Enhancement of Intestinal Immune Function in Mice by β-D-Glucan from Aureobasidium Pullulans ADK-34

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Abstract

β-Glucans, glucose polymers that are the main constituents of the outer cell walls of micro-organisms such as fungi and yeast, are known to play an immunostimulatory role. We prepared β-glucan (β-(1,3),(1,6)-D-glucan) from an edible cultured fungus through fermentation techniques using a strain of Aureobasidium pullulans ADK-34. The purity of this β-glucan preparation (AP-FBG) was demonstrated to be high through various instrumental analyses. We then examined the effects of AP-FBG on intestinal immune systems. We prepared Peyer’s patch (PP) cells and measured interleukin (IL)-5, IL-6, and IgA production in culture media with AP-FBG. We found that both cytokines and IgA increased; furthermore, IL-6 secreted by PP dendritic cells (PPDCs) cultured in the presence of AP-FBG significantly increased. We tested IgA production after oral administration of AP-FBG for 2 weeks and found that AP-FBG tended to promote the production of IgA in the small intestine. Interestingly, we observed a significant increase in IgA production in the small intestines of mice treated with cyclophosphamide (CY; an immunosuppressant) after oral administration of AP-FBG diet compared with CY-treated and control diet mice. Production of IL-6 and IgA by PP cells and IL-6 production by PPDCs in AP-FBG-fed and CY-treated mice also increased. These results demonstrate that AP-FBG has the ability to activate PPDC and induce IL-6 production and IgA secretion in PP cells. These abilities were more clearly expressed when AP-FBG was orally administered in a CY-induced immunosuppressed condition. Therefore, AP-FBG may be a useful ingredient for preparing functional foods with immunomodulatory activities.

Introduction

β-Glucans are glucose polymers that are primarily used to construct the outer cell walls of plants and micro-organisms, such as fungi, mushrooms and yeast. Recent reports have also shown that various β-glucans from food sources, including mushrooms, yeast and cereals, play a significant role in enhancing immunological response, possess antitumour and immunomodulating properties, reduce allergic reactions and prevent infectious diseases [1, 2].

Certain strains of Aureobasidium pullulans, a black yeast-like fungus, produce various polysaccharides, including β-glucan, pullulan and a glucon having an acidy residue [3–5]. This β-glucan (β-(1,3),(1,6)-D-glucan) consists of a β-(1-3)-linked backbone with β-(1-6)-linked side chains of varying lengths and distributions; it has a similar structure to β-glucans obtained from mushroom fruiting bodies. These β-glucans are also well known to have several health benefits [6]. There have been several studies on the efficacy of β-glucan from A. pullulans demonstrating, for example antitumour effects [7], promotion of DNA synthesis by human peripheral blood mononuclear cells (PBMCs) [8], prevention of food allergies [9] and activation of antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells, monocytes and neutrophils [10]. Lately, some new benefits have been reported, for example reduction in restraint stress [11], anti-osteoporotic effects [12] and reduction in acute xylene-induced inflammation [13].

We have succeeded in isolating a strain, ADK-34 (FERM BP-8391), that produces considerable quantities of β-(1,3), (1,6)-D-glucan with high solubility compared with previously reported β-glucans, and is being industrially manufactured at present [14]. We have termed this β-glucan AP-FBG in this study. We previously reported that AP-FBG is a biological response modifier (BRM); for example, when injected i.p., AP-FBG was able to accumulate leucocytes and neutrophils and promote production of the Th1-type cytokines TNF-α, IL-12 and IFN-γ, Ns from peritoneal exudate cells (PEC) [14, 15].
As more attention is paid to the healthy food market, there is considerable interest in food ingredients having the potential to stimulate the immune system, as a health food supplement for prevention of cancer, infectious diseases and allergic reactions, and β-glucan is one of the major candidates.

Gut-associated lymphoid tissues (GALT) is the largest immune organ in the body and contains an enormous quantity and variety of antigens. Upon ingestion, the first immune site in the ingested materials contact is the GALT. Therefore, evaluation of effects on intestinal immune response is important for oral ingestion. The inductive tissue of the immune organs in the gut is the Peyer’s patch (PP); therefore, we hypothesized that AP-FBG could stimulate the PP via certain mechanisms and induce immune responses.

In this study, we demonstrated the high purity of AP-FBG, which has good stability, high homogeneity and solubility in water, through various instrumental analyses. We also assessed the effects of AP-FBG on the intestinal immune systems in normal mice and immunosuppressed mice by cyclophosphamide (CY). The results demonstrate that A. pullulans-cultured β-glucan (AP-FBG) acts as a stimulating agent on the intestinal immune system.

Materials and methods

**Experimental animals.** Male and female 5–6-week-old BALB/c mice were purchased from CLEA Japan (Tokyo, Japan) and were housed in a room at 23–25 °C and 50–60% relative humidity with a 12-h light/dark cycle. The mice were provided with an experimental diet and water ad libitum. Animal experiments in the present study followed the animal experimentation guidelines of ADEKA Corp.

**Preparation of AP-FBG.** AP-FBG (A. pullulans ADK-34-fermented β-D-glucan) was industrially manufactured by extraction of extracellular β-glucan produced by the black yeast A. pullulans ADK-34 (FERM BP-8391) and was refined and powdered in our laboratory [14]. For instrumental analysis and in vitro experiments, we used 96% pure β-glucan which was prepared from the cultured containing AP-FBG by ethanol precipitation, and for in vivo experiments, the β-glucan in 73% purity prepared from the cultured containing AP-FBG by freeze-dry, was used. The rest components in AP-FBG by freeze-dry were minerals and fructose that was degraded product of sucrose of culture media, these components were derived from the culture media for AP-FBG production. The β-glucan was measured for endotoxin contamination, using the Endosafe-PTS (Charles River Laboratories International, Inc., Wilmington, MA, USA), which was approved for pharmaceutical evaluation by the U. S. Food and Drug Administration (FDA). The endotoxin level was 0.034 EU/ml when the β-glucan concentration of the sample was 250 μg/ml, which was the concentration added in our in vitro experiments. We confirmed that IL-6 was not detected when PP DCs were added 1 ng/ml lipopolysaccharide (4.9 EU/ml measured by Endosafe-PTS) derived from Escherichia coli B55:O4 (Sigma-Aldrich Co., St. Louis, MO, USA; data not shown). So, we regard that this endotoxin level included in β-glucan was low and had no influence in the results of our assays.

**Fourier transform infrared spectrometer (FT-IR) analysis.** Beta bonds were analysed using a Fourier transform infrared spectrometer (FT-IR; JASCO Corporation, Tokyo, Japan) with a KBr tablet containing the AP-FBG powder was analysed.

**High performance size-exclusion chromatography (HPSEC) analysis.** The purity of the β-glucan was analysed using high performance size-exclusion chromatography (HPSEC) using a Shodex SB-800 HQ series column (OHpak SB-802 HQ column; SHOWA DENKO K.K., Tokyo, Japan) combined with a TSK gel G6000PWXL column (TOSO K.K., Tokyo, Japan), maintained at 60 °C. The mobile phase was water, and the flow rate was 0.5 ml/min. A sample containing 0.1% AP-FBG in a volume of 30 μl was injected into the column and analysed using the refractive index (RI) detector. The β-glucan purity of the AP-FBG sample was determined using the area of the high-molecular weight peak of the β-glucan standard.

**Electrical conductivity, spectrum and gas chromatography-mass spectroscopy (GC-MS) analyses.** Various metallic ions were analysed with an electrical conductivity metre, using a 0.1% AP-FBG solution. Various organic compounds were analysed using spectrum analysis. AP-FBG (20 mg/ml) in 70% ethanol solution (v/v) was analysed at wavelengths of 340 nm, 260 nm and 210 nm. The primary compounds that have 340-nm absorbance are various proteins, the 260-nm absorbance include those that have benzene rings. The remaining ethanol (EtOH) content was analysed by gas chromatography-mass spectroscopy (GC-MS) analyses (Shimadzu Corp., Kyoto, Japan) with a 0.25-mm-i.d. TC-WAX capillary column (GL Sciences, Tokyo, Japan) using helium as the carrier gas. The sample was a 0.1% AP-FBG solution.

**Analysis of water content, protein content and lipid content.** The water content was determined by the drying loss method. The sample was dried by heating under ordinary pressure (105 °C, 2 h), following the Standard Methods of Analysis for Hygienic Chemists approved by the Pharmaceutical Society of Japan. Protein concentrations were determined using the conventional Kjeldahl method [16]. Lipid concentrations were determined using the conventional Soxhlet extraction method [17].

