Analysis of specific antibodies and immune biomarkers in healthy subjects after consumption of Sophy β-glucan (β-1,3-1,6 glucan) as a health food supplement

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Abstract

In this clinical study, we investigated changes in the levels of β-1,3-1,6 glucan (BG)-, lipopolysaccharide (LPS)-, and ovalbumin (OVA)-specific antibodies (IgG, IgA2, and IgM), interleukin-6 (IL-6), soluble CD44 (sCD44), and granulocyte-colony stimulating factor (G-CSF) in the sera and saliva of 15 healthy subjects after consuming commercially produced Sophy β-glucan (main ingredient: BG) for 1-2 weeks as a health food supplement. The results showed significant increases in BG-specific IgG (9.51 ± 4.37%) and IgM (16.51 ± 8.87%), but not in BG-specific IgA2. We observed no changes in LPS- and OVA-specific IgG, IgA2, or IgM between before and after consumption of the supplement. Clear increases in serum IL-6 (39.37 ± 16.81%), sCD44 (15.88 ± 8.39%), and G-CSF (15.04 ± 8.43%) were also observed after consuming Sophy β-glucan. Increases in IL-6 (8.33 ± 3.82%), BG-IgA2 (11.27 ± 4.63%), and sCD44 (14.12 ± 9.54%) in the subjects' saliva were also observed after consuming Sophy β-glucan for 1 week. Taken together, our data suggest that the consumption of Sophy β-glucan clearly enhanced the BG-specific immune network system in the whole body.

Key words : β-1,3-1,6 glucan (BG), BG-specific antibody, immune biomarker, immune network system

Introduction

Glucans are mainly found in extracts of some species of mushrooms and in certain microbes, such as black yeast, and they have several unique immunological activities. In particular, β-glucans have been shown to activate cytotoxic activity against cancer cells accompanied by increased production of interleukin-2 (IL-2), IL-6, IL-12, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)[1, 2]. These findings strongly indicate that β-glucans enhance the immune system underlying the activation of lymphocytes, monocytes, macrophages, granulocytes, and natural killer (NK) cells via the β-glucan receptor, Dectin-1[3].

We established and succeeded in purifying β-1,3-1,6 glucan (BG) produced by the black yeast *Aureobasidium pullulans* (*A. pullulans*) strain AFO-202 by using the latest culture technology as a health food supplement (Sophy β-glucan), which contains about ten-fold the quantity of BG in mushroom extracts.

It has recently been reported that human and animal sera contain BG-specific antibodies[4], and we have reported finding an immunological relationship between BG-specific antibodies and immune biomarkers, including various cytokines and soluble-form molecules[5]. However, the rates of changes or kinetics of these BG-specific antibodies and other immune biomarkers after consuming Sophy β-glucan (main ingredient: BG) as a health food supplement had never been investigated.

In this clinical study we investigated changes in the levels of BG-specific antibodies (IgG, IgA2, and IgM) and other immune biomarkers, IL-6, soluble-form CD44 (sCD44), and granulocyte-colony stimulating factor

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(G-CSF), in the sera and saliva of 15 healthy subjects after consuming Sophy β-glucan.

Materials and Methods

Reagents

Sophy β-glucan was produced by Sophy Co. (Kochi-city, Kochi) from *A. pullulans* strain AFO-202, by using the latest biological culture and preparation techniques, and it is currently available commercially as a health food supplement. The super-purified BG derived from Sophy β-glucan described above that was used as an antigen in this study was kindly provided by the Sophy Co. Enzyme immunoassay (EIA) kits for IL-6 and sCD44 were purchased from Diaclone Laboratories Co. (USA). An EIA kit for G-CSF was purchased from IBL Laboratories Co. (Fujioka, Gunma). Lipopolysaccharide (LPS) and ovalbumin (OVA) were purchased from Sigma Co. (USA). Horseradish peroxidase (HRPOD)-conjugated rabbit anti-human IgG, HRPOD-conjugated rabbit anti-human IgM and HRPOD-conjugated mouse anti-human IgA2 monoclonal antibody (mAb) were purchased from MBL Co. (Nagoya).

