

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

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Abstract

There have been a number of reports showing that the crude β -glucan fraction prepared from various kinds of Basidiomycetes mushrooms acts as anti-cancer and anti-allergic reagent through stimulation of IFN- γ production. It has been reported, however, that the exposure of the airway to β -(1,3) D-glucan, contained in house dust, indoor moulds and some bacteria, potentiates the airway allergic response. It seems likely that the discrepant effects on immune function may be related to such factors as differences of the administration route, average molecular weight and water solubility. We isolated a new low-molecular-weight (about 100 kDa) β -glucan from *Aureobasidium pullulans* 1A1 strain of black yeast, and found that it had low viscosity and was water-soluble. In this study, we examined the effects of water-soluble low-molecular-weight β -(1 $\rightarrow$ 3) and 50–80% branched β -(1 $\rightarrow$ 6) glucan (LMW- β -Glucan) isolated from *A. pullulans* on the ova-albumin (OVA)-treated allergic reaction in mice. Feeding standard laboratory diets containing 0.5 and 1% LMW- β -Glucan significantly inhibited the OVA-specific IgE elevation compared to that in OVA-sensitized mice fed standard laboratory diet alone (control). Furthermore, feeding standard laboratory diets containing 0.5 and 1% LMW- β -Glucan inhibited the reduction of IL-12 and IFN- γ production from splenocytes and the reduction of CD8- and IFN- γ -positive cell number in the small intestine of the OVA-sensitized mice. These findings suggest that anti-food allergic action of LMW- β -Glucan may be due to the inducing IFN- γ production in the small intestine and splenocytes.

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1. Introduction

There are a number of reports regarding the polysaccharide (β -glucan) fraction prepared from various kinds of Basidiomycetes mushrooms as anti-cancer agents having the biological response modifier (BRM) effect [1–3]. On the other hand, it has been reported that

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exposure of the airway to β -(1,3) D-glucan contained in house dust, indoor moulds and some bacteria potentiates airway allergic responses [4–8]. Conversely, Miyamaoto et al. [9] reported that the oral administration of polysaccharide fractions prepared from the Basidiomycete *Agaricus blazei* might clinically improve the symptoms of bronchitis through elevation of the interferon (IFN)- γ level. Xie et al. [10] reported that the oral administration of a polysaccharide isolated from the fruiting body of *Cryptoporus vlovatus* prevented the ova-albumin (OVA)-induced allergic rhinitis through the inhibition of eotaxin mRNA expression in nasal mucosa and lung tissues. Carrageenan (which consists of alternating copolymers of β -(1 $\rightarrow$ 3)-D-galactose and (1 $\rightarrow$ 4)-3,6-anhydro-D- or L-galactose) isolated from the red seaweed has been used a reagent to induce non-specific inflammatory reactions, which may be a consequence of activation of innate immunity through pattern recognition receptors. Footpad oedema and pleurisy are commonly used model systems induced by either subcutaneous or intrapleural administration of carrageenan. Tsuji et al. [11] reported that the oral administration of λ -carrageenan to immunized mice successfully decreased OVA-specific immunoglobulin (Ig) E. A β -glucan (Pullulan[®]) prepared from *Aureobasidium pullulans* K-1 strain is approved by the Ministry of Health, Labour and Welfare in Japan as a safe food additive and widely used as a thickening polysaccharide. However, there are a number of reports that the inhalation of β -glucan into the airway causes allergic reactions with the elevation of IgE [4–6]. Conversely, the oral administration of polysaccharides including β -glucan prepared from Basidiomycetes mushrooms and medicinal plants prevents allergic reactions through the decrease of IgE elevation and stimulation of IFN- γ production [9,10]. Thus, the discrepant findings reported for polysaccharides including β -glucan most likely reflect different dosages, routes of administration, average molecular weights and water solubility. Food allergy is a common allergic disease with a substantial risk of life-threatening anaphylactic reactions. It usually manifests with 1 h after ingestion of the food allergen and is thought to be mediated by allergen-specific IgE. It has been reported that the intestinal permeability is increased after oral challenge and consequently that an increase in intestinal permeability may facilitate the absorption of allergen into the circulation and lead to allergic systemic reactions [12,13].

It seems likely that (1 $\rightarrow$ 3) or (1 $\rightarrow$ 6) β -glucans isolated from Basidiomycetes mushrooms have high viscosity and high molecular weight (over 2000 kDa) and are water-insoluble [14]. In addition, β -glucan

easily forms gels containing high-order structures of single spirals or triplet spirals due to its unique primary structure; therefore, the purification was extremely difficult, and consequently crude β -glucan fractions were used in various experiments rather than purified β -glucan [14,15]. The β -(1,3–1,6) glucan prepared from *A. pullulans* K-1 strain was isolated and shown to have high viscosity and a high molecular weight (approximately 1000 to 2000 kDa) [16]. Recently, we succeeded in the isolation and industrial production of water-soluble low-molecular-weight β -(1,3–1,6) D-glucan from *A. pullulans* GM-NH-1A1 strain (black yeast, a mutant of the strain K-1). Many reports regarding β -glucan of Basidiomycetes mushrooms indicated that it appeared to modify immune responses in mice [9,10,14,17]. Therefore, in this study, we characterized the β -glucan isolated from *A. pullulans* GM-NH-1A1 strain and examined its usefulness as a prophylactic dietary supplements administered by the oral administration of water-soluble low-molecular-weight β -glucan in order to prevent food allergic reactions in mice.