**Metal analysis.** Arsenic (AS₂O₃) was analysed using hydride-generation atomic absorption spectrometry, following the Standard Methods of Analysis for Hygienic Chemists approved by the Pharmaceutical Society of Japan. Heavy metal (lead; Pb) was analysed by absorption spectrochemical analysis.

**Preparation of whole Peyer's patch (PP) cells and PP dendritic cells.** Peyer’s patch cells and PP dendritic cells (PPDCs) were

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prepared from naïve female 6–8-week-old mice, according to Sato et al. [18]. The PP was digested with collagenase type 1 (1 mg/ml; Sigma-Aldrich, St. Louis, MO, USA) and a single-cell suspension was prepared. The cells were resuspended in Roswell Park Memorial Institute (RPMI) complete medium solution containing 10% foetal calf serum (FCS), 1% glutamate and 100 units of penicillin-streptomycin, and cell numbers were adjusted to 1 × 10^6 cells/well for cytokines and 3 × 10^6 cells/well for IgA antibodies. The cell suspension was plated on 96-well multi-plates and then cultured with AP-FBG (250 μg/ml) for 3 days (cytokines) or 7 days (IgA) under 37 °C, 5% CO_2 conditions. The cytokine content of the culture medium was measured using sandwich enzyme-linked immunosorbent assay (ELISA).

Peyer’s patch dendritic cells were purified from the single-PP-cell suspension by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) using anti-mouse CD11c-coated magnetic beads and LS columns. The cells were adjusted to 1 × 10^6 cells/well and cultured with AP-FBG (250 μg/ml) for 3 days, and then IL-6 and IL-12 were measured.

Measurement of cytokines (IL-5 and IL-6) and IgA antibodies after oral administration of AP-FBG. For oral administration of AP-FBG to mice, feed containing 2.5% AP-FBG in a nutritionally complete diet (MF, Oriental Yeast Co. Ltd., Tokyo, Japan) was prepared. The diet was orally administered ad libitum to 5-week-old male mice for 2 weeks. On day 15, the mice were sacrificed by cervical dislocation after gentle anaesthesia by diethyl ether to isolate the PP as described above. PP cells were prepared to 1 × 10^6 cells/well. The cell suspension was plated on 48-well multiplates then cultured for 5 days, and the cytokine content of the culture medium was measured using ELISA.

IgA antibody detection in the small intestine after oral administration of AP-FBG. After the PP was retrieved, the duodenum was aseptically removed and a 4-cm-long piece of the duodenum from the side of the stomach was separated, washed and homogenized in phosphate-buffered saline (PBS) containing protease inhibitor (Roche Diagnostic, Basel, Switzerland). The suspension was centrifuged, and the extract was isolated. The concentration of IgA antibodies in the extract was measured using ELISA as described above. The amount of IgA in the duodenum extract was expressed as mg weight per mg protein in the extract. The concentration of the protein in the extract was measured using the Bradford method with a Protein Assay kit (Bio-Rad Laboratories Inc, Hercules, CA, USA).

Cytokine and IgA antibody production of mice administered AP-FBG feed and cyclophosphamide (CY) treatment after oral administration of AP-FBG. Five-week-old male mice were administered either the control diet (MF, Oriental Yeast, Tokyo, Japan) or one containing 2.5% AP-FBG for 1 week ad libitum, then were injected i.p. with 100 mg/kg CY. Control mice were administered the control diet and then injected i.p. with saline. Four and 8 days after the CY or saline injection, the mice, fed either the control or experimental diet during that time, were sacrificed. The PP and duodenum were prepared as described above, and cytokine and IgA levels were measured using ELISA. Following the CY and control treatments described above, 4 days after CY or saline injection, the PP cells were separated, and PPDCs were prepared using the methods described above. PPDCs were adjusted to 2.5 × 10^6 cells/well and cultured for 3 days. IL-6 production in the culture supernatant was measured using ELISA.

