Effect of oral administration of β -glucans derived from *Aureobasidium pullulans* SM-2001 in model mice and rat with atopic dermatitis-like phenotypes

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Abstract In this study, we investigated the anti-atopic dermatitis (AD) activity of β -glucans derived from *Aureobasidium pullulans* SM-2001 (β GdAP). β GdAP was orally administered to AD animal models such as vasodilation, allergic pruritus and contact dermatitis. Administration of β GdAP attenuated the amount of Evans blue solution on vasodilation rat. Scratching behaviors, secretion of histamine and ear thickness were significantly ($p < 0.05$) attenuated in the β GdAP-treated mouse groups. Interestingly, transcriptional expression of T-bet, a transcription factor for Th1 reactions, was increased, but that of GATA-3, a transcription factor for Th2 reactions, was attenuated in the β GdAP-treated groups ($p < 0.05$). In addition, we found that reduced transcriptional expression of forkhead box P3 and galectin-9, regulators of regulatory T cells, was recovered in the β GdAP-treated groups ($p < 0.05$). Taken together, these data indicate that

administration of β GdAP could effectively attenuate AD-like phenotypes via regulation of Th1/Th2 transcriptional activity and Treg activation.

Keywords β -Glucan · *Aureobasidium pullulans* SM-2001 · Atopic dermatitis · Th1/Th2 · FOXP3 · Galectin-9

Introduction

Atopic dermatitis (AD) is a chronic relapsing disease that is characterized by xeroderma, pruritus, and inflammation [1]. Although the detailed mechanisms of AD disease are still not clear, various factors such as air pollution, allergens in housing environment, and stress have been suggested as critical factors that exacerbating AD symptoms [2]. From the immunological perspective, AD is induced by an imbalance of Th1/Th2 cells that is caused by inhibition of the proliferation and differentiation of Th1 cells along with the release of diverse Th2 cytokines such as interleukin-4 (IL-4), IL-5, and IL-13 [3, 4]. In addition, regulatory T cells (Tregs) that are positive for foxhead box P3 (FOXP3) have been reported to play critical roles in immunosuppressive function [5]. FOXP3 is a well-known transcription factor that is associated with the activation of Tregs [6]. Moreover, expression of galectin-9 in intestinal epithelial cells has been reported to play an immunomodulatory role in Treg polarization [7]. Therefore, agents that activate FOXP3 and galectin-9 expression may be useful for alleviating the symptoms of AD.

AD is primarily treated with local steroids including calcineurin inhibitors, antihistamines, γ -linolenic acid, cyclosporine, and interferon gamma (IFN- γ). However, these therapies often result in relapse when discontinued, or cause side effects when used for a long time [8]. Therefore,

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there is growing interest in natural remedies for AD that can regulate inflammation-related cytokine expression and have minimal side effects. One such compound that has attracted attention is β -glucan, which is a biologically active polysaccharide present in the cell wall of yeasts, mushrooms, cereals, algae, and a few bacteria [9–11]. β -Glucan can activate the immune functions of normal human cellular tissues to inhibit the proliferation of cancer cells, prevent relapse of cancer, and promote the production of IFN- γ and IL, which are known to indirectly inhibit the proliferation of cancer cells [12, 13]. β -Glucan has been reported to have role in innate immune responses through activating B-lymphocytes [14]. β -Glucan has also been found to have anti-hyperlipidemic effects [15], anti-obesity effects via inhibition of the formation and accumulation of body fat [16], anti-oxidative activity [17], and arthritis-alleviating effects [18]; thus, it can be used as a health food ingredient and a food additive.

The exopolymer of *Aureobasidium pullulans* SM-2001 is mainly composed of β -1,3- and β -1,6-glucans and has been reported to have anti-osteoporotic effects [19] and inhibitory effects against UVB-induced skin damage [20]. However, despite there being evidence for the diverse effects of β -glucans derived from *A. pullulans* SM-2001 (β GdAP), there is not enough information about its immunomodulatory effects in atopic dermatitis disease models. Therefore, in the present study, several AD-like phenotypes were induced in rats and mice, and β GdAP was orally administered in order to investigate its immunomodulatory effects.

Materials and methods

Reagents

The compound 48/80 (COM), Evans blue solution, dinitrofluorobenzene (DNFB), and aluminum hydroxide gel were obtained from Sigma-Aldrich (CA, USA), and dinitrophenyl-derivatized ovalbumin (DNP-OVA) were purchased from Alpha diagnostic Intl. Inc., USA.

Animals and treatment

Six-week-old Sprague–Dawley (SD) male rats from Samtako (Osan, Korea) and six-week-old male ddY mice from Central Lab. Animal Inc. (Seoul, Korea) were purchased for the experiment. During the acclimation and experimental periods, a room temperature of $22 \pm 1^\circ\text{C}$, humidity of $60 \pm 10\%$, and a 12-h light/12-h dark cycle were maintained. The animals were given free access to pellet-type solid feed AIN-76A (Choongang Co., Seoul, Korea) and water. After an acclimation period of 1 week, the

experimental animals were divided into six groups of ten animals each: (1) Control group (C: normal diet), (2) Negative control group (N: normal diet + AD induction), (3) Positive control group (P: normal diet + 0.2 mg/kg body weight of Zyrtec + AD induction), (iv) β GdAP treatment group 1 (T1: normal diet + 1 mg/kg body weight of β GdAP + AD induction), (v) β GdAP treatment group 2 (T2: normal diet + 10 mg/kg body weight of β GdAP + AD induction), and (vi) β GdAP treatment group 3 (T3: normal diet + 20 mg/kg body weight of β GdAP + AD induction). Zyrtec (cetirizine) (UCB FARCHIM S.A., Switzerland) was used to attenuate the AD-like symptoms. Macromolecular β GdAP polymers externally secreted from the black yeast strain *A. pullulans* SM-2001 with an average molecular weight of 2.6×10^5 Da were obtained from Glucan Co. (Seoul, Korea) [21]. The doses of β GdAP, which used in this study, were determined by considering the guideline for adults from Glucan Co. (250 mg/60 kg body weight). The purity of the β -1,3- and β -1,6-glucan used in the present experiment was 15%. The animal experiment was performed with the approval of the Institutional Animal Care Board of Gyeongnam National University of Science and Technology (Jinju, Korea) (Approval No. 2014-04).

Histamine-induced vasodilation

The compound 48/80 (COM), which promotes histamine secretion, was used to induce the AD-like phenotype in the SD rats according to the method of Ishiguro et al. [22]. Briefly, Zyrtec and β GdAP were orally administered to the positive control (P) and β GdAP groups (T1, T2, and T3) respectively. At 1 h after Zyrtec and β GdAP administration, COM (10 $\mu\text{g/mL}$) at a dose of 50 μL was intra-dermally injected into the shaved dorsal dermis of rats once every 7 days. At 30 min after the COM injection, 200 μL of Evans blue solution was injected into the tail vein, and all the animals were sacrificed 30 min later. The dorsal dermis was cut out 30 min later to measure the diameters of the spots (in millimeters) representing extravasation of the blue dye due to vasodilation, using a digital caliper (Bluebird, NA500-150S). To measure the amount of leaked dye, the dorsal dermis of each animal was cut in a 10-mm diameter circle, and the cut pieces were kept in a 1.0 N KOH (1.0 mL) solution at 37°C for 48 h to initiate the reaction. Then, 0.6 N H_3PO_4 (2.5 mL) and acetone (6.5 mL) were added to the reaction mixture, which was shaken and centrifuged at $900 \times g$ for 10 min. The absorbance of the supernatant was determined at 620 nm by spectrophotometric analysis (iMAX microplate reader, Biorad co., Korea) to measure the amount of blue dye that had leaked in milligrams per site. To measure the level of serum histamine, the animals were sacrificed at 30 min after the

administration of Evans blue, and blood samples taken from the vena cava were centrifuged for 15 min at $1300\times g$ to separate the serum. The level of histamine in the separated serum was measured using Histamine EIA Kits (LDN, Germany).

Mouse model of allergic pruritus

The histamine secretion promoter COM (3 mg/kg body weight) was subcutaneously injected into the shaved dorsal dermis of ddY mice to induce the AD-like phenotype. Zyrtec and β GdAP were orally administered once a day for 7 days. At 30 min after COM administration, the number of reactions (expressed in the form of pruritus) in the entire body was counted for 20 min. When the animals were sacrificed 30 min after Evans blue administration, blood samples taken from the vena cava were centrifuged for 15 min at $1300\times g$ to separate the serum. The level of histamine in the separated serum was measured using Histamine EIA Kits (LDN, Germany).

Mouse model of contact dermatitis

To induce contact dermatitis, which is an AD-like phenotype, the ddY mice were sensitized by intraperitoneal injection of 0.2 mL of saline solution containing 10 μ g DNP-OVA and 1 mg of aluminum hydroxide gel. Seven days after sensitization, 10 μ L of 0.1% 2,4-DNFB dissolved in ethanol was applied to the ear and paw regions. Ear thickness was measured before DNFB application and 1 h and 24 h after DNFB application using a digital caliper (Bluebird, NA500-150S). Immediately after the DNFB application, the number of reactions (in the form of pruritus) on the entire body within a span of 1 h was counted according to a previously reported method [23].

RNA isolation and analysis

To analyze the immunomodulatory effects of β GdAP, rats with vasodilation were sacrificed, and their mesenteric lymph nodes were resected. The separated mesenteric lymph nodes were placed in Trizol[®] Reagent (Ambion, CA, USA) and homogenized using SilentCrusher M (Heidolph, Schwabach, Germany). RNA was isolated using the total RNA isolation kit (Intronbio Co., Sungnam, Korea) and stored at -20°C until use for cDNA synthesis (TaKaRa Co., Tokyo, Japan). Quantitation was performed using a CFX connect real-time system (Bio-Rad Co., CA, USA) with the SYBR Green real-time master mix (Toyobo Co., Tokyo, Japan). Relative expression of each gene was determined by using the comparative Ct method and normalized to the expression of GAPDH. The sequences of the T-bet primers are 5'-CCACCCAGAAGACTGTGGAT-3'

(forward) and 5'-CACATTGGGGGTAGGAACAC-3' (reverse); GATA-3 primers, 5'-CATTACCACCTATCCGCCCTATG-3' (forward) and 5'-CACACACTCCCTGCCTTC TGT-3' (reverse); FOXP3 primers, 5'-CCCATCCCCAG GAGTCTTG-3' (forward) and 5'-CCATGACTAGGGGC ACTGTA-3' (reverse); galectin-9 primers, 5'-GAGAGGA AGACACACATGCCTTTC-3' (forward) and 5'-GACCA CAGCATTCTCATCAAAACG-3' (reverse); GAPDH primers, 5'-CCACCCAGAAGACTGTGGAT-3' (forward) and 5'-CACATTGGGGGTAGGAACAC-3' (reverse).

Statistical analysis

The variance of the data obtained through repeated experiments was analyzed using SPSS 12.0 (SPSS Inc., IL, USA), and the results are represented as the mean $\pm$ standard deviation values. The significance was determined by analysis of variance followed by Duncan's multiple range test at the level of $p < 0.05$.

Results and discussion

Effects of β GdAP on COM-induced vasodilation

To analyze the effects of β GdAP on vasodilation, an AD-like phenotype, rats in which vasodilation was induced were orally administered β GdAP (one group of control rats was not administered β GdAP). The degree of vasodilation was determined by measuring the size of the spots containing leaked Evans blue. As shown in Fig. 1(A), there was no dye leakage in the control group (C), and the largest blue-dye spots (largest diameter, 5.31 mm) were observed in the COM-treated groups (N). The diameter of the spots significantly ($p < 0.05$) decreased on Zyrtec administration in the positive control groups. Among the β GdAP groups, T1 had the smallest blue-dye spot diameter of 3.81 mm, which indicates the lowest degree of vasodilation and therefore the greatest degree of inhibition against vasodilation. To determine the amount of Evans blue dye that had leaked, the dorsal dermis of the rats was resected and dissolved in an alkaline solution, and the absorbance of the supernatant was determined through spectrophotometric analysis. The positive control group (P) had the lowest amount of dye (0.024 mg/site), while the negative control groups (N) had the highest amount of dye (0.248 mg/site) and exhibited remarkable AD-like symptoms [Fig. 1(B)]. Histamine, which is mainly secreted by mast cells and basophilic cells, is the main factor that causes vasodilation in AD. To further investigate the effects of β GdAP on vasodilation, the concentration of serum histamine was measured in each group. As shown in Fig. 1(C), the COM-treated negative control group (N) showed the highest level

Fig. 1 Effects of β GdAP on vasodilation induced by COM. Administration of β GdAP significantly attenuated vasodilation induced by COM, as evident from (A) the diameter of the blue-dye spots and (B) the amount of leaked dye in mice from the different groups. (C) Serum histamine level in rats with vasodilation induced by COM. C Control, N Negative control (COM-induced AD), P Positive control

(COM-induced AD + Zyrtec), T1 COM-induced AD + 1 mg/kg body weight of β GdAP, T2 COM-induced AD + 10 mg/kg body weight of β GdAP, T3 COM-induced AD + 20 mg/kg body weight of β GdAP. ^{a–d}Significant difference at $p < 0.05$. Data represent the mean $\pm$ SD values from five replicates

of serum histamine (28.09 ng/mL), and the positive control (P) and β GdAP groups (T1, T2, and T3) showed lower levels of serum histamine than the N group ($p < 0.05$). In an experiment conducted using type I dermatitis models with passive cutaneous anaphylaxis, it was reported that remarkable extravasation of the blue dye due to vasodilation occurred 30 min after exposure to the antigen [24]. The significant decreases in the diameter of the leaked dye spots and the amount of leaked blue dye shown in the present experiment can be considered as direct evidence of the effects of β GdAP on the inhibition of vasodilation, which represents the acute inflammatory changes caused by AD. Taken together, these data indicate that administration of β GdAP was effective for attenuating vasodilation, an AD-like phenotype.

Effects of β GdAP on allergic pruritus

To further confirm the effects of β GdAP on AD-like phenotypes, scratching behaviors were examined using a mouse model of allergic pruritus. As shown in Fig. 2(A), the COM-treated mouse group (N) showed the most frequent scratching behaviors, while the other mouse groups (P, T1, T2, and T3) showed significantly ($p < 0.05$) less frequent scratching behaviors than the COM-treated mouse group (N). Figure 2(B) shows the levels of serum histamine, which is a factor that causes allergic pruritus, in the different groups. The N group showed significantly higher levels of histamine than the other groups ($p < 0.05$). In the case of AD, mast cells sensitized by IgE secrete histamine, and this leads to the development of symptoms such as edema, erythema, and pruritus [25]. In particular, histamine secretion induced by COM leads to edematous changes [26]. In the present experiment, the significant decrease

observed in the serum histamine levels in the treatment and P groups compared to the N group was direct evidence of the effects of β GdAP on the inhibition of the series of reactions caused by AD. These findings indicate that β GdAP alleviates inflammation. COM is known to promote histamine secretion from mast cells. In addition, COM promotes histamine secretion to induce scratching behaviors in mice [27]. The decrease in scratching behaviors in AD mice treated with β GdAP indicates that β GdAP has a positive effect on allergic pruritus [28]. In the present experiment, scratching behaviors significantly decreased in the AD group in which β GdAP was orally administered compared to the AD group that was not treated with β GdAP. Therefore, β GdAP appears to have inhibitory effects on COM-induced pruritus. These results indicate that administration of β GdAP can attenuate allergic pruritus, which is an AD-like phenotype.

Effects of β GdAP on contact dermatitis

Finally, we tested the effects of β GdAP on AD-like phenotypes, using the DNP-OVA- and DNFB-induced mouse model of contact dermatitis. Ear thicknesses were measured twice, at 1 h and 24 h after DNFB treatment. The ear thicknesses of the DNP-OVA- and DNFB-treated mouse group (N) dramatically increased compared to the control mouse group (C) ($p < 0.05$). However, it was significantly decreased in the P and β GdAP-treated mouse groups (T1, T2, and T3) ($p < 0.05$) [Fig. 3(A)]. To investigate pruritus associated with contact dermatitis induced by DNP-OVA and DNFB, signs of pruritus were observed for 1 h after DNFB treatment. In the P and β GdAP-treated groups (T1, T2, and T3), pruritus was significantly ($p < 0.05$) alleviated compared to the DNFB-treated mouse group

Fig. 2 Effects of β GdAP on allergic pruritus induced by COM. Administration of β GdAP significantly attenuated acute pruritus on mice induced by COM, as evident from (A) the frequency of scratching behaviors and (B) the serum histamine level. C Control, N Negative control (COM-induced AD), P Positive control (COM-induced

AD + Zyrtec), T1 COM-induced AD + 1 mg/kg body weight of β GdAP, T2 COM-induced AD + 10 mg/kg body weight of β GdAP, T3 COM-induced AD + 20 mg/kg body weight of β GdAP. ^{a-c}Significant differences between means within the same row at $p < 0.05$. Data represent the mean $\pm$ SD values from five replicates

Fig. 3 Effects of β GdAP on contact dermatitis induced by DNP-OVA and DNFB. Administration of β GdAP significantly attenuated the contact dermatitis phenotype, as evident from (A) ear thickness and (B) the frequency of scratching behaviors in the contact dermatitis mouse model induced by DNP-OVA and DNFB. C Control, N Negative control (COM-induced AD), P Positive control (COM-

induced AD + Zyrtec), T1 COM-induced AD + 1 mg/kg body weight of β GdAP, T2 COM-induced AD + 10 mg/kg body weight of β GdAP, T3 COM-induced AD + 20 mg/kg body weight of β GdAP. ^{a-c}Significant differences between means within the same row at $p < 0.05$. Data represent the mean $\pm$ SD values from five replicates

(N) [Fig. 3(B)]. When DNFB was repeatedly applied to the ears of the mice, the increase in the level of serum IgE induced an increase in the thickness of the ears. Similarly, it has been reported that repeated DNFB application induces epidermal thickening, scab formation, and inflammatory cell infiltration [29]. DNFB-induced contact dermatitis models are useful for the evaluation of type I allergic dermatitis. When DNFB is applied to the ears of mice sensitized with DNP-OVA, two types of edema are induced: the edema that occurs 1 h after DNFB application is called the immediate-phase response (IPR), and the edema that occurs 24 h after DNFB application is called the late-phase response (LPR) [30]. After DNFB application, scratching behaviors are observed during IPR, which

is an inflammatory reaction caused by the chemical mediators released from mast cells. In the present experiment, β GdAP inhibited edema in both IPR and LPR and significantly inhibited scratching behaviors in IPR. Therefore, the present data indicate that administration of β GdAP can alleviate the DNFB-induced contact dermatitis phenotype.

Changes in transcriptional activity in β GdAP-administered rat groups

To investigate the mechanisms through which β GdAP alleviates AD-like phenotypes, the expression of T-bet and GATA-3, as transcription factors of Th1 and Th2 cells,

respectively, was examined in the mesenteric lymph node tissues of rats with vasodilation. As shown in Fig. 4, T-bet, a transcription factor associated with Th1 reactions, showed significantly ($p < 0.05$) higher expression in the β GdAP groups (T1, T2 and T3) than in the negative control group (N). However, GATA-3, a transcription factor associated with Th2 reactions, showed significantly ($p < 0.05$) lower expression in the β GdAP groups (T1, T2 and T3) than in the negative control group (N). Based on the T-bet/GATA-3 expression ratio, the Th1/Th2 transcriptional ratio was found to be significantly ($p < 0.05$)

decreased in the AD-induced negative control group (N), but it dramatically recovered in the β GdAP-treated groups (T1, T2 and T3) [Fig. 4(C)]. Cytokines are substances produced mostly by immune cells that are closely related to immune regulation. Th1 cell cytokines are known to enhance cellular immune responses and inhibit allergy-related cellular immune responses [31]. Further, Th2 cells secrete cytokines to promote the inflow and activation of allergy-related cells, such as B cells, mast cells, and eosinophils, and cause damage to tissues through fibrosis [32]. Activated immune cells release IgE, histamine, and cytotoxic substances to

Fig. 4 Effects of oral administration of β GdAP on the expression of Th1 and Th2. T cell polarization in mesenteric lymph node (MLN) tissues of vasodilation rats was evaluated by analyzing the expression of T-bet (Th1, **A**) and GATA-3 (Th2, **B**), and the Tbet/GATA-3 ratio (Th1/Th2, **C**). C Control, N Negative control (COM-induced AD), P Positive control (COM-induced AD + Zyrtec), T1 COM-induced

AD + 1 mg/kg body weight of β GdAP, T2 COM-induced AD + 10 mg/kg body weight of β GdAP, T3 COM-induced AD + 20 mg/kg body weight of β GdAP. ^{a–f}Significant differences between means at $p < 0.05$. Data represent the mean $\pm$ SD values from four replicates

Fig. 5 Effects of β GdAP on Treg activation. Total RNA was isolated from the mesenteric lymph node (MLN) tissues of rats with vasodilation, and transcriptional expression of FOXP3 (**A**) and galectin-9 (**B**) was analyzed by qRT-PCR. C Control, N Negative control (COM-induced AD), P Positive control (COM-induced

AD + Zyrtec), T1 COM-induced AD + 1 mg/kg body weight of β GdAP, T2 COM-induced AD + 10 mg/kg body weight of β GdAP, T3 COM-induced AD + 20 mg/kg body weight of β GdAP. ^{a–c}Significant differences between means at $p < 0.05$. Data represent the mean $\pm$ SD values from four replicates

intensify the inflammatory responses and induce clinical signs [33]. Therefore, inhibiting Th2 cell action that is central to allergic reactions is essential for the treatment of allergic diseases. In the present study, β GdAP inhibited Th2 cell reactions that are central to allergic reactions that have immunomodulatory effects.

Stimulation of FOXP3 and galectin-9 expression by administration of β GdAP

To determine the effects of β GdAP on the activation of Tregs, transcriptional changes in FOXP3, a transcription factor of Tregs, and galectin-9, which is known as a stimulator of Treg activation, were investigated in the mesenteric lymph nodes of rats with vasodilation. As shown in Fig. 5, the expression levels of FOXP3 and galectin-9 were significantly ($p < 0.05$) attenuated in the AD-induced group (N) compared with the control group (C). However, FOXP3 and galectin-9 expression was dramatically recovered in the β GdAP groups (T1, T2 and T3) ($p < 0.05$). FOXP3 is a well-known specific marker of Tregs, and FOXP3-positive Tregs have been reported to play an important role in immune tolerance [34]. Further, activation of FOXP3-positive Tregs was effective in reducing disease severity in inflammatory bowel disease and animal models of asthma [35]. Dietary synbiotics such as galacto- and fructo-oligosaccharides have been reported to have suppressive effects on allergic symptoms in mice and humans via upregulation of the expression of galectin-9 [36]. Since activation of Treg and galectin-9 expression may be useful for suppressing the immune responses in AD, our data indicate that administration of β GdAP may activate Tregs via stimulation of the transcriptional expression of FOXP3 and galectin-9, which may result in the attenuation of AD-like phenotypes.

Overall, in the present study, we investigated the effects of β GdAP against AD-like phenotypes by using three AD animal models. Oral administration of β GdAP in a COM-induced AD rat model significantly reduced vasodilation, which is an acute inflammatory change caused by AD, and allergic pruritus. We found that contact dermatitis was also significantly attenuated with β GdAP treatment. Taken together, we believe that these results, to the best of our knowledge, are the first to demonstrate the anti-AD effects of β GdAP via regulation of the expression of Th1/Th2 and activators of Tregs.

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Compliance with ethical standards

Conflict of interest We certify that there is no conflict of interest in the manuscript.

References

1. Leung DYM, Boguniewicz M, Howell MD, Nomura I, and Hamid QA. New insights into atopic dermatitis. *J. Clin. Invest.* 113: 651-657 (2004)
2. Sim TK, Ko DK, Kim HC, Baek YJ, Lee JS, and Yoo HS. Effects of *Amyda sinensis* on allergic inflammation mechanism related atopy dermatitis. *Daejeon University Oriental Medicine*, 20: 69-83 (2011)
3. Meagher LJ, Wines NY, Cooper AJ. Atopic dermatitis; review of immunopathogenesis and advances in immunosuppressive therapy. *Aust. J. Dermatol.* 43: 247-254 (2002)
4. Blaser K, Ring J, Capron. T cell regulation in allergy, asthma and atopic skin diseases. *S. Karger Pub.* 121-137 (2008).
5. Tao JH, Cheng M, Tang JP, Liu Q, Pan F, and Li XP. Foxp3, Regulatory T cell, and autoimmune diseases. *Inflammation*. 40: 328-339 (2017)
6. Kanamori M, Nakatsukasa H, Okada M, Lu Q, Yoshimura A. Induced regulatory T cells: their development, stability, and applications. *Trends Immunol.* 37: 803-811 (2016)
7. de Kivit S, Kraneveld DA, Knippels LMJ, van Kooyk Y, Garssen J, and Willemsen LEM. Intestinal epithelium-derived galectin-9 is involved in the immunomodulating effects of nondigestible oligosaccharides. *J. Innate Immun.* 5: 625-638 (2013)
8. Terada Y, Tsubota M, Sugo H, Wakitani K, Sekiguchi F, Wada K, Takada M, Oita A, and Kawabata A. Tacrolimus triggers transient receptor potential vanilloid-1-dependent relapse of pancreatitis-related pain in mice. *Pharmacology*. 99: 281-285 (2017)
9. Delaney B, Nicolosi RJ, Wilson TA. Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters. *J. Nutr.* 133: 468-475. (2003)
10. Babicek K, Cechova I, Simon RR. Toxicological assessment of a particulate yeast (1, 3/1,6)-beta-D-glucan in rats. *Food Chem. Toxicol.* 45: 1719-1730 (2007)
11. McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1 $\rightarrow$ 3)-beta-D-glucans. *Appl. Microbiol. Biot.* 68: 163-173 (2005)
12. Sandula J, Kogan G, Kacurakova M, and Machova E. Microbial (1 $\rightarrow$ 3)- β -D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity. *Carbohydr. Polym.* 38: 247-253 (1999)
13. Ohno N, Miura T, Miura NN, Adachi Y, and Yadomae T. Structure and biological activities of hypochlorite oxidized zymosan. *Carbohydr. Polym.* 44: 339-349 (2001)
14. Ali MF, Driscoll CB, Walters PR, Limper AH, and Carmona EM. β -Glucan-activated human B lymphocytes participate in innate immune responses by releasing proinflammatory cytokines and stimulating neutrophil chemotaxis. *J. Immunol.* 1: 5318-5326 (2015).
15. Kim YH, Han KI, Jeon M, Hwang SG, Jung EG, Kwon HJ, and Han MD. Anti hyperlipidemic activities of a chemically engineered sulfated mushroom β -glucan on high fat dietary-induced hyperlipidemia in Sprague-Dawley rats. *J. Life Sci.* 24: 1209-1216 (2014)
16. Ingeborg B, Yokoyama W, Davis P, Hudson C, Backus R, Richter D, Knuckles B, and Schneeman BO. Phstprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. *Am. J. Clin. Nutr.* 69: 55-63 (1999)
17. Hui YF, Den ES, Chi TH. Antioxidant and free radical scavenging activities of edible mushrooms. *J. Food Lipids.* 9: 35-43 (2002)

18. Kim JW, Cho HR, Kim KY, Ku SK, and Lee HS. Effect of beta-glucan on the collagen-induced rheumatoid arthritis. *J. Vet. Clin.* 27: 315-324 (2010)
19. Jung MY, Kim JW, Kim KY, Choi SH, and Ku SK. Polycan, a β -glucan from *Aureobasidium pullulans* SM-2001 mitigates ovariectomy-induced osteoporosis in rats. *Exp. Ther. Med.* 12: 1251-1262 (2016)
20. Kim KH, Park SJ, Lee YJ, Lee JE, Song CH, Choi SH, Ku SK, and Kang SJ. Inhibition of UVB-induced skin damage by exopolymers from *Aureobasidium pullulans* SM-2001 in hairless mice. *Basic Clin. Pharmacol.* 116: 73-86 (2015)
21. Seo HP, Kim JM, Shin HD, Kim TK, Chang HJ, Park BR, and Lee JW. Production of β -1,3/1,6-glucan by *Aureobasidium pullulans* SM-2001. *Korean J. Biotechnol. Bioeng.* 17: 376-380 (2002)
22. Ishiguro K, Oku H, Saitani A, and Yamamoto Y. Effects of conjugated linoleic acid on anaphylaxis and allergic pruritus. *Biol. Pharm. Bull.* 25: 1655-1657 (2002).
23. Matsuda H, Tomohiro N, Ido Y, and Kubo M. Anti-allergic effects of cnidii monnieri fructus (dried fruits of *Cnidium monnieri*) and its major component, osthol. *Biol. Pharm. Bull.* 25: 809-812 (2002)
24. Ryu JC, Lee JG, Ku SK, and Jee SY. Effect of sopung-san extracts on the passive cutaneous anaphylaxis of wistar rats (type I allergic dermatitis). *J. Korean Med. Ophthalmol. Otolaryngol. Dermatol.* 22: 1-10 (2009)
25. Byun DG, and Kim JW. Influence of cytokines on the development of T cell subsets. *Pediat. Allergy Respir. Dis.* 6: 20-32 (1996)
26. Bunker CB, Foreman JC, Dowd PM. Vascular responses to histamine at low temperatures in normal digital skin and Raynaud's phenomenon. *Agents Actions.* 33: 197-199 (1991)
27. Sugimoto Y, Umakoshi K, Nojiri N, and Kamei C. Effects of histamine H1 receptor antagonists on compound 48/80-induced scratching behavior in mice. *Eur. J. Pharmacol.* 351: 1-5 (1998)
28. Shinmei Y, Hossen MA, Okihara K, Sugimoto H, Yamada H, and Kamei C. Effect of Brazilian propolis on scratching behavior induced by compound 48/80 and histamine in mice. *Int. Immunopharmacol.* 4: 1431-1436 (2004)
29. Ueda Y, Sone T, Inagaki N, and Nagai H. Effects of prednisolone on the cutaneous reaction and skin barrier function in mice treated with a hapten. *Biol. Pharm. Bull.* 26: 618-621 (2003).
30. Watanabe C, Satoh T, Tahara E, Murakami K, Hayashi K, Hase K, Andoh T, Kuraishi Y, Kadota Y, Nagai H, and Saiki I. Inhibitory mechanisms of glycoprotein fraction derived from *Miscanthus sinensis* for the immediate phase response of an IgE-mediated cutaneous reaction. *Biol. Pharm. Bull.* 22: 26-30 (1999)
31. Hatanaka H, Abe Y, Kamiya T, Morino F, Nagata J, Tokunaga T, Oshika Y, Suemizu H, Kijima H, Tsuchida T, Yamazaki H, Inoue H, Nakamura M, and Ueyama Y. Clinical implications of interleukin (IL)-10 induced by non-small-cell lung cancer. *Ann. Oncol.* 11: 815-819 (2000)
32. Romagnani S. The role of lymphocytes in allergic disease. *J. Allergy Clin. Immun.* 105: 399-408 (2000)
33. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immun.* 125: 73-80 (2010)
34. Ohki H, Martin C, Corbel C, Coltey M, and Le Douarin NM. Tolerance induced by thymic epithelial grafts in birds. *Science.* 237: 1032-1035 (1987)
35. Suri-Payer E, and Fritzsching B. Regulatory T cells in experimental autoimmune disease. *Springer Semi. Immun.* 28: 3-16 (2006)
36. de Kivit S, Saeland E, Kraneveld AD, van de Kant HJ, Schouten B, van Esch BC, Knol J, Sprickelman AB, van der Aa LB, Knippels LM, Garssen J, van Kooyk Y, and Willemsen LE. Galectin-9 induced by dietary synbiotics is involved in suppression of allergic symptoms in mice and humans. *Allergy.* 67: 343-352 (2012)