2. Materials and methods

All experimental protocols were approved by the Graduate School of Medicine, Ehime University, Animal Care and Use Committee.

2.1. Animals

Male Balb/c mice (10 weeks old) were purchased from Clea Japan Co. (Osaka, Japan). They were housed for 1 week in a room with controlled temperature and humidity and given free access to food and water before animal studies were performed. The mice were treated according to the ethical guidelines of the Animal Center, Graduate School of Medicine, Ehime University. The Animal Studies Committee of Ehime University approved the experimental protocol.

2.2. Purification of low-molecular-weight β -glucan from *A. pullulans* GM-NH-1A1 strain

A. pullulans GM-NH-1A1 strain was obtained from the strain K-1 by a general mutation-inducing treatment [18], and then cultured in the following medium (0.3% ascorbate sodium, 3% sucrose, 0.001% FeSO₄·7H₂O, 0.05% MgSO₄·7H₂O, 0.1% KCl, 0.1% K₂HPO₄ and 0.2% NaHCO₃) (3 L) for 10 days at 25 to 30 °C. The culture-conditioned medium was filtered using an ultra membrane (UF membrane, Nitto Denko Co., Tokyo, Japan) to remove the low molecular substances and salts, and then the ultrafiltered supernatant was adjusted to pH 3.5 with citric acid. The β -glucan fractions obtained thus were precipitated by adding 70% ethanol, and then the precipitates were freeze-dried and dissolved in sterile 0.9% NaCl solution. The viscosity of β -glucan measured using the BM-

type rotary viscometer (Tokimec Inc., Tokyo, Japan). The solubility of β -glucan (2 mg/mL) was determined under the various alkaline and temperature conditions.

2.3. Determination of average molecular weight and structural analysis of β -glucan purified from *A. pullulans* GM-NH-1A1 strain

The average molecular weight was measured by Toyoperyl-Gel chromatography (HW-650, Tosoh Co., Tokyo, Japan) with 0.1 M NaOH (pH 12) using standard polysaccharides (Pullulans®) with average molecular weight 5900 to 1,600,000 (Shodex STANDARD D-82, Showa Denko Co., Tokyo, Japan). ^1H NMR (499.83 Hz) and ^{13}C NMR (125.68 Hz) spectra were recorded in D_2O using a Varian Unity Inva 500 spectrometer (Tosoh Co, Tokyo, Japan). The structure and purity of the isolated β -glucan was determined based on the ^1H and ^{13}C NMR spectral analysis. Furthermore, β -glucan was treated with the *exo*- β -(1,3)-glucanase (Kitalase, KI Chemical Industry Co. Ltd., Shizuoka, Japan) at 37 °C for 24 h, and the reaction mixture was concentrated under reduced pressure. The residue was dissolved in a small amount of distilled water, applied to silica gel TLC plates (Silica Gel 60, Merck Co. Germany), and developed with 2-propanol–acetone–0.1 M lactate (4:4:2, v/v). The products were identified as glucose and gentiobiose, respectively, by direct comparison of authentic samples.

2.4. Ova-albumin (OVA)-induced allergy mouse model

Male Balb/c mice (10 weeks old) were divided into five groups that were matched for body weight, after 1 week of being fed laboratory powder chow ad libitum. The normal and control groups continued to be fed laboratory powder chow ad libitum. The remaining mice were fed laboratory chow powder containing 0.25%, 0.5% or 1.0% β -(1,3–1,6) D-glucan purified from *A. pullulans* GM-NH-1A1 strain for 37 days. The mice were sensitized by an intraperitoneal injection of OVA (Sigma, MO, USA) (3 μg) with alum (Sigma, MO, USA) (2 mg) as an adjuvant on days 16 and 30 and then OVA (15 mg/kg body weight) was administered orally on day 37 to the OVA-sensitized mice. Body weight and food intake were measured weekly. Blood samples were taken from each mouse by venous puncture under anesthesia with diethylether 24 h after the oral administration of OVA, and the mice were killed with an overdose of diethylether. Experiments were performed in a ventilated room. The serum was prepared and frozen at –20 °C until analysis. The liver, spleen, thymus and small intestine were removed from the body using sterile treatments, and the liver, spleen and thymus were weighed.

2.5. Measurement of OVA-specific immunoglobulins in sera

To detect OVA-specific immunoglobulin (Ig) G, IgG₁ and IgA, 96-well microtitre plates (Becton Dickinson, NJ, USA) were coated with 100 μL of OVA at 200 $\mu\text{g}/\text{mL}$ in 0.1 mM