Measurement of cytokines and IgA secretion by ELISA. Cytokines were assayed using a specific sandwich ELISA. IL-5, IL-6 and IL-12 were measured using the OptEIA set (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). For detection of IgA antibodies, alkaline phosphatase-conjugated monoclonal anti-mouse IgA (Zymed, San Francisco, CA, USA) was used. The plate was then washed and the substrate (3,3′,5,5′-tetramethylbenzidine; TMB) was added. Absorbance was determined at a wavelength of 405 nm.

Statistics analysis. Data are represented as means ± SDs. Differences between two groups were evaluated using Student’s t-test and three groups using ANOVA followed by Fisher’s PLSD. Differences were considered statistically significant for values of P < 0.05.

Results

Purity of AP-FBG

Beta bonds were analysed by FT-IR analysis of a KBr tablet containing the sample (Fig. 1A). Absorption at 889–890/cm was detected for AP-FBG, but no absorption was detected at 850/cm or 920/cm, indicating that AP-FBG contained β bonds, but not α bonds, in the molecules. The purity of the sample was determined using HPSEC and refractive index (RI) detectors (Fig. 1B). There were no peaks other than the high molecular weight peak for β-glucan, that is, there was no contamination by sucrose (the raw material), glucose or fructose (constituent sugar of sucrose).

The results of various analyses are shown in Table 1, indicating that arsenic and heavy metals (Pb) were not present, and metallic ions and certain organic substances were sufficiently low, based on electrical conductivity and EtOH extract spectrum results. Detected compounds other than β-glucan were water, protein and lipid, but in sufficiently low quantities. These results demonstrate that the AP-FBG sample had quite high β-glucan purity.

Cytokine and IgA antibody production using PP cells cultured invitro

IgA antibodies have an important role in mucosa in preventing bacterial infection and invasion of allergenic molecules. Enhanced IgA production in mucosa is widely considered to be advantageous for the maintenance of health. Therefore, we investigated the capacity of AP-FBG to
promote immunity in intestinal mucosa. It is well known that the PP is the major inductive site for IgA antibody responses in the gut [19]. After stimulation, B cells differentiate into IgA-producing B cells in the PP and migrate to the intestinal mucosa, and differentiate into the IgA-secreting plasma cells in response to cytokines such as IL-5 and IL-6 [20]. We previously reported that IL-6 secreted from PPDC-induced IgA secretion from B cells [18]. We also showed that IL-5 secreting cells in PP were capable of enhancing IgA production [21]. PP cell cultures may be considered to resemble the process of IgA production in the intestine; thus, we measured the secretion of IL-5, IL-6 and IgA from PP cells stimulated by AP-FBG.

Peyer’s patch cells were stimulated with AP-FBG in vitro, and the amount of IgA produced significantly increased compared with the control group (Fig. 2). The amounts of IL-5 and IL-6 produced also significantly increased (Fig. 2).

Cytokine production by DCs isolated from PP cultures in vitro

We then examined IL-6 and IL-12 production by PPDCs. The PPDCs were stimulated with AP-FBG in vitro, and the amount of the above cytokines was measured in the culture supernatant. The amount of IL-6 increased significantly in the AP-FBG cultures compared with the control group (Fig. 3). This result suggests that the increase in the level of IL-6 secreted by whole PP cells was due to stimulation of PPDCs by AP-FBG, and that IL-6 promotes the secretion of IgA from B cells. On the other hand, there was no significant difference in the IL-12 levels between the two groups (Fig. 3).

IgA antibody and cytokine production in the duodenum and PP cells after oral administration of AP-FBG

To investigate the ability of AP-FBG to promote the immunity of intestinal mucosa, AP-FBG was administered to mice for 2 weeks, and the amount of IgA in the duodenum was measured. As shown in Fig. 4, mice fed AP-FBG tended to enhance IgA antibody production (P = 0.089). The PP of AP-FBG-fed and control mice was excised to evaluate cytokines (IL-5 and IL-6); however, IL-5 and IL-6 production could not be detected in either the control or the AP-FBG diet groups (data not shown).
IgA antibody production in the duodenum of CY-treated mice after feeding AP-FBG

We next examined IgA level on CY-treatment mice to observe the effect of AP-FBG on immunosuppressed mice. We examined IgA production in the small intestine obtained from mice fed the experimental diet for 1 week and injected with CY or saline. In the homogenized extracts from the duodenum, IgA was lower on day 4 and day 8 after CY treatment (Fig. 5, $P < 0.01$). On day 8, the IgA production of CY-treated mice had increased, approaching the control diet, compared to the results on day 4. In the group of CY-treated mice fed the AP-FBG diet, the IgA level was higher than in the group of CY-treated mice fed the control diet (Fig. 5, $P < 0.05$) on day 8. These results indicate that AP-FBG contributed to the recovery of IgA production reduced by CY treatment, rather than inhibiting the initial effects of CY.