Ethics statement

The study protocol was approved by the institutional review boards (IRBs) of Kyushu University of Health and Welfare and Chikamori Hospital, and the IRB numbers were 18-024 and 161, respectively. Informed consent was obtained from all of the subjects prior to their participation in this study.

Consumption of Sophy β-glucan as a health food supplement and preparation of serum and saliva samples

Fifteen healthy subjects with no abnormalities of the oral cavity (5 males, age 38.2 ± 9.5 yr; 10 females, age 28.7 ± 5.2 yr) in Chikamori Hospital (Kochi-city, Kochi) consumed Sophy β-glucan, 40 mg/day for 1-2 weeks, as a health food supplement. Blood collection and serum preparation were performed according to the standard methods. To collect saliva, subjects were asked to gargle and rinse their mouth (oral cavity) 15 times with water, and about 40 min later a saliva specimen was collected in a 15-mL sterile tube for 2 min. All serum and saliva samples were centrifuged at 3000 rpm for 30 min, and the supernatants were harvested and stored at -80°C until assayed.

Measurement of BG-, LPS-, and OVA-specific antibodies in the serum and saliva samples

EIA plates (Sumitomo Co., Tokyo) were coated with 1 μg/mL of BG, LPS, and OVA in carbonate bicarbonate buffer (0.010 M $NaCO_3$, 0.035 M $NaHCO_3$, pH9.6) for 24 hr at 4°C. The wells were washed 4 times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBST) and blocked with PBST containing 2% BSA (BSA-PBST) for 60 min at room temperature, then washed 3 times with PBST. The sera (x1000 dilution for IgG, x50 dilution for IgA2 and x500 dilution for IgM) and the saliva (x5 dilution for IgA2) were then added to each well, and the plate was incubated for 60 min at room temperature with shaking. The wells were then washed 5 times with PBST, and after adding HRPOD-conjugated rabbit anti-human IgG (x10000 dilution), HRPOD-conjugated mouse anti-human IgA2 mAb (x3000 dilution), or HRPOD conjugated rabbit anti-human IgM (x5000 dilution) to each well, the plate was incubated for 60 min at room temperature with shaking. The wells were then washed 10 times with PBST, and after adding substrate chromogen (TMB; Cosmo Bio Co.) to each well, the plate was incubated for 20 min at room temperature with gentle shaking. The reaction was stopped by the addition of 0.5 M-HCl, and optical density (O.D.) was read at 450 nm with a multichannel EIA-microplate reader (TOSHO Co.). The experiment was repeated 5 times.

Measurement of IL-6, sCD44, and G-CSF in the serum and saliva samples

The levels of IL-6, sCD44, and G-CSF in the serum and saliva samples were measured with EIA kits. Each of the measurements was repeated 3 times.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The data were statistically analyzed by performing the unpaired *t*-test. A P value <0.05 was considered evidence of a statistically significant difference.

Results and Discussion

First, we examined the changes in BG-, LPS-, OVA-specific IgG, IgA2, and IgM and the immune biomarkers IL-6, sCD44, and G-CSF in the serum and saliva samples of the 15 healthy subjects after consuming Sophy β-glucan for 1-2 weeks.

As shown in Figure 1, clear increases in BG-specific IgG (9.51 ± 4.37%) and IgM (16.51 ± 8.87%), but not in IgA2 (1.12 ± 0.67%), were observed in the sera of the healthy subjects after consuming Sophy β-glucan, but no changes in LPS- or OVA-specific IgG, IgA2, or IgM were seen in their sera under the same conditions. The BG-specific IgG and IgM were statistically significant ($P < 0.01$) compared with the percentage increases in LPS-specific IgG and IgM and OVA-specific IgG and IgM, respectively.

Next, we also examined the changes in IL-6, sCD44, and G-CSF in the sera after consuming Sophy β-glucan. Figure 2 shows that there were marked increases in IL-6 (39.37 ± 16.81%), sCD44 (15.88 ± 8.39%), and G-CSF (15.04 ± 8.43%) in their sera. We also examined the changes in some immune biomarkers in saliva after consuming Sophy β-glucan for 1 week, and clear increases in IL-6 (8.33 ± 3.82%), sCD44 (14.12 ± 9.54%), and BG-IgA2 (11.27 ± 4.63%) were observed.

In this study increases in BG-specific IgG and IgM were observed in the sera after consuming Sophy β-glucan in comparison with before consumption, and the increases in BG-specific IgM were marked. It is well known that IgM is the first antibody to appear as part of the immunological response. It may be surmised that the increase in BG-specific IgM after consuming Sophy β-glucan plays an important role in some immunobiological response network. Although we did not establish a placebo group in this study because of the short period of consumption of Sophy β-glucan, the fact that there were no significant increases in LPS-, OVA-specific IgG, IgA2, and IgM after consuming Sophy β-glucan, suggested that the BG-specific immune reaction was effectively appeared in the whole body.

IL-6 is a cytokine produced by T-cells, B-cells, monocytes, fibroblasts, endothelial cells, etc., and plays an important role in both natural and acquired immune responses, in particularly by regulating the growth and maturation of B-cells to produce antibodies [6]. Interestingly, the results of this study showed increased IL-6 in both serum and saliva after consuming Sophy β-glucan. Thus, it is likely that the increases in BG-specific IgG and IgM in the sera and BG-specific IgA2 in the saliva were directly or indirectly influenced by IL-6 action.

CD44 is expressed on lymphocytes, macrophages, granulocytes, fibroblasts, endothelial cells, NK cells, etc., and it plays an important role in stimulating NK-cell and lymphokine-activated killer cell (LAK) functions underlying IFN-γ production. The marked increases in sCD44 in both sera and saliva after consuming Sophy β-glucan suggest that the enhancement of NK-cell and LAK- cell functions in the whole body [7].

G-CSF is a glycoprotein that stimulates the bone marrow to produce granulocytes (neutrophils) and release them into the bloodstream, and it prolongs the survival and stimulates the proliferation and differentiation, and function of neutrophil precursors and mature neutrophils [8]. In this study we observed an increase in G-CSF production in the sera after consuming Sophy β-glucan. Sophy β-glucan consumption probably stimulates the proliferation and differentiation of neutrophils in the immune response and protects against microbial infection.

Although the exact mechanism(s) underlying the increase and immunological roles of BG-specific antibodies and immune biomarkers IL-6, sCD44, and G-CSF in the sera and saliva of healthy subjects after consuming Sophy β-glucan remains unclear, we are vigorously investigating the mechanism(s) by using molecular immunological techniques. Since β-glucan has been reported to stimulate intestinal immunity [9], it is possible that the action of intestinal bacteria flora promotes the increase in BG-specific antibodies and some immune biomarkers after Sophy β-glucan

consumption, and more effectively regulates the BG-related immune network system in the whole body as a biological response modifier (BRM). Further analyses at the cellular and molecular levels in an *in vitro* model are needed to identify the exact mechanism(s) responsible for the increase in BG-specific antibodies and some immune biomarkers after consuming Sophy β-glucan.

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Disclosure

None of the authors has any conflicts of interest (COI) to disclose.

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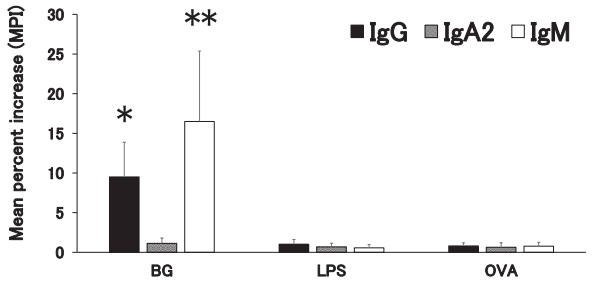

Figure 1. Measurement of BG-, LPS-, and OVA-specific IgG, IgA2 and IgM in the sera of 15 healthy subjects after consuming Sophy β-glucan. The measurements were performed using an EIA system (See Materials and Methods for details). Each measurement was repeated 4 times. The percentage increases in each subject were calculated as follows. Percent increase (%) = {δOD value after consumption - δOD value before consumption)/δOD value before consumption} x 100, and the average values for the entire population (n = 15) were then calculated.
* P < 0.01 (BG-specific IgG vs. LPS-specific IgG or OVA-specific IgG), ** P < 0.01 (BG-specific IgM vs. LPS-specific IgM or OVA-specific IgM).

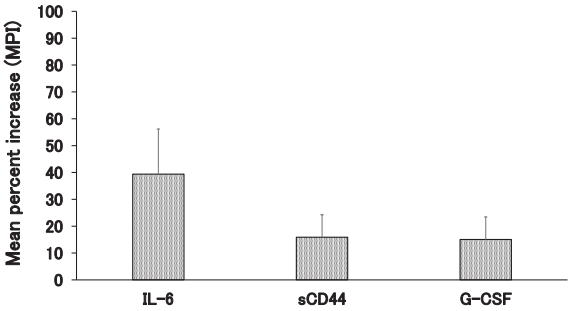

Figure 2. Measurement of IL-6, sCD44, and G-CSF in the sera of 15 healthy subjects after consuming Sophy β-glucan. The measurements were performed using an EIA system (See Materials and Methods for details). Each measurement was repeated 4 times. The percentage increases in each subject were calculated as follows. Percent increase (%) = {amount after consumption - amount before consumption)/amount before consumption} x 100, and the average values for the entire population (n = 15) were then calculated.

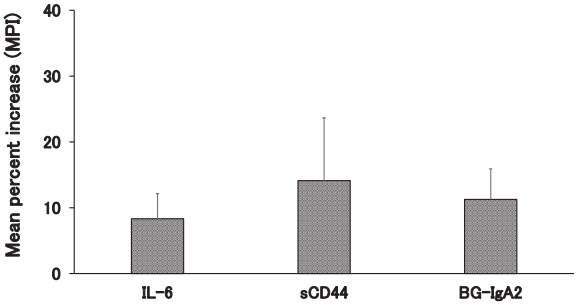

Figure 3. Measurement of IL-6, sCD44 and BG-IgA2 in the saliva of 15 healthy subjects after consuming Sophy β-glucan. The measurements were performed using an EIA system (See Materials and Methods for details). Each measurement was repeated 4 times. The percentage increases in each subject were calculated as follows. Percent increase (%) = {amount or δOD value after consumption - amount or δOD value before consumption)/amount or δOD value before consumption} x 100, and the average values for the entire population (n = 15) were then calculated.

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Sophy β-glucan（β-1,3-1,6 glucan）摂取後の特異抗体と免疫バイオマーカーの解析

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要旨

Sophy β-glucan（β-1,3-1,6 glucan）摂取後の健康人血清中および唾液中のβ-1,3-1,6 glucan（BG）特異抗体と免疫バイオマーカーの動態を酵素抗体法で検討した。その結果、Sophy β-glucan 摂取 2 週間後、摂取前に対して血清中に BG 特異 IgG(9.51 ± 4.37%) と IgM(16.51 ± 8.87%) の増加が認められた。一方、lipopolysaccharide (LPS) および ovalbumin (OVA) 特異 IgG､IgA2 および IgM の増加は認められなかった。さらに、Sophy β-glucan 摂取 2 週間後、摂取前に対して血清中に IL-6(39.37 ± 16.81%)、sCD44(15.88 ± 8.39%)、G-CSF(15.04 ± 8.43%) の増加が認められた。また、Sophy β-glucan 摂取 1 週間後、摂取前に対して唾液中に IL-6(8.33 ± 3.82%)、BG-IgA2(11.27 ± 4.63%)、sCD44(14.12 ± 9.54%) の増加が認められた。以上の結果は、Sophy β-glucan を摂取後、血清中または唾液中の BG 特異 IgG、IgA2 および IgM、さらに IL-6、sCD44、G-CSF が増加することから BG 特異的な生体免疫システムが増強されたことがわかった。

キーワード：β-1,3-1,6 glucan(BG)、BG 特異抗体、免疫バイオマーカー、免疫ネットワークシステム