carbonate/bicarbonate buffer (pH 9.6) at 4 °C overnight. After washing with phosphate buffered saline (PBS) containing 0.05% Tween 20, 150 μL of PBS containing 1% bovine serum albumin (BSA) were added and the plates were incubated for 2 h at room temperature. The plates were washed three times with PBS containing 0.05% Tween 20, and then sera (100 μL) diluted in PBS containing 1% BSA were added and incubated for 2 h at room temperature. After washing with PBS containing 0.05% Tween 20, biotinylated rabbit anti-mouse IgG, IgG₁ (Zymed Laboratories Inc., CA, USA) or IgA (Rockland, PA, USA) was added and incubated for 2 h at room temperature. The plates were then washed twice, and peroxidase-conjugated streptavidin (DakoCytomation, Kyoto, Japan) was added. After washing, the plates were incubated at room temperature with 2,2'-azino-bis(3-ethylbenzoline-6-sulfonic acid) (ABTS) substrate (VECTOR Laboratories, CA, USA), and then optical density (OD) was measured at 405 nm in a microplate reader (Nulge Nunc International, Tokyo, Japan). The assays of serum OVA-specific IgG_{2a} and IgE were performed as follows: briefly, 96-well microtitre plates were coated with rat anti-mouse IgG_{2a} (10 $\mu\text{g}/\text{mL}$) or IgE (5 $\mu\text{g}/\text{mL}$) antibodies (Serotec, Oxford, UK) in 0.1 mM carbonate/bicarbonate buffer (pH 9.6) at 4 °C overnight. The subsequent procedures were performed using the same methods described above. After washing, 100 μL of sera diluted in 10% inactivated fetal bovine serum (FBS) and biotinylated OVA prepared using a biotinylation kit (Sigma, MO, USA) were added and incubated for 2 h at room temperature. Then the ABTS reagent was added and absorbance was measured using a microplate reader. To measure total IgE, 96-well microtitre plates were coated with rat anti-mouse IgE (5 $\mu\text{g}/\text{mL}$) antibody (Serotec, Oxford, UK) in 0.1 mM carbonate/bicarbonate buffer (pH 9.6) at 4 °C overnight. The subsequent procedures for the detection of total IgE were performed using the same methods described above.

2.6. Measurement of cytokine production in splenocytes

The spleen was gently teased to release the cells in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 $\mu\text{g}/\text{mL}$) and amphotericin B (0.25 $\mu\text{g}/\text{mL}$), and then the cell suspension was passed through a glass-wool column to remove cell debris and adherent cells. Isolated splenocytes were placed in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin and amphotericin B at 2 to 5×10^6 cells per well in 48-well culture plates (Corning, NY, USA) and incubated for 1 h. Then OVA (75 $\mu\text{g}/\text{mL}$) or concanavalin A (Con A) (10 $\mu\text{g}/\text{mL}$) was added to the splenocytes and they were cultured for 48 h. After the incubation period, the culture medium was centrifuged at 4 °C and $1500 \times g$ for 10 min to remove the cells. The supernatants from the centrifugation were stored at –80 °C until they were analyzed for their content of interleukin (IL)-4, IL-12 and interferon (IFN)- γ , using mouse IL-4 (R & D Systems Inc., MN, USA), IL-12 (Pierce Biotechnology Inc., IL, USA) and IFN- γ (R & D Systems Inc., MN, USA) ELISA kits.

2.7. Histological examination (in vivo)

All small intestines were fixed in 10% buffered formalin for at least 24 h, progressively dehydrated in solutions con-

taining an increasing percentage of ethanol (70, 80, 95 and 100%, v/v) cleared in HistoClear (FUME HOOD, AS-ONE, Tokyo, Japan), embedded in paraffin under a vacuum, sectioned into 5- μ m-thick sections, de-paraffinized, and stained

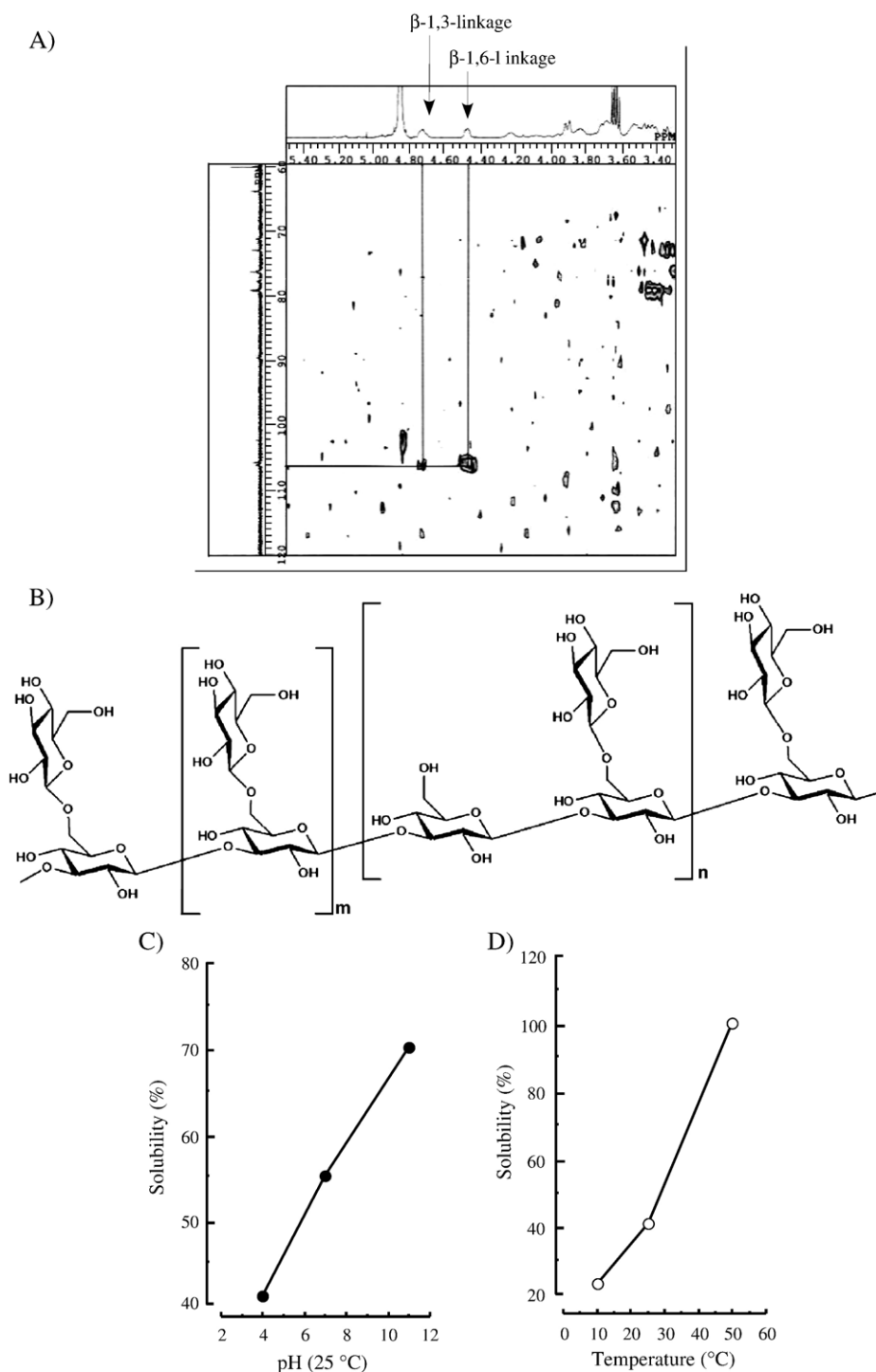


Fig. 1. ^{13}C – ^1H Cosy NMR spectrum (A), structure (B) and solubility (C and D) of β -glucan isolated from *Aureobasidium pullulans* 1A1 strain.

with Harris' hematoxylin and eosin. The CD8- and IFN- γ -positive cells in the small intestine were assessed by the immunoperoxidase technique using mouse anti-mouse CD8 α antibody (Santa Cruz, CA, USA) and rat anti-mouse IFN- γ antibody (PBL Biochemical Lab., NJ, USA), respectively.

2.8. Statistical analysis

All values are expressed as means $\pm$ S.E. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Fisher's protected least significant difference multiple-comparison test. Differences were considered significant at $P<0.05$.

3. Results

3.1. The structure and characteristics of β -glucan purified from *A. pullulans* GM-NH-1H1 strain

As shown in Fig. 1A and Table 1, the ^1H NMR spectrum (in D_2O , δ ppm) of β -glucan isolated from *A. pullulans* GM-NH-1A1 strain exhibited signals of C-1 position corresponding to $\beta(1,3)$ - and $\beta(1,6)$ -linkage at 4.73 (broad singlet) and 4.47 (broad singlet), respectively. In the ^{13}C NMR spectrum (in D_2O , δ ppm) of β -glucan, the signals at 103.94 and 104.46 were corresponded to the $\beta(1,3)$ - and $\beta(1,6)$ -linkage at C-1 position, respectively. The signals at 71.43 and 62.16 ppm in the ^{13}C NMR spectrum were assigned to the $\beta(1,6)$ -linkage at C-6 position and free C-6 position, respectively. Furthermore, when β -glucan isolated from *A. pullulans* GM-NH-1A1 strain was incubated with the *exo*- $\beta(1,3)$ -glucanase, glucose and gentiobiose were produced. Therefore, the structure of β -glucan isolated from *A. pullulans* GM-NH-1A1 strain was elucidated to be $\beta(1\rightarrow3)$ D-glucan with 50–80% branches $\beta(1\rightarrow6)$ by analysis of the ^1H and ^{13}C NMR spectra and enzymatic (*exo*- $\beta(1,3)$ -glucanase) reaction (Fig. 1B). The average molecular weight of β -glucan was determined to be approximately 100 kDa by the direct comparison of water-soluble standard marker Pullulan $^{\text{®}}$ with molecular weight 5900

Table 1
 ^1H and ^{13}C NMR data for LMW- β -Glucan isolated from *Aureobasidium pullulans* 1A1 strain

Carbon no.	LMW- β -Glucan	
	^1H NMR (δ ppm in D_2O)	^{13}C NMR (δ ppm in D_2O)
1 $\beta(1\rightarrow3)$	4.73 (brs)	103.94
1 $\beta(1\rightarrow6)$	4.47 (brs)	104.46
2	4.23, 3.91 or 3.65 ^a (brm)	77.26, 76.07 or 74.71 ^b
3 $\beta(1\rightarrow3)$	4.23, 3.91 or 3.65 ^a (brm)	87.72
4	4.23, 3.91 or 3.65 ^a (brm)	77.26, 76.07 or 74.71 ^b
5	4.23, 3.91 or 3.65 ^a (brm)	77.26, 76.07 or 74.71 ^b
6 $\beta(1\rightarrow6)$	3.65 or 3.47 ^a (brm)	71.43
6	3.65 or 3.47 ^a (brm)	62.16

brs, broad singlet; brm, broad multiplet.

^a Overlapping signals and values may be interchanged.

^b Values may be interchanged.

Table 2

Effects of LMW- β -Glucan on food intake in mice sensitized by OVA

Treatment	Food intake (g/mouse/day)		
	First OVA (ip)	Second OVA (ip)	Final OVA (po)
Untreated mice (Normal)	3.41 $\pm$ 0.30*	3.39 $\pm$ 0.30*	3.70 $\pm$ 0.22*
OVA-sensitized mice (Control)	2.05 $\pm$ 0.18	1.48 $\pm$ 0.10	2.10 $\pm$ 0.10
+LMW- β -Glucan (0.25%)	1.60 $\pm$ 0.11	2.07 $\pm$ 0.14*	1.90 $\pm$ 0.10
(0.5%)	1.67 $\pm$ 0.11	2.67 $\pm$ 0.22*	1.63 $\pm$ 0.14
(1.0%)	2.51 $\pm$ 0.12*	1.91 $\pm$ 0.11	1.95 $\pm$ 0.11

Values are means $\pm$ S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P<0.05$.

to 1,600,000 (Shodex, STANDARD P-82, Showa Denko Co., Tokyo, Japan) by gel chromatography. The viscosity of β -glucan (2 mg/mL) was less than 20 mPa s (cp) at 30 $^{\circ}\text{C}$ using the rotary viscometer. The solubility of β -glucan (2 mg/mL) increased under the alkaline and high temperature conditions (Fig. 1C and D). In this study, the $\beta(1,3\rightarrow1,6)$ D-glucan isolated from *A. pullulans* GM-NH-1A1 strain is hereafter abbreviated as LMW- β -Glucan.

3.2. Food intake, diarrheal incidence, the weights of body, liver, spleen and thymus in OVA-sensitized Balb/c mice

The food intake of mice was decreased by the first and second intraperitoneal injections of OVA compared to that of untreated mice (normal) (Table 2). In the first OVA challenge, the food intake in the OVA-sensitized mice fed the laboratory chow diet containing 1% LMW- β -Glucan was increased compared to that of OVA-sensitized mice fed standard laboratory chow diet alone (control). In the second OVA challenge, the decrease of food intake in OVA-sensitized mice was slightly restored by the feeding the laboratory chow diet containing 0.5% LMW- β -Glucan. In the final sensitization using orally administered OVA, there was no significant difference in food intake between OVA-sensitized mice fed standard laboratory chow diet alone (control) and OVA-sensitized mice fed the laboratory chow diet containing LMW- β -Glucan (0.25, 0.5 or 1%) (Table 2). The mice fed a laboratory chow diet caused a 100% diarrheal incidence by the final sensitization using orally administered OVA. In the final oral OVA challenge, the incidence of diarrhea in OVA-sensitized mice was prevented by 43% by the feeding the laboratory chow diet containing 1% LMW- β -Glucan. The diarrheal incidence induced by the final oral OVA was not prevented by the feeding the laboratory chow diet containing 0.25 or 0.5% LMW- β -Glucan (data not shown).

There was no significant difference in the final weight of the body or liver among untreated mice (normal), OVA-sensitized mice fed standard laboratory chow diet alone (control), and OVA-sensitized mice fed the laboratory chow

Table 3

Effects of LMW- β -Glucan on the weights of body, spleen and thymus in mice sensitized by OVA

Treatment	Body (g)	Liver (g)	Spleen (mg)	Thymus (mg)
Untreated mice (Normal)	31.3 $\pm$ 0.46	1.774 $\pm$ 0.056	107.1 $\pm$ 6.95*	32.3 $\pm$ 1.63
OVA-sensitized mice (Control)	29.6 $\pm$ 0.58	1.622 $\pm$ 0.042	135.2 $\pm$ 7.15	33.6 $\pm$ 0.98
+LMW- β -Glucan (0.25%)	29.6 $\pm$ 0.50	1.593 $\pm$ 0.044	132.7 $\pm$ 8.87	34.1 $\pm$ 1.18
(0.5%)	30.6 $\pm$ 0.46	1.603 $\pm$ 0.042	138.0 $\pm$ 7.70	32.9 $\pm$ 0.83
(1.0%)	30.0 $\pm$ 0.61	1.618 $\pm$ 0.068	157.0 $\pm$ 8.21	29.3 $\pm$ 1.30*

Values are means $\pm$ S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P<0.05$.

diets containing 0.25, 0.5 or 1% LMW- β -Glucan (Table 3). The thymus weight of OVA-sensitized mice fed the laboratory chow diet containing 1% LMW- β -Glucan was significantly lower than that of OVA-sensitized mice (control). The spleen weight of OVA-sensitized mice (control) was greater than that of the normal mice; however, there was no significant difference in the spleen weight between control mice and OVA-sensitized mice fed the LMW- β -Glucan (0.25, 0.5 or 1%) (Table 3).

3.3. Effects of LMW- β -Glucan on serum OVA-specific immunoglobulin production in OVA-sensitized allergic mice

The OVA-specific IgG, IgG₁, IgG_{2a}, IgA and IgE levels were increased by sensitization with OVA (Table 4 and Fig. 2). Feeding the laboratory diet containing 1% LMW- β -Glucan significantly inhibited the elevation of OVA-specific IgG in the OVA-treated mice. Feeding the laboratory diets containing LMW- β -Glucan (0.25, 0.5 and 1%) had no effect on the

Table 4

Effects of LMW- β -Glucan on blood OVA-specific immunoglobulins (IgG, IgG₁, IgG_{2a} and IgA) in mice sensitized by OVA

Treatment	OVA-specific immunoglobulins (OD $\times$ 100)			
	IgG	IgG ₁	IgG _{2a}	IgA
Untreated mice (Normal)	5.3 $\pm$ 1.1*	6.0 $\pm$ 2.6*	0.3 $\pm$ 0.1*	20.5 $\pm$ 2.8*
OVA-sensitized mice (Control)	81.0 $\pm$ 0.8	99.2 $\pm$ 0.7	1.5 $\pm$ 0.2	48.5 $\pm$ 4.8
+LMW- β -Glucan (0.25%)	80.4 $\pm$ 1.3	98.0 $\pm$ 2.0	1.1 $\pm$ 0.2	41.0 $\pm$ 3.3
(0.5%)	79.0 $\pm$ 1.0	98.4 $\pm$ 2.0	1.1 $\pm$ 0.2	43.4 $\pm$ 3.6
(1.0%)	76.8 $\pm$ 1.3*	98.8 $\pm$ 1.5	1.0 $\pm$ 0.3	41.0 $\pm$ 3.6

Values are means $\pm$ S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P<0.05$.

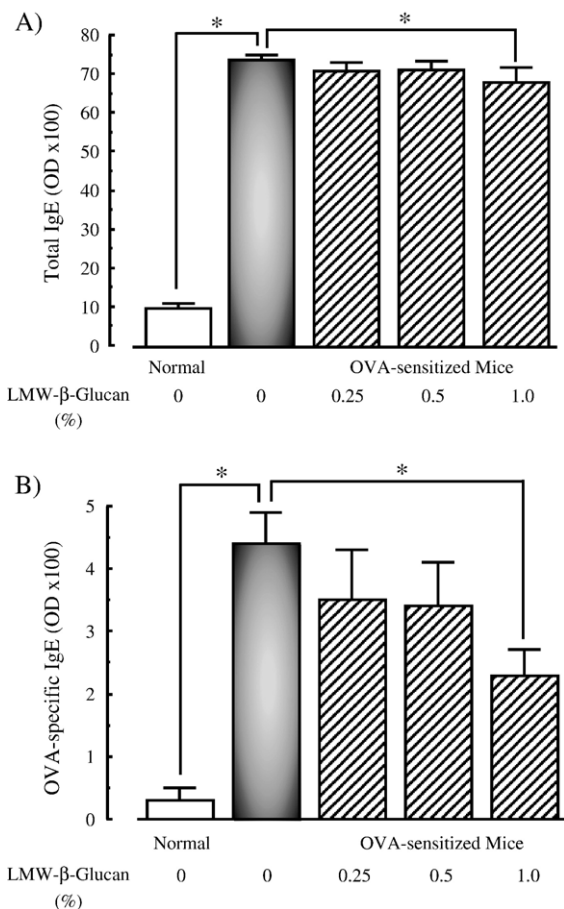


Fig. 2. Effects of LMW- β -Glucan isolated from *A. pullulans* 1A1 strain on total IgE (A) and OVA-specific IgE (B) in mice sensitized by OVA. Values are means $\pm$ S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P<0.05$.

elevation of OVA-specific IgG₁ and IgG_{2a} in the OVA-sensitized mice (Table 3). The elevation of total IgE and OVA-specific IgE was significantly inhibited by feeding the laboratory diet containing 1% LMW- β -Glucan in the OVA-treated mice (Fig. 2).

3.4. Effects of LMW- β -Glucan on cytokine production from spleen in OVA-sensitized allergic mice

There was no significant difference in Con A-induced IL-4 production between normal mice and OVA-sensitized mice fed the laboratory diet alone (control). On the other hand, the IFN- γ and IL-12 production from splenocytes induced by Con A in the OVA-sensitized mice was significantly lower than that in normal mice (Fig. 3). The Con A-induced IL-4 production from splenocytes in the OVA-sensitized mice was significantly increased by feeding the laboratory diets containing 0.5 and 1% LMW- β -Glucan. Furthermore, the Con A-induced IFN- γ and IL-12 production in the OVA-sensitized mice was also

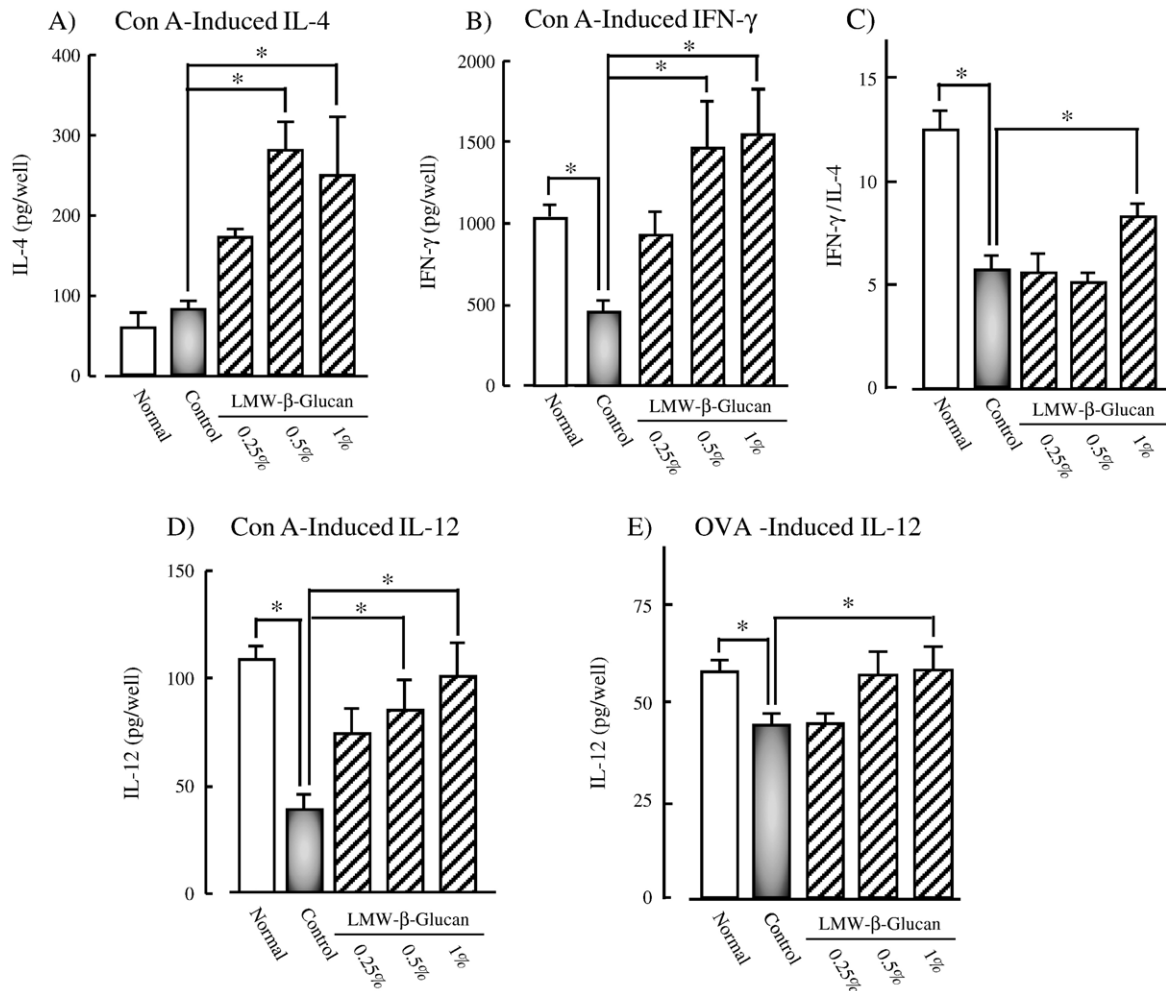


Fig. 3. Effects of LMW-β-Glucan isolated from *A. pullulans* 1A1 strain on Con A-induced IL4 (A), IFN-γ (B), the ratio of IFN-γ/IL-4 (C) and IL-12 (D), and OVA-induced IL-12 (E) from splenocytes in mice sensitized by OVA. Values are means ± S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P < 0.05$.

significantly increased by feeding the laboratory diets containing 0.5 and 1% LMW-β-Glucan (Fig. 3). The ratio of IFN-γ/IL-4 production from splenocytes in the OVA-sensitized mice fed the laboratory diets containing 1% LMW-β-Glucan was greater than that in the OVA-sensitized mice (control) (Fig. 3).

3.5. Effects of LMW-β-Glucan on small intestinal immune functions (immunohistochemistry) in OVA-sensitized allergic mice

The numbers of CD8 T- and IFN-γ-positive cells in the small intestine in the OVA-sensitized mice (control) tended to be reduced ($P < 0.08$ and $P < 0.07$) compared with those in the normal mice (Fig. 4). The CD8 T- and IFN-γ-positive cell numbers in the small intestine were significantly increased by feeding the laboratory diets containing 0.5 and 1% LMW-β-Glucan compared to those in control mice (Fig. 4).

4. Discussion

It has been reported that food allergy is particularly frequent during childhood and may affect children's final height and weight [19]. Furthermore, food allergy and other types of allergic diseases such as allergic rhinitis and asthma are increasing in both developed and developing countries [20]. Food allergy is most frequently the result of an IgE-mediated hypersensitivity reaction [21]. The production of IgE is promoted by Th₂ cells and their cytokines, such as IL-4 and IL-5, while IFN-γ secreted by Th₁ cells can inhibit IgE production [22]. Furthermore, antigen-specific IgG_{2a} antibody is induced by Th₁ cells and Th₁ cytokines, whereas IgE and IgG₁ antibody production is induced by Th₂ cells and cytokines [23–25]. It has been reported that the oral

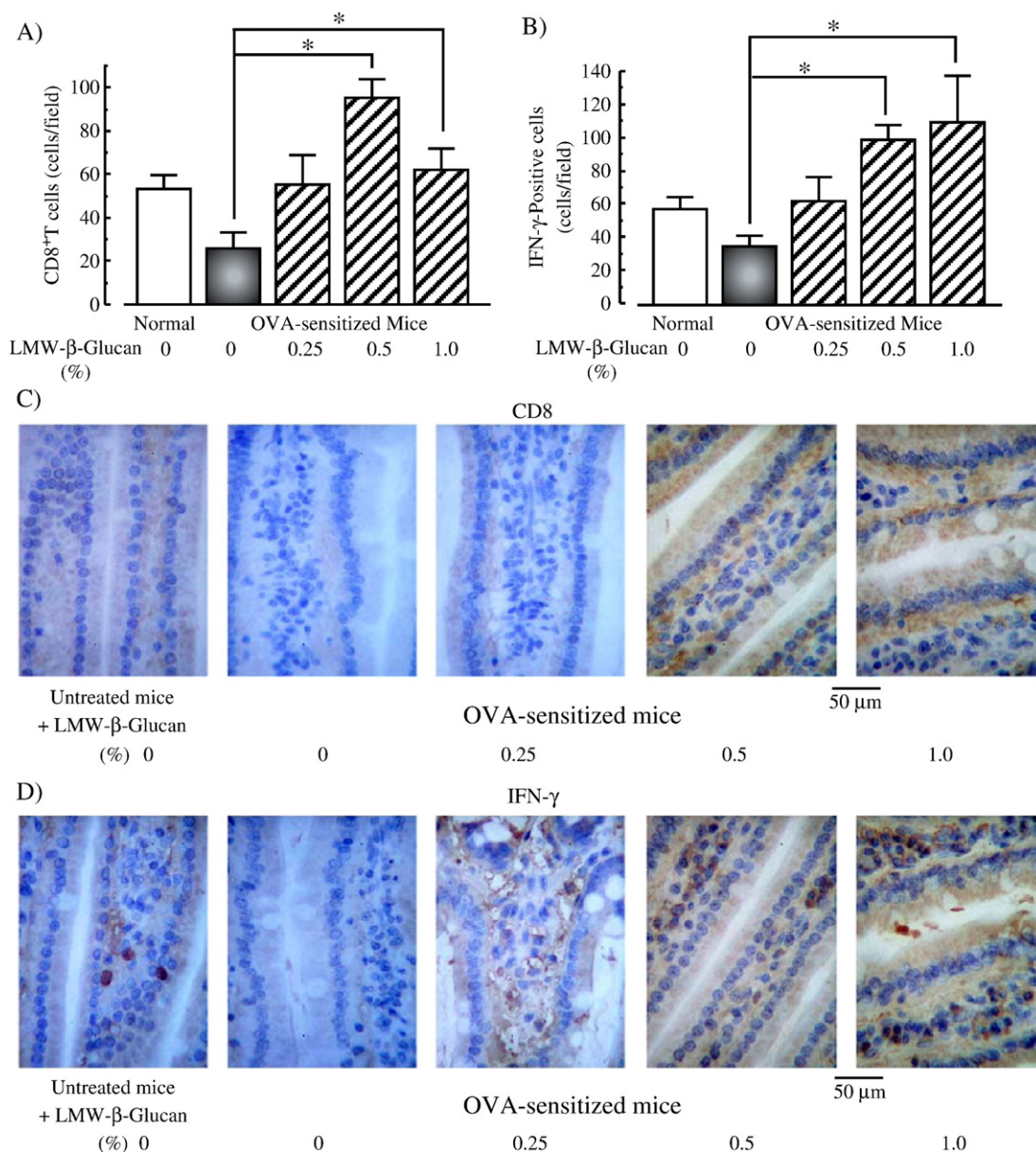


Fig. 4. Effects of LMW-β-Glucan on CD8- and IFN-γ-positive cells in small intestine of mice sensitized by OVA. (A) and (B): values are means ± S.E. of 7 mice. *Significantly different from OVA-sensitized mice (control), $P < 0.05$. (C) and (D) Light micrographs of CD8⁺ T and IFN-γ-positive cells in small intestine stained by the immunoperoxidase technique using mouse anti-mouse CD8α antibody and rat anti-mouse IFN-γ rat antibody, respectively.

administration of β-glucan fractions prepared from Basidiomycetes mushrooms exert potent antitumor actions through the stimulation of IFN-γ production in the spleen and small intestine [9,26]. There are some reports that β-glucan in house dust and indoor moulds caused air allergic responses [4–8]. However, Instanes et al. [6] reported that mould extracts contained less than 2% β-1,3-glucan; therefore, the potentiation of the OVA-specific immune response in a Th₂-dependent

manner (OVA-specific IgE elevation) suggests that mould also contains other and more potent adjuvants than β-1,3-glucan that are active at lower concentrations. Thus, the experimental findings about β-glucan suggest that it may have opposite effects on the allergic reactions that probably depend on the differences of dosage, route of administration, average molecular weight, purity and water-solubility of β-glucan that may be contributed to the immune response, although

this has not been proven yet. In the present study, we found that the serum OVA-specific IgG, IgG₁, IgG_{2a}, IgE and IgA were increased by sensitization with OVA. The elevation of serum total IgE and OVA-specific IgE was prevented by feeding control diets containing 1% LMW- β -Glucan purified from *A. pullulans* 1A1 strain in OVA-sensitized mice. IL-12 is a heterodimeric cytokine consisting of glycosylated subunits of 35 and 40 kDa, linked by disulphide bonds [27]. IL-12 is primarily secreted by macrophages, monocytes, dendritic cells and splenocytes in response to a variety of microbial factors [28–30]. IL-12 plays a central role in the initiation of cell-mediated immunity both directly and through its induction of IFN- γ [31,32]. It has been reported that IL-12 plays an important role in the regulation of mucosal immunity [33,34]. It was found that the numbers of IFN- γ and CD8-positive cells in the small intestine were reduced by sensitization with OVA. Feeding control diets containing 0.5 and 1% LMW- β -Glucan prevented the reduction of IFN- γ - and CD8-positive cells in the small intestine in the OVA-sensitized mice. There are some reports that the intestinal intra-epithelial lymphocytes (IELs) can secrete IL-2, IL-3, TNF- α and IFN- γ [35,36], and that the majority of IELs are CD4⁺CD8⁺ T cells and the CD4⁺CD8⁺ T cells in the IEL population consist of 2 subpopulations, one expressing CD8 $\alpha\alpha$ homodimers and the other expressing CD8 $\alpha\beta$ heterodimers [37]. It has been demonstrated that IFN- γ is secreted from the IEL CD8 $\alpha\beta$ subpopulation in response to stimulation by antigens derived from food and microorganisms [38–41]. From these results, it seems likely that the inhibitory effects of LMW- β -Glucan purified from *A. pullulans* 1A1 strain on food-induced allergic reactions might be due to the inhibition of food-antigen-specific IgE elevation through the inhibition of IL-12 and IFN- γ reduction induced by food antigens in the spleen cells and/or CD8-positive cells of the small intestine. In the present study, LMW- β -Glucan could not be detected in the blood after the oral administration of LMW- β -Glucan (data not shown); therefore, it seems likely that LMW- β -Glucan cannot be absorbed from the gut. Therefore, these findings suggest that the anti-food allergic action of LMW- β -Glucan may be due to the stimulation of immune function in the small intestinal lymphocytes.

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