

ORIGINAL PAPER

Oral administration of pulverized Konjac glucomannan prevents the increase of plasma immunoglobulin E and immunoglobulin G levels induced by the injection of syngeneic keratinocyte extracts in BALB/c mice

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Clinical and Experimental Allergy

Summary

Background Immunoglobulin (Ig) E plays a key role in the pathogenesis of atopic diseases, such as asthma, atopic dermatitis and allergic rhinitis. Oral administration of pulverized Konjac glucomannan (PKGGM) has recently been demonstrated to prevent both plasma IgE elevation and developing dermatitis in NC/Nga mice, a model of atopic dermatitis.

Objective To clarify the direct effect of PKGGM on the increase of plasma IgE, we employed the system of BALB/c mouse that increases IgE level without developing dermatitis in response to continuous injection of the extract of syngeneic keratinocytes, PAM 212 cells (PAM extract).

Methods Three weeks after the start of feeding with either control or PKGGM diet, mice were injected subcutaneously with PAM extract bi-weekly for 10 weeks. The levels of plasma Igs were measured by enzyme-linked immunosorbent assay every 2 weeks after the injection. The levels of ϵ germline transcription and the amounts of mRNA for IL-4, IFN- γ , GATA-3 and T bet gene in the spleen were evaluated by real-time RT-PCR at the end of the experiment.

Results On the one hand, PKGGM prevented the increase of plasma IgE and IgG (IgG1, IgG2b) induced by PAM extract, and on the other hand, it enhanced the levels of plasma IgG3. However, it did not affect the level of plasma IgM. PKGGM also reduced the levels of plasma ovalbumin (OVA)-specific IgE in OVA-sensitized mice. Moreover, PKGGM attenuated the induction of ϵ germline transcription and expression levels of mRNA for IL-4, IFN- γ and GATA-3 in the spleen of PAM extract-injected mice. PKGGM also attenuated the induction of ϵ germline transcription and mRNA for IFN- γ and T bet in the spleen of phosphate-buffered saline-injected control mice.

Conclusions These results suggested that oral administration of PKGGM prevents the elevation of plasma IgE by suppressing IgE class switching in B cells and/or the commitment development of naive lymphocytes to both T-helper type 1 (Th1) and Th2.

Keywords ϵ germline transcription, konjac glucomannan, oral administration, plasma IgE, Th1/Th2 balance

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Introduction

A high serum immunoglobulin (Ig) E concentration is one of the critical characteristics of atopic diseases, such as atopic dermatitis, asthma and allergic rhinitis [1–5]. Clinical observations have suggested a correlation between serum IgE levels and the extent and/or the severity of a disease, especially with cutaneous lesions [4–6]. The antigen-

specific IgE on Fc ϵ receptors in mast cells induce the release of inflammatory mediators, such as histamine, leukotrienes and prostaglandins, in response to the binding of multivalent antigens, which subsequently cross-link the Fc ϵ receptor. In case of skin, these factors cause the immediate hypersensitivity reaction and the late phase inflammatory reaction involving T cells and eosinophils followed by the development of dermatitis [1, 7]. Recent

studies have shown that the ligation of IgE itself on Fcε receptors without antigen can stimulate the release of such inflammatory mediators from mast cells [8, 9]. Hence, the inhibition of IgE production is an important therapeutic target for atopic dermatitis.

IgE production is associated with a predominant T-helper type 2 (Th2) cellular response [10]. Naive CD4⁺ T cells stimulated with allergen in the presence of IL-4 express GATA-3, a member of the GATA family transcription factors, and develop into Th2 cells that secrete cytokines such as IL-4 and IL-13 [11]. On the other hand, naive CD4⁺ T cells stimulated with allergen in the presence of IL-12 express T bet, a T-box family transcription factor, and preferentially differentiate into Th1 cells that secrete cytokines such as IL-2 and IFN-γ [12]. Furthermore, either Th1 or Th2 effectors have the ability to down-regulate the other one to each other.

Konjac glucomannan is a highly viscous water-soluble and high molecular weight polysaccharide, consisting of a single chain of D-glucose and D-mannose joined by β-1, 4-linkage with some branches [13], and is rich in Konjac, a Japanese traditional food, obtained from the tubers of the potato-like plant *Amorphophallus konjac*. Recently, Onishi et al. [14] showed that oral administration of pulverized Konjac glucomannan (PKGM), that is reduced to a size of less than 105 μm in average diameter by means of a pulverizer, prevented the elevation of plasma IgE, as well as the development of eczematous lesions and scratching behaviour in NC/Nga mice, a model for atopic dermatitis. However, the mechanisms of PKGM action on the suppression of plasma IgE levels and/or the development of dermatitis remained unclear, especially whether PKGM directly suppresses the production of IgE.

