection against both experimental candidasis and aspergillosis by mechanisms that involve the direct antifungal properties of antibody.[34] The incidence of deep mycosis has been increasing with improvements in chemotherapy for malignant diseases and the popularization of marrow transplant and organ transplant medical care. These results suggested that the oral administration of *A. brasiliensis* extracts modifies the host immune system and prevents fungal infections. The functional role of the anti-BG antibody in the biological activity of BG as a BRM is little understood. We previously

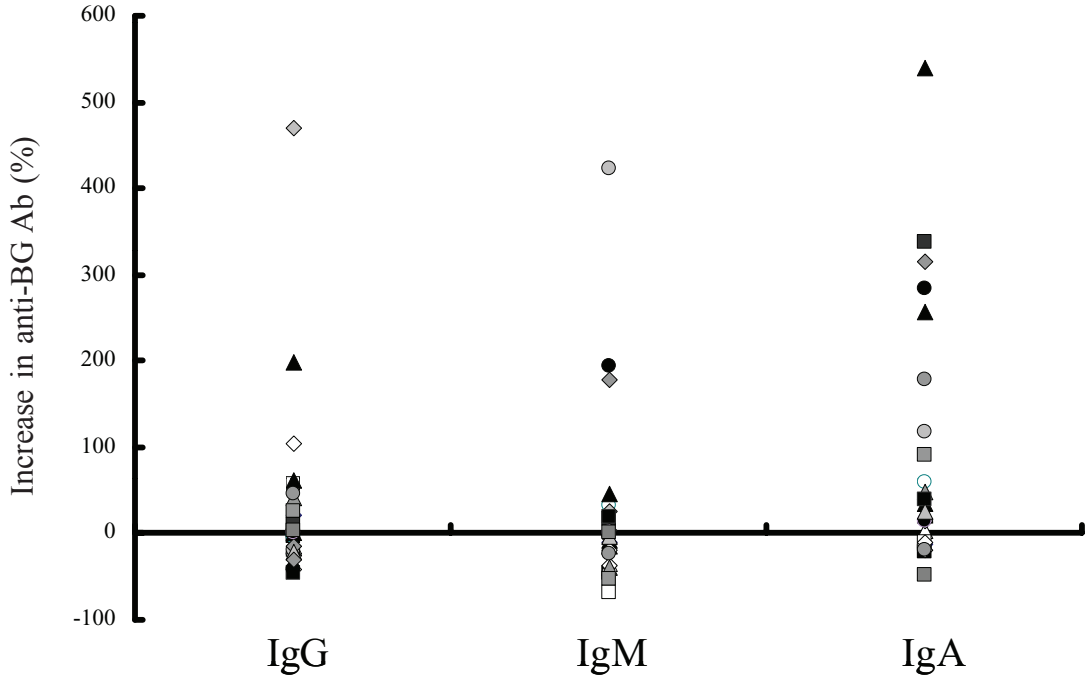


FIGURE 9. Comparison of the increase in the anti-BG IgG, IgM, or IgA antibody titer in the *Agaricus brasiliensis* group. The anti-BG IgG, IgM, or IgA antibody titer was measured by ELISA using CSBG-coated plates. The rate of increase in the titer was calculated.

reported that an anti-BG antibody isolated from an immunoglobulin preparation enhanced the response of human macrophages derived from THP-1 to *C. albicans in vitro*. Rachini et al.[35] also reported that anti-BG antibody promoted phagocytosis of *Cryptococcus neoformans*. The BG high-responder strain DBA/2 had a higher anti-BG antibody titer than the other strains of mice.[31] The difference in the anti-BG antibody titer might be related to the responsiveness to BG. This antibody is an important functional molecule in acquired immunity. It enhanced complement activation, NK-cell function, and phagocytosis via the Fc receptor dependent on class. We examined the class of the anti-BG antibody in human sera. Anti-BG antibodies of the IgG, IgM, and IgA class were detected, and the IgG class had the highest titer. It is generally accepted that IgG possesses activity to bind the Fc receptor and is as opsonic. This result was consistent with the report that the anti-BG antibody was detected in human immunoglobulin G preparations. With the taking of *A. brasiliensis*, the anti-BG antibody titer of each class increased in every individual. Also, an increase of the IgA class was noticeable. It was suggested that mucosal immunity is activated by the taking of *A. brasiliensis*. It is also possible to show the action of the anti-BG antibody as one of the molecules recognizing BG in cooperation with other receptors. A part of the functional role of the anti-BG antibody might be figured out by identifying the class of the anti-BG antibody in human sera.

In this study, we first demonstrated the clinical effect of oral administration of *A. brasiliensis* on the anti-BG antibody titer in humans. The results suggested that *A. brasiliensis* induced a BG-specific response, and individual differences existed in this response. The resulting production of anti-BG antibody could be useful as an index of the immune response to BG in humans.

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