Cytokine and IgA antibody production by the PP and PPDCs isolated from mice administered AP-FBG feed and CY treatment

IgA in mucosal tissue of the small intestine is presumably secreted by B cells homing from the PP. Therefore, we examined the PP on day 4 after CY or saline injection, rather than on day 8, considering a time-lag prior to production of IgA in the gut based on the time for homing of B cells [22] from the PP. The PP cells were cultured for 3 days and the amounts of IL-5, IL-6 and IgA were measured. By day 4, secretion of IgA by PP cells from CY-treated mice had decreased, while IgA production in the CY-treated and AP-FBG-administered group tended to be higher than in the CY-treated control feed group (Fig. 6A). These results resembled the results for IgA levels in the duodenum on day 8. Production of IL-6 increased with CY treatment and was greater in the

Figure 2 Cytokine and antibody production induced by AP-FBG in Peyer’s patch (PP) cells in vitro. The PP cells derived from mice ($n = 5$) were adjusted to $1 \times 10^6$ cells/well, and cultured for 3 or 7 days. Cytokine and IgA production in the culture supernatant was measured using enzyme-linked immunosorbent assay (ELISA). Data represent one of two independent experiments. Values represent means ± SDs, *$p < 0.05$, **$p < 0.01$, significantly different from the mean of the control group.

Figure 3 Cytokine production induced by dendritic cells (DCs) isolated from Peyer’s patch (PP) cells cultured in vitro. Magnetic cell sorting (MACS)-purified CD11c+ DCs from the PP of mice fed control feed ($n = 3$) were incubated ($1 \times 10^5$ cells/well) for 3 days in the presence of AP-FBG (250 µg/ml). Cytokine production in the culture supernatant was measured using enzyme-linked immunosorbent assay (ELISA). Data represent one of two independent experiments. Values represent means ± SDs, *$p < 0.05$, **$p < 0.01$, significantly different from the mean of the control group.

Figure 4 IgA production in the duodenum after oral administration of AP-FBG. After oral administration of AP-FBG ($n = 4$) or control feed ($n = 4$) for 2 weeks to normal mice, we isolated the intestine and spleen. After removal of Peyer’s patch (PP), a 4-cm-long piece of the duodenum was separated, homogenized, the extract was isolated. The concentration of IgA antibodies in the extracts was measured using enzyme-linked immunosorbent assay (ELISA). Results are shown for mg IgA per mg protein in the extract. Data represent one of three independent experiments. Values represent means ± SDs, *$p < 0.05$, significantly different from the mean of the control group.
CY-treated and AP-FBG-administered group than in the CY-treated control feed group (Fig. 6A). In addition, IL-6 production by PPDCs increased in the CY-treated and AP-FBG-administered group (Fig. 6B). These results suggest that oral administration of AP-FBG activated PPDC and induced secretion of IL-6 and production of IgA, promoting recovery from the immunosuppressive effects of CY. IL-5 production by the PP cells was the same in all groups.

Discussion

In this study, we demonstrated that the β-glucan purity of AP-FBG was high through various instrumental analyses. In our previous study [14], we elucidated the primary structure of AP-FBG by NMR spectroscopy, and its structure was shown to be a mixture of a 1,3-β-D-glucan backbone with a 1,6-β-D-glucopyranosyl side branching unit every two residues (major structure) and a 1,3-β-D-glucan backbone with a 1,6-β-D-glucopyranosyl side branching unit every three residues (minor structure).

The results of FT-IR in this study showed that AP-FBG has β bonds, but no α bonds in the molecules; therefore, there was only β-glucan in the AP-FBG sample and no other sugars that have an α bond. The results of HPSEC demonstrated that there was no contamination by oligosaccharides, for example by sucrose as a raw material, or by glucose and fructose, constituent sugars of sucrose. Furthermore, from the results of electrical conductivity and the EtOH extract spectrum, arsenic and heavy metals were not present, and metallic ions and certain organic...
substances were present only in very low concentrations. These results demonstrate that the purity of the AP-FBG sample was quite high, and the compounds other than β-glucan (water, protein and lipid) were present in only low amounts.

We examined the effects of AP-FBG on intestinal immune function in mice. PP cells were stimulated with AP-FBG in vitro, and the amount of IgA significantly increased compared with the control group. The amount of IL-5 and IL-6, important cytokines for the development of IgA-producing B cells, also significantly increased. In particular, AP-FBG markedly and selectively enhanced IL-6 secretion of PP cells (Fig. 2). These results are consistent with a previous report using a different strain of A. pullulans [23]. As mentioned previously, we have shown that DCs, which are the major antigen-presenting cell population in the PP, secrete IL-6, and IL-6 secreted from DCs promotes IgA secretion from B cells [18]. In the PPDCs stimulated with AP-FBG in vitro, the amount of IL-6 increased significantly in the AP-FBG groups compared with the control group. This observation suggests that the increase in IL-6 was due to secretion from PPDCs stimulated by AP-FBG, and that IL-6 promotes secretion of IgA from B cells. Several molecules have been suggested as β-glucan receptors, such as complement receptor type 3 (CR3)[24, 25], and dectin-1 [1, 26, 27]. Although the AP-FBG receptor of PPDC has not been elucidated [28], we hypothesize that IL-6 secretion was induced by stimulation of PPDCs by AP-FBG via some kind of receptor.

We examined the production of IL-5, IL-6 and IgA by PP cells obtained from mice fed AP-FBG for 2 weeks. The IgA production in the small intestine tended to increase (Fig. 4). Nevertheless, the effect of AP-FBG on intestinal IgA responses was more striking in CY-treated immunosuppressed mice.

Cyclophosphamide is an anticancer agent, and it is well known that the number of peripheral blood leukocytes, particularly B cells, is decreased by CY treatment. It has been reported that the number of leukocytes reaches a minimum on approximately on day 4 and recovers on days 7–10 [22, 29–31]. We measured IgA production in the small intestine (duodenum) from mice administered either a control diet or an AP-FBG diet for 1 week and injected with CY or saline, on day 4 and day 8 after CY treatment. IgA had decreased by day 8 after CY administration (Fig. 5). Of the few reports concerning the effects of CY on the intestinal immune system [22, 32, 33], Cozon et al. [22] have shown that the amount of IgA in small intestine decreases and subsequently recovers after administration of CY. The reason for the IgA decrease is attributed to a decrease in the number of B cells in the PP and the blood.

In the present study, by day 8 the IgA level in the group of CY-treated mice administered the AP-FBG diet had significantly increased compared with the CY-treated mice administered the control diet (Fig. 5). However, on day 4, the IgA production of the CY-treated AP-FBG diet mice had decreased to the same extent as the CY-treated mice fed the control diet. These results indicate that AP-FBG contributed to the recovery of IgA production that had been reduced by CY treatment, and did not prevent the initial inhibition of IgA production by CY. These results further suggest that AP-FBG intake will not have negative effects on anticancer therapy with CY.

On day 4, IgA production by PP cells from CY-treated mice had decreased, while IgA production in the CY-treated and AP-FBG-administered group was tended to be larger than that of the CY-treated control feed group (Fig. 6). These results were similar to those for IgA in the duodenum on day 8. As mentioned previously, it is well known that PP is the inductive site for IgA production [19], that is, B cells, stimulated by various antigens in the PP, migrate to intestinal mucosa, differentiate into plasma cells and secrete IgA [20]. Therefore, there is a time-lag prior to production of IgA in the gut based on the homing time of B cells [22] from the PP. Thus, we expect that the results for cytokines and IgA from the PP on day 4 reflected the same processes affecting the induction of IgA in the duodenum on day 8.

The amount of IL-6 in the PP on day 4 was increased by CY treatment, and that in the CY-treated and AP-FBG-administered group was greater than in the CY-treated control feed group (Fig. 6). In addition, IL-6 production by PPDCs increased in the CY-treated and AP-FBG-administered group. Thus, we conclude that oral administration of AP-FBG activated PPDCs and induced them to secrete IL-6 and produce IgA, and AP-FBG promoted recovery from the immunosuppressive effects of CY. On the other hand, we measured the amount of TGF-β in the PP cell cultures on day 4, and found no significant differences among three groups (data not shown).

In this study, we have elucidated the effects of AP-FBG intake on intestinal immune systems and suggested a role for IL-6 from PPDCs in enhancing IgA production under normal conditions and in CY-treated immunosuppressed mice. Intake of AP-FBG induced a slight increase in intestinal immunity under normal conditions, and under CY-treated immunosuppressed conditions, promoted a remarkable recovery of intestinal immunity. IgA antibodies have an important role in mucosa in preventing the invasion of bacteria; thus, AP-FBG may be capable of enhancing protection against oral infection, especially in compromised individuals.

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References

1. Borchers AT, Keen CL, Gershwin ME. Mushrooms, tumors, and immunity: an update. Exp Biol Med 2004;229:393–406.
2. Ross GD, Cain JA, Myson BL, Newman SL, Lachmann PJ. Specificity of membrane complement receptor type three (CR3) for β-glucans. Complement 1987;4:61–74.
3. Catley BJ, Whelan WJ. Observations on the structure of pullulan. Arch Biochem Biophys 1971;143:138–42.
4. Hamada N, Tsujioka Y. The structure of the carbohydrate moiety of an acidic polysaccharide produced by Aureobasidium sp. K-1. Agric Biol Chem 1983;47:1167–72.
5. Deshpande MS, Rale VB, Lynch JM. Aureobasidium pullulans in applied microbiology: a status report. Enzyme Microb Technol 1992;14:314–27.
6. Bohn JA, BeMiller JN. (1-→3)-β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polym 1995;28:3–14.
7. Kimura Y, Sumiyoshi M, Suzuki T, Sakanaka M. Antitumor and antimetastatic activity of a novel water-soluble low molecular weight (1,3-1,6)-D-glucan from Aureobasidium pullulans 1A1 strain black yeast. Anticancer Res 2006;26:4131–42.
8. Ikewaki N, Fujii N, Onaka T, Ikewaki S, Inoko H. Immunological actions of Sophy beta-glucan, currently available commercially as a health food supplement. Microbiol Immunol 2007;51:861–73.
9. Kimura Y, Sumiyoshi M, Suzuki T, Sakanaka M. Inhibitory effects of water-soluble low-molecular-weight β-(1,3-1,6) D-glucan purified from Aureobasidium pullulans GM-NH-1A1 strain on food allergic reactions in mice. Int Immunopharmacol 2007;7:965–72.
10. Vetvicka V, Thornton BP, Ross GD. Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J Clin Invest 1996;98:50–61.
11. Kimura Y, Sumiyoshi M, Suzuki T, Sakanaka M. Effects of water-soluble low-molecular-weight beta-1, 3-D-glucan (branch beta-1, 6) isolated from Aureobasidium pullulans 1A1 strain black yeast on restraint stress in mice. J Pharm Pharmacol 2007;59:137–44.
12. Shin HD, Yang KJ, Park BR, Son CW, Jang HJ, Ku SK. Antiestroporanetic effect of Polycan, beta-glucan from Aureobasidium, in ovarectomized osteoporotic mice. Nutrition 2007:23:853–60.
13. Kim HD, Cho HR, Moon SB et al. Effects of beta-glucan from Aureobasidium pullulans on acute inflammation in mice. Arch Pharm Res 2007;30:325–8.
14. Tada R, Tanioka A, Iwasawa H et al. Structural characterization and biological activities of a unique type β-D-glucan obtained from Aureobasidium pullulans. Glycoconj J 2008;25:851–61.
15. Tsubaki K, Tanioka A, Hatashima K, Shoji Y, Iwasawa H, Yamazaki M. Immuno-modulation activities of new β-glucan from cereal and fermentation food. In: Yagasaki K, Yamazaki M, eds. Bromocarcinology: Pharmacology of Foods and Their Components. Kerala: Research Signpost, 2008:147–69. ISBN: 978-81-308-0287-9.
16. The Japanese Standards of Quasi-Drug Ingredients 2006; Method of determination, 2006; Chapter 64: 2-68–69. ISBN: 978-4-89808-0907-8.
17. Official Methods of Analysis of AOAC International (15th edn.): AOAC. Official Method 920.85: 1990; Chapter 32: 780. ISBN: 978-0935584424 or 0-935584-42-0.