Several reports have proposed the crucial involvement of endogenous substances in the initiation of immunological reactions by dendritic cells and T cells [15, 16]. According to this hypothesis, destruction or stress of the skin may be more important than exposure to exogenous antigens in atopic dermatitis. Indeed, we have demonstrated that continual injection of syngeneic keratinocyte (PAM-212 cells) extracts (PAM extract) without adjuvant induced the increase of serum IgE, IgG1 and IgG2b levels without developing skin lesions in BALB/c mouse [17]. This result suggested that PAM extract might initiate the increase of serum Ig levels by a Th2-dominant mechanism. The Ig-inducing factor(s) in PAM extract has not been identified, but is distinct from known factors such as IL-4, IL-13, IL-18 and prostaglandin E2 (Morimoto et al., manuscript in preparation). In this study, we aimed to clarify the effect of PKGM on the increase of plasma IgE and IgE class switching in B cells by evaluating the expression of ε germline transcription by this system. Moreover, we measured the levels of IL-4, IFN-γ, GATA-3 and T bet gene expression in the spleen to examine the

effect of PKGM on the differentiation of naive T cells to Th1 and Th2 effector cells.

Materials and methods

Animals

BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). Mice were 4- or 5-weeks old at the start of the experiments. They were maintained in a specific pathogen-free environment in closed racks with free access to food and water in the Institute of Laboratory Animal Science, Hiroshima University. This study was carried out in accordance with the Guideline for Animal Experiment in Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University.

Feeding of pulverized Konjac glucomannan diet and injection of keratinocyte extracts into the mice

The mice (male) were fed either with a commercial rodent diet, MF (Oriental Yeast, Tokyo, Japan), or 5% PKGM-containing MF (w/w), which inhibited the development of dermatitis and the increase of plasma IgE levels in NC/Nga mice [14, 18]. PKGM was a gift from Shimizu Chemical (Mihara, Japan), and its mean particle sizes were estimated to be about 77 μm. Keratinocyte cellular extract was prepared as described previously [17]. Briefly, PAM 212 cells, a murine keratinocyte cell line derived from BALB/c mouse [19], were cultured and harvested at subconfluence. Cells were washed with calcium- and magnesium-free phosphate-buffered saline (PBS), centrifuged and finally resuspended in five volumes of ice-cold PBS to the weight of the pellet, followed by sonication and centrifugation at 105 000 g for 60 min. The supernatants were collected as cellular extracts (PAM extract) and stored at -20 °C until use. Three weeks after the start of feeding with either control or PKGM diet, we started to inject mice with either PBS or PAM extract every 2 weeks for six times in total. For each mouse, an aliquot of 250 μL solution was injected subcutaneously into bilateral inguinal regions (total 500 μL/mouse). Blood was obtained by retro-orbital bleeding. Blood from each mouse was placed in a heparinized plastic tube and centrifuged at 10 000 g for 10 min. After centrifugation, plasma was collected and stored at -80 °C for analysis.

The plasma concentrations of IgE, IgM, IgG (total IgG, IgG1, IgG2a, IgG2b, IgG3) and IgA in either plasma or culture media were determined by enzyme-linked immunosorbent assay (ELISA) kits purchased from Bethyl Laboratories (Montgomery, TX, USA), following the manufacturer's instructions.

Ovalbumin immunization

Three weeks after starting the administration of either control or PKGM diet, the mice (female) were immunized intraperitoneally with ovalbumin (OVA) (Sigma, St Louis, MO, USA, 2 µg/mouse) with an alum adjuvant (LSL, Tokyo, Japan). Two and four weeks after the first immunization, the mice were given boosters using the same doses of the antigen. After the first challenge, and 1 week after the last challenge, plasma was obtained by as described above.

Plasma concentrations of total IgE antibody were determined by ELISA as previously described [14]. For the measurement of OVA-specific plasma IgE, microtitre plates were coated with OVA (100 µg/mL), incubated with plasma, and then with a biotinylated rat anti-mouse IgE (BD Bioscience, San Jose, CA, USA, 1:250 dilution). Finally, AttoPhos Substrate (Boehringer, Mannheim GmbH, Mannheim, Germany) was added after adding streptavidin-labelled alkaline phosphatase (BD Bioscience, 1:1000 dilution), and the fluorescence intensity was monitored by CytoFluor (Excitation: 485/20, Emission: 590/35, Applied Biosystems, Foster City, CA, USA). Serial dilutions of mouse IgE standard (BD Bioscience) were used for a standard curve and were measured in the same way as plasma samples except for using the plate coated with anti-mouse IgE (BD Bioscience). One unit was regarded as equivalent to 1 ng/mL mouse IgE from the standard curve.

Measurement of gene expression levels by real-time reverse transcriptase-polymerase chain reaction

Spleens from control-fed and PKGM-fed mice injected with either PBS or PAM extract six times were isolated one week after the last injection. The levels of ϵ germline transcription, IL-4, IFN- γ , GATA-3 and T bet expression in the spleen were evaluated by quantitative real-time RT-PCR (TaqMan assay) [20]. The assays were performed with ABI PRISM 7700 sequence detector (Applied Biosystems). Total RNA from each spleen was isolated with RNeasy Mini kit (QIAGEN K.K., Tokyo, Japan). Total RNA was subjected to the first-strand complementary DNA (cDNA) synthesis by a Superscript II first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA, USA). Primers and probe for ϵ germline transcription were synthesized by QIAGEN Custom Oligo (Tokyo, Japan), as detailed in a previous report [21]. Primers and TaqMan minor groove binder probes for IL-4, IFN- γ , GATA-3 and T bet genes were obtained from Applied Biosystems (TaqMan Gene Expression Assays). The measurement of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA levels, as an internal standard for calibration, was performed with TaqMan Rodent GAPDH Control Reagents (Applied Biosystems). The gene expressions were quantified by relating the PCR threshold cycle obtained from tissue samples to amplicon-specific standard curves. Amplification was

performed for one cycle of a sequential incubation at 50 °C for 2 min and 95 °C for 10 min, and subsequent 60 cycles of consecutive incubations were performed at 95 °C for 15 s and 60 °C for 1 min.

Statistics

Data were presented as means $\pm$ SEM. The significance of differences between treated groups, and that among different time points in each group were determined by two-way ANOVA, and one-way ANOVA, respectively. Post hoc analyses were performed by Bonferroni's post-test. The significance of differences in gene expression was determined by Student's *t*-test. A value of *P* < 0.05 was considered significant.

Results

The effect of pulverized Konjac glucomannan on the levels of plasma immunoglobulin in BALB/c mice

To investigate the role of PKGM in the modulation of humoral immune responses *in vivo*, we examined plasma concentrations of Igs in BALB/c mice which were fed either mice diet containing 5% PKGM (w/w) or an isocaloric diet, *ad libitum*, and injected with PAM extract or PBS every 2 weeks.

After 14-weeks feeding of either control or PKGM diet, there was no difference in the appearance and body weight among the groups (30.2 $\pm$ 0.5 g for PBS-injected mice fed with control diet, 29.5 $\pm$ 0.4 g for PBS-injected mice fed with PKGM diet, 30.7 $\pm$ 0.3 g for PAM extract-injected mice fed with control diet, 30.0 $\pm$ 0.3 g for PAM-extract-injected mice fed with KGM diet). In accordance with our previous report [17], continual injection of PAM extract induced the increase of plasma IgG and IgE levels during the period of the experiment in mice fed with control diet. The levels of plasma IgG and IgE in mice fed with PKGM were significantly lower than those in mice fed with control diet: IgG at 5 and 11 weeks (Fig. 1b) and IgE at 9 and 11 weeks (Fig. 1c). However, they were still significantly higher than those in mice injected with PBS. Neither PAM extract injection nor PKGM diet affected the plasma levels of IgM and IgA (Figs 1a and d). To further study the effect of PKGM on IgG, the levels of IgG subclass (IgG1, IgG2a, IgG2b, IgG3) in each group were measured by IgG subclass-specific ELISAs. The increase of plasma IgG1, which was enhanced to the greatest extent by the injections with PAM extract in accordance with our previous report, was totally abolished in mice fed with PKGM diet (Fig. 2a). The levels of IgG2b in mice injected with PAM extract were also suppressed to some extent in PKGM-fed mice injected with PAM extract at 11 weeks (Fig. 2c). Plasma IgG3 levels in PKGM-fed mice were significantly higher than in control-fed mice (Fig. 2d). Neither PAM extract nor PKGM diet affected the levels of plasma IgG2a (Fig. 2b).

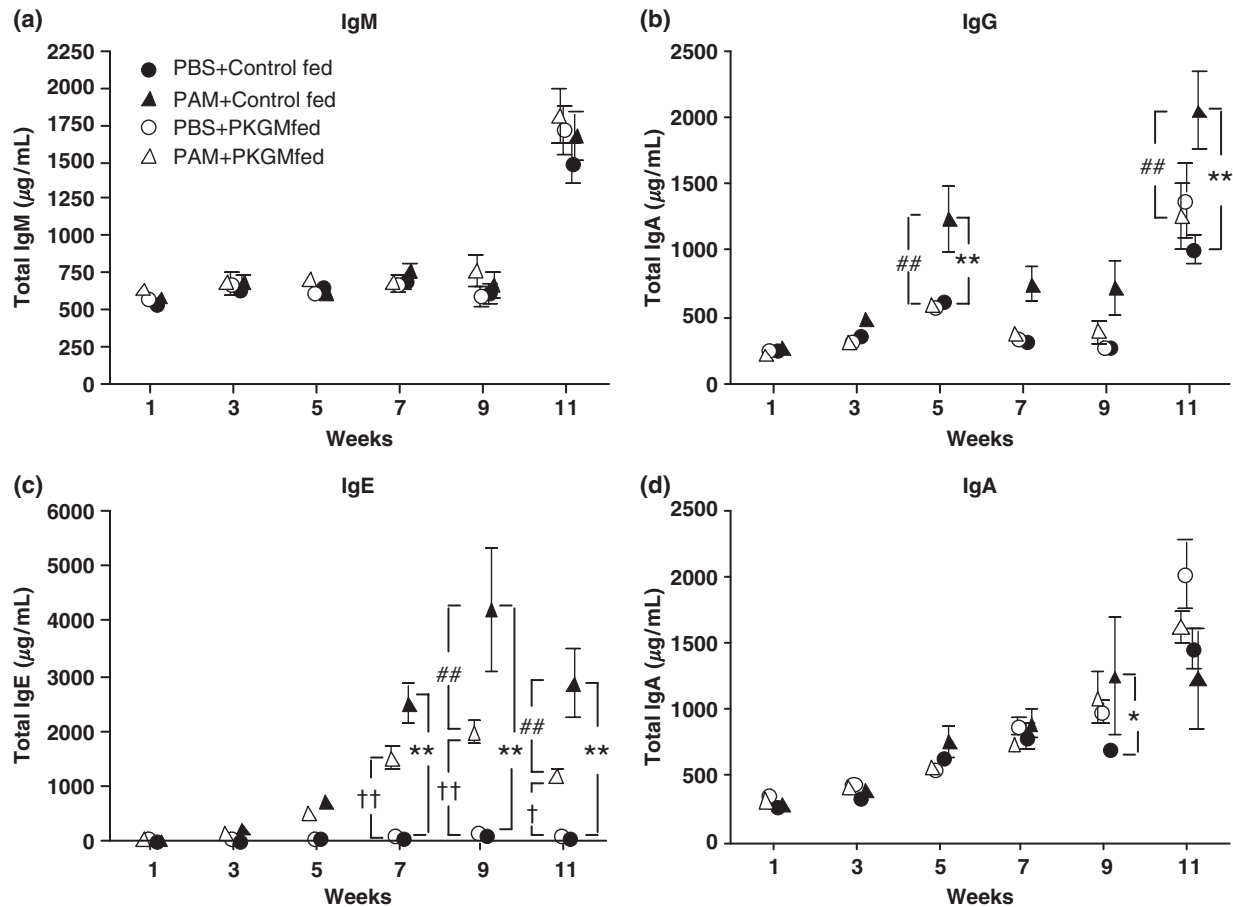


Fig. 1. Effect of pulverized Konjac glucomannan (PKGM) on the levels of plasma immunoglobulins (Igs) in BALB/c mice injected with PAM-extract. BALB/c mice, fed with control diet (closed circle: $n=9$; closed triangle: $n=8$) or PKGM-containing diet (5%, w/w; open circle: $n=10$; open triangle: $n=8$) were injected with either PAM extractor PBS every 2 weeks. Plasma Ig levels were measured by ELISA every one week after each injection. Injections of PAM extract increased the plasma levels of IgE (c), IgG (b) and IgA (d), but not IgM (a), in the mice fed with control diet (closed circle vs. closed triangle). However, plasma concentrations of IgE (c) and IgG (b) in PKGM-fed mice (open triangle) were significantly lower than those in control-fed mice (closed triangle) in spite of the injection of PAM extract. Data are means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ PBS+control-fed group vs. PAM+control-fed group. ## $P < 0.01$ PAM+control-fed group vs. PAM+PKGM-fed group. † $P < 0.05$, †† $P < 0.01$ PBS+PKGM-fed group vs. PAM+PKGM-fed group.

To confirm the effect of PKGM on antigen-specific IgE, we examined the plasma concentrations of total and OVA-specific IgE in BALB/c mice that were sensitized with OVA and fed with either PKGM or control diet. Both total IgE and OVA-specific IgE in PKGM-fed mice were significantly lower than those in control-fed mice (Table 1), indicating that PKGM inhibited antigen-induced immunoresponses.

The effect of pulverized Konjac glucomannan on immunoglobulin E class switching

To study the effect of PKGM on IgE class switching, the amount of ϵ germline transcript in spleen of each group of mice at 11 weeks after the start of injections with either PAM extract or PBS were determined by quantitative real-time RT-PCR. No obvious difference was observed in the size of the spleen among groups (data not shown).

The level of ϵ germline transcript in mice injected with PAM extract was attenuated in mice fed with PKGM diet

(Fig. 3). Administration of PKGM also inhibited ϵ germline transcription in mice injected with PBS. There was no difference of ϵ germline expression between mice injected with PAM extract and those with PBS, when mice were fed with PKGM diet. This result indicated that PKGM prevented not only the increase of plasma IgE, but also the class switching in B cells to produce IgE.

The effect of pulverized Konjac glucomannan on the expression of mRNA for interleukin-4, interferon- γ , GATA-3 and T bet in the spleen of BALB/c mice injected with PAM-extract

The expression levels of mRNA for IL-4, IFN- γ , GATA-3 and T bet in the spleen of mice injected with PAM extract at 11 weeks were determined by quantitative real-time RT-PCR. As expected, both the levels of mRNA for IL-4 and GATA-3 in mice injected with PAM extract were significantly suppressed when mice were fed with PKGM

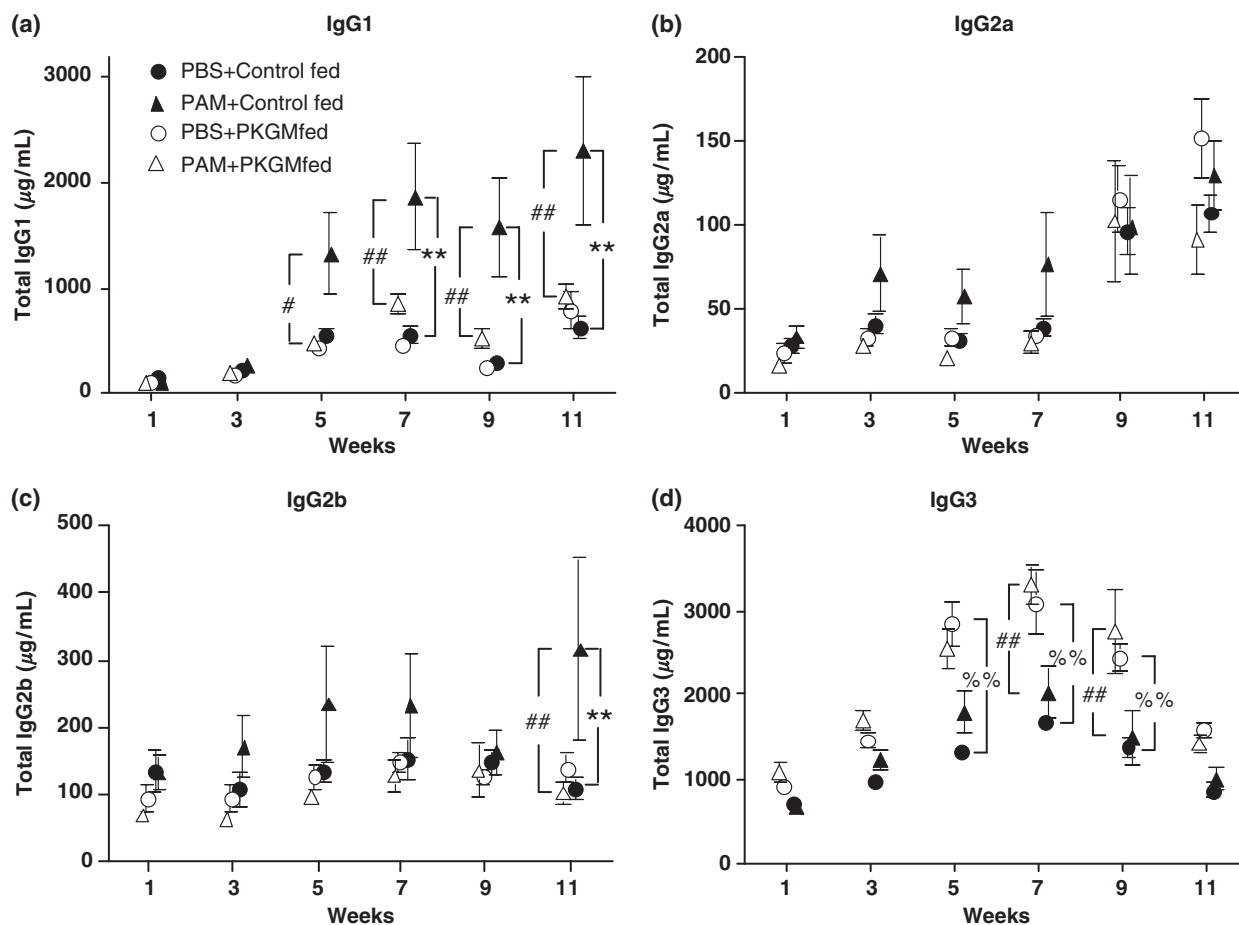


Fig. 2. Effect of pulverized Konjac glucomannan (PKGM) on the levels of plasma immunoglobulin (Ig)G subclasses in BALB/c mice injected with PAM-extract. The levels of plasma IgG subclasses of mice described in Fig. 1 were measured by ELISA, and are shown with symbols as described in Fig. 1. Among the IgG subclasses, the increase of IgG1 concentration was the most dramatically inhibited and the difference between that of control-fed mice and that of PKGM-fed mice became significant at 5 weeks and then after (a). The increases of plasma IgG2b in control-fed mice injected with PAM extract (closed triangle) were significantly inhibited in PKGM-fed mice (open triangle) at 11 weeks (c). The levels of IgG3 in mice given by PKGM administration were higher than those by control chaw administration either when injected with PAM extractor phosphate-buffered saline (PBS) (d). No significant effects of PKGM feeding on the plasma IgG2a were observed (b). ** $P < 0.01$ PBS+control-fed group vs. PAM+control-fed group. # $P < 0.05$, ## $P < 0.01$ PAM+control-fed group vs. PAM+PKGM-fed group. % $P < 0.01$ PBS+control-fed group vs. PBS+PKGM-fed group.

diet (Figs 4a and c). However, the expression of mRNA for IFN- γ either in mice injected with PBS or those with PAM extract was also suppressed when the mice were fed with PKGM diet (Fig. 4b). Moreover, the expression of mRNA for T bet in mice fed with PKGM diet was lower than that in mice fed with control diet, especially in the group injected with PBS (Fig. 4d). There was no difference of the levels of the four genes between mice injected with PAM extract and those with PBS, when mice were fed with PKGM diet. These results suggested that PKGM suppressed not only Th2 response but also Th1 response of the lymphocytes.

Discussion

In this study, we demonstrated that the administration of PKGM diet reduced the increase of plasma IgE, IgG1 and

IgG2b induced by the injection of the soluble fraction of syngeneic keratinocytes (PAM-extract) in BALB/c mice. Neither PAM extract nor PKGM diet affected the level of plasma IgM. Moreover, PKGM suppressed not only the plasma levels of total IgE, but also that of OVA-specific IgE in OVA-sensitized mice. The amount of ϵ germline transcript in the spleen of PKGM-fed mice was significantly lower than that in the spleen of control-diet-fed mice at 11 weeks after the injections. The increases of splenic gene expression for IL-4 and GATA-3 in mice induced by the injection with PAM extract were attenuated when mice were fed with PKGM diet. Interestingly, the expression of mRNA for IFN- γ in mice fed with PKGM diet was also suppressed, regardless of the injection with PAM extract or that with PBS.

The induction of isotype switching to a particular heavy chain in B cell correlates with the transcriptional

Table 1. Effect of PKGM on the plasma level of OVA-specific IgE in mice sensitized with OVA

Weeks	PBS+control fed	PBS+PKGM fed	OVA+control fed	OVA+PKGM fed
<i>Total IgE (ng/mL)</i>				
0	135 ± 27	75.4 ± 8.7	89.7 ± 11	80.1 ± 8.0
5	168 ± 66	108 ± 28	2089 ± 349**	725 ± 206 ^{††##}
<i>OVA-specific IgE (U)</i>				
0	36.3 ± 0.50	38.8 ± 1.1	36.2 ± 0.41	36.1 ± 0.54
5	136 ± 0.61	138 ± 0.61	2249 ± 307**	917 ± 38 ^{††##}

Plasma levels of OVA-specific and total IgE in mice injected with OVA or PBS were measured by ELISA. Values are presented as means ± SEM, $n = 6$ per group.

** $P < 0.01$ PBS+control-fed group vs OVA+control-fed group. ^{##} $P < 0.01$ OVA+control-fed group vs OVA+PKGM-fed group. ^{††} $P < 0.01$ PBS+PKGM-fed group vs. OVA+PKGM-fed group.

PKGM, pulverized Konjac glucomannan; OVA, ovalbumin; IgE, immunoglobulin E; PBS, phosphate-buffered saline.

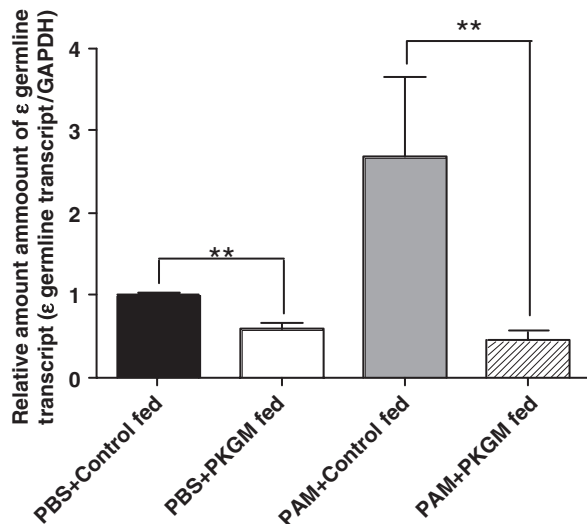


Fig. 3. Effect of pulverized Konjac glucomannan (PKGM) on the level of ϵ germline transcription in spleen of BALB/c mice injected with PAM-extract. Control- or PKGM-fed mice were injected with PAM extract or phosphate-buffered saline (PBS) as described in Fig. 1. Total RNA was isolated from spleens of the mice 1 week after the final injection at 11th weeks. Epsilon germline transcripts were measured with semi-quantitative real-time RT-PCR. The level of ϵ germline was normalized by GAPDH, and expressed as a ratio to that in control-fed mice injected with PBS (relative value, 1.0). The level of ϵ germline transcript in control-fed mice injected with PAM extract (shaded bar; $n = 7$) was dramatically suppressed in mice fed with PKGM (hatched bar; $n = 7$). The level of ϵ germline transcript in control-fed mice injected with PBS (closed bar; $n = 7$) was also suppressed with PKGM (open bar; $n = 9$). Data are means ± SEM. ** $P < 0.01$ by Student's t -test.

activation of the corresponding gene in germline configuration [22]. In the case of IgE, ϵ germline transcription is essential for IgE class switching [23, 24]. In this study, we have demonstrated that the ϵ germline transcription in the spleen was suppressed in mice fed with PKGM. Hence, the

IgE class switching in splenic B cells was inhibited by the administration of PKGM. Although we have not assessed the effect of PKGM on $\gamma 1$ germline transcription, PKGM likely inhibits the class switching to IgG1 as well, since switching to IgG1 and switching to IgE are potently stimulated by IL-4 [10, 25].

In general, Th2 cells drive B cells to differentiate and produce Igs by secretion of cytokines such as IL-4 and IL-13. In addition, our previous study has shown that the injections of PAM extract consistently increased the levels of serum IgE, IgG1 and IgG2b [17], suggesting the enhancement of Th2 cellular responses. Thus, the results of this study suggested that PKGM suppresses Th2 cellular responses. However, the studies of mRNA expression in the spleen for IFN- γ , IL-4, GATA-3 and T bet suggest rather bi-directional suppression of helper T cells. Recent molecular-biological studies regarding differentiation of helper T cells have revealed that GATA-3 is a potent transactivator of the gene of IL-4 and functions as a Th2 lineage determinant [26, 27]. On the other hand, the expression of T bet mRNA correlates with the production of IFN- γ by Th1 cells [12]. The present results demonstrated that oral administration of PKGM attenuated the splenic gene expressions not only for IL-4 and GATA-3, but also for IFN- γ in mice injected with PAM extract. Moreover, the expressions of both IFN- γ and T bet mRNA were suppressed in mice injected with PBS and fed with PKGM. Onishi et al. [14] have reported the decrease of IFN- γ in plasma of NC/Nga mice fed with PKGM. Taking into account the increase of IFN- γ in chronic stage of atopic dermatitis [28, 29], such bi-directional effects of PKGM may be more beneficial from the view point of the treatment of atopic diseases.

The effects of PKGM are not likely because of a non-specific immunosuppression, because of the following observations. First, PKGM suppressed neither the mRNA expression for IL-4 nor that for GATA-3 in mice injected with PBS. Second, the levels of all plasma Igs in mice fed with PKGM were not different from those in mice fed with control diet, when they were not injected with PAM extract. Third, there was no difference of plasma corticosteroid concentrations between mice fed with control diet and those fed with PKGM (data not shown). Thus, the effect of PKGM is likely a selective modification of helper T cell activities and consequent subsidence of B cell hyper-reactivity rather than a non-specific immunosuppression.

The essential structure in PKGM for immunomodulatory function and its direct target of PKGM is a matter of the discussion. Mice and humans do not have enzymes to digest and metabolize KGM by themselves. However, intestinal bacteria that can degrade KGM were found in humans [30], and may ferment KGM to mono/oligosaccharides in the intestine. Moreover, Onishi et al. [14] have shown that non-pulverized KGM did not inhibit

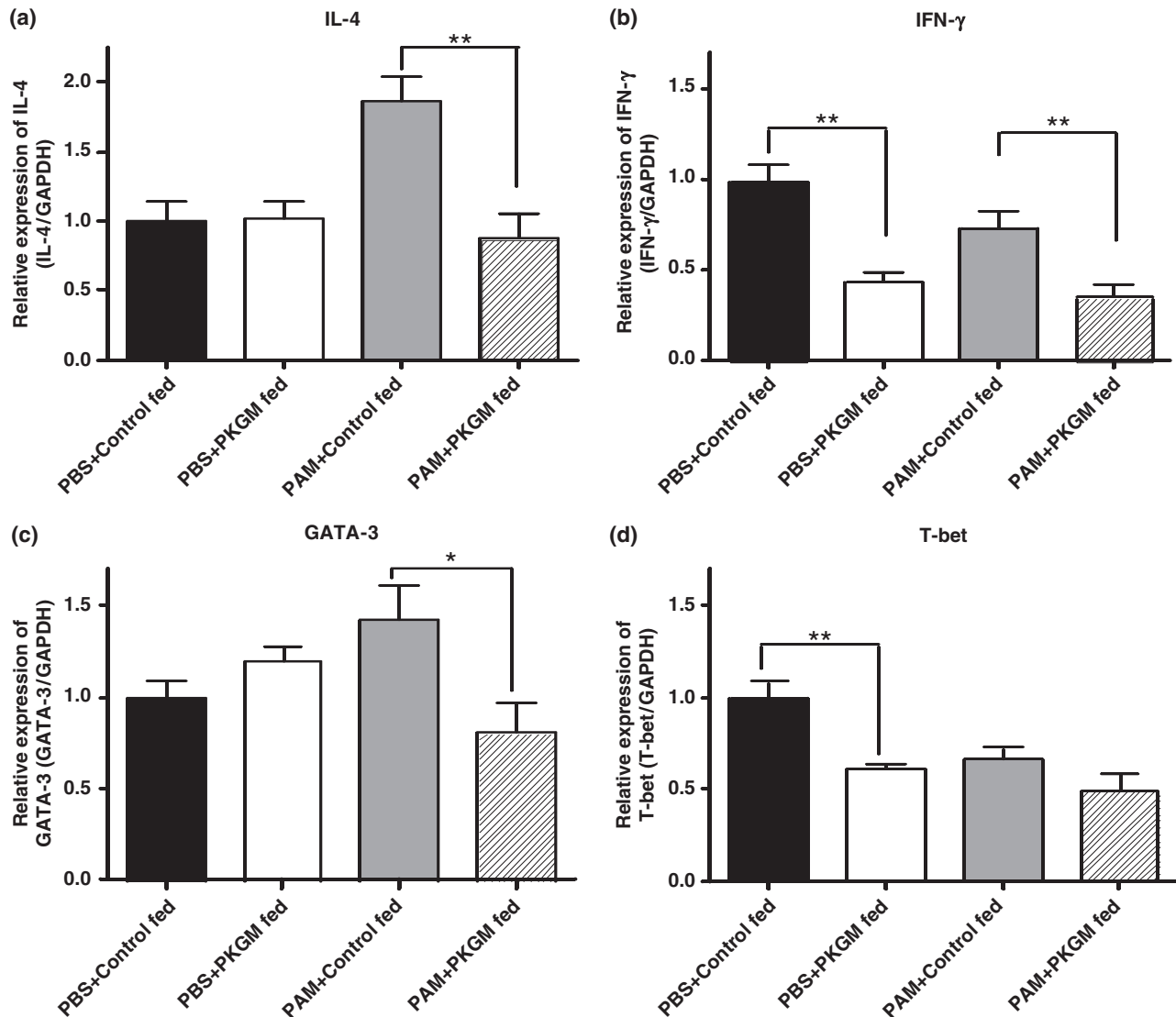


Fig. 4. Effect of pulverized Konjac glucomannan (PKGM) on the levels of mRNA for IL-4, IFN- γ , GATA-3 and T bet in spleen of BALB/c mice injected with PAM-extract. The levels of gene expression were measured as described for Fig. 3 and are shown with bars as described in Fig. 3. The levels of IL-4 (a), IFN- γ (b) and GATA-3 (c) mRNA in control-fed mice injected with PAM extract were significantly suppressed in PKGM-fed mice injected with PAM-extract. The levels of IFN- γ (b) and T bet (d) mRNA in control-fed mice injected with phosphate-buffered saline (PBS) were also significantly suppressed in PKGM-fed mice injected with PBS. Data are means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ by Student's *t*-test.

the increase of IgE, suggesting that the pulverization of KGM improved the efficiency of KGM degradation by intestinal bacteria, and enabled KGM to be absorbed by gut mucosae.

The differentiation of naive CD4⁺ T cells into Th1 and/or Th2 cells is induced by the antigen presentation by antigen-presenting cells (APC), which are essential for the initiation of GATA-3 and T bet gene expression [11, 31]. These processes of differentiation and Th1 and Th2 cell functions are negatively regulated by CD4⁺ CD25⁺ regulatory T cells [32]. Therefore, PKGM may influence (APC) or regulatory T cells to inhibit the commitment of splenic lymphocytes to Th1 or Th2. On the other hand, PKGM significantly decreased ϵ germline transcription without

suppressing a IL-4 and GATA-3 expression in control-diet-fed mice injected with PBS, suggesting direct effect of PKGM on B cells. Moreover, IgE production by B cells treated by IL-4 and anti-CD40 antibodies was weakly inhibited in the presence of solubilized KGM, *in vitro* (Suzuki et al., manuscript in preparation). Taken together, PKGM may act on both helper T cells and B cells to reduce the IgE production from B cells.

The level of only IgG3 was increased among those in plasma IgG subtypes by PKGM administration. Recent studies have revealed that IL-21 stimulates both IgG3 production and $\gamma 3$ germline transcription [33], and prevents antigen-induced IgE production and ϵ germline transcription [34, 35]. These effects are similar to those of

PKGM observed in this study. However, IL-21 also stimulates the production of IgG1 [33], which was suppressed by PKGM. Likewise, we could not attribute the whole effect of PKGM to any single cytokine, including IL-21. *In vitro* studies for PKGM action on IgE production should elucidate more precise mechanism.

In conclusion, we revealed that oral administration of PKGM suppressed the increase in plasma IgE (both total and antigen specific), IgG1, IgG2b and IgE class switching induced by the injection of syngeneic keratinocyte extracts. PKGM also attenuated the increase of mRNAs for IL-4 and GATA-3 and suppressed the expression of IFN- γ and T bet to some extent. Thus, PKGM may have a profound therapeutic potential against diseases mediated by the increase of IgE and/or the imbalance of helper T cell functions.

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