

Hydrolyzed Konjac Glucomannan Suppresses IgE Production in Mice B Cells

Hidenori Suzuki^a Souichi Oomizu^{a,d} Yuhki Yanase^a Nobukazu Onishi^c
Kazue Uchida^a Shoji Mihara^a Kazuhisa Ono^b Yoshikazu Kameyoshi^a
Michihiro Hide^a

^aDepartment of Dermatology, Division of Molecular Medical Science, Graduate School of Biomedical Sciences, and

^bDepartment of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter,

Hiroshima University, ^cDepartment of Research and Development, Nishikawa Rubber Co. Ltd., Hiroshima, and

^dDepartment of Immunology and Immunopathology, Faculty of Medicine, Kagawa University, Kagawa, Japan

Key Words

Plasma IgE · Konjac glucomannan · ϵ -Germline transcription · T helper type 1/T helper type 2

Abstract

Background: Oral administration of pulverized Konjac glucomannan (KGM) reduces increased plasma IgE and the amount of ϵ -germline transcript (ϵ GT) in the spleen, as well as preventing the development of dermatitis in mice. To elucidate the mechanism of action of pulverized KGM, we solubilized KGM and studied its effect on IgE in vitro and in vivo.

Methods: Solubilized KGM was prepared by acid hydrolysis, and we analyzed the effective molecular size for the suppression of IgE production and ϵ GT in vitro and the level of plasma IgE induced by immunization with ovalbumin in BALB/c mice. **Results:** The production of IgE and ϵ GT in splenic cells, but not purified B cells, was inhibited by hydrolyzed KGM (KGM hydrolyzed with 0.25 N HCl; H-KGM) at the optimal size of between 10 and 500 kDa. However, no effect was observed when H-KGM was substituted with unhydrolyzed KGM in vitro. IgE production from purified B cells cocultured with purified monocytes, but not with purified T cells, was inhibited by H-KGM. The release of IFN γ in cultures

of monocytes but in purified B cells with or without T cells was enhanced in the presence of H-KGM. Injection of mice with H-KGM also suppressed the production of plasma IgE and IgG1 but not IgG2a in vivo. **Conclusion:** KGM at an optimal size prevents germline class-switching and IgE production both in vitro and in vivo. H-KGM may be useful as a tool to study the mechanism of action of KGM and as a dietary supplement to prevent atopic diseases.

Copyright © 2009 S. Karger AG, Basel

Introduction

A high serum IgE concentration is one of the critical characteristics of atopic diseases, such as atopic dermatitis, asthma and allergic rhinitis [1–3]. Clinical observations have suggested a mild correlation between serum IgE levels and the extent and/or severity of disease, especially with cutaneous lesions [4, 5]. The antigen-specific IgE bound to Fc ϵ receptors expressed on mast cells induces the release of inflammatory mediators, such as histamine, leukotrienes, prostaglandins and cytokines, in response to the binding of multivalent antigens, which subsequently cross-links the Fc ϵ receptors. These factors

cause the immediate hypersensitivity reaction and the late-phase inflammatory reaction involving T cells and eosinophils [6]. Moreover, clinical trials of omalizumab, an anti-IgE monoclonal antibody that prevents the binding of IgE to Fcε receptors and reduces the amount of circulating IgE, have revealed its efficacy in atopic diseases, including asthma [7], allergic rhinitis [8] and atopic dermatitis [9]. Thus, the inhibition of IgE production is an important therapeutic target in atopic diseases.

IgE production is associated with a predominant T helper type 2 (Th2) cellular response [10]. Naive CD40⁺ T cells stimulated with allergen in the presence of IL-4 express GATA-3 and develop into Th2 cells that secrete cytokines such as IL-4 and IL-13 [11]. On the other hand, naive CD40⁺ T cells stimulated with allergen in the presence of IL-12 express T bet and preferentially differentiate into Th1 cells that secrete cytokines such as IL-2 and IFNγ [12]. Furthermore, both Th1 and Th2 effectors have the ability to downregulate each other.

Konjac glucomannan (KGM) is a highly viscous 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], which is abundant in the potato-like plant *Amorphophallus konjac*. Recently, Onishi et al. [14] showed that oral administration of pulverized KGM prevented the elevation of plasma IgE, as well as the development of eczematous lesions and scratching behavior, in NC/Nga mice, a model for atopic dermatitis. In our previous studies [15, 16], we also demonstrated that the administration of a pulverized KGM diet reduced the increase in plasma IgE, IgG1 and IgG2b, but not IgG2a, and the amount of ε-germline transcript (εGT) in the spleen induced by the injection of ovalbumin (OVA) and/or the injection of extract of syngeneic keratinocytes in BALB/c mice. However, the expression of mRNA for IFNγ was also suppressed in mice fed with the pulverized KGM diet. Therefore, the mechanism of action of pulverized KGM on IgE production involves the inhibition of IgE class-switching in splenic B cells, but not the augmentation of Th1 reactions, which suppress Th2 reactions. To elucidate the mechanism of action of pulverized KGM more precisely, in vitro studies with solubilized KGM are essential. However, because of its high viscosity, the maximum concentration of pulverized KGM in aqueous solutions such as culture media for IgE production is limited, and the solutions are not easy to handle. In this study, we solubilized KGM by acid hydrolysis and discovered an effective molecular size of KGM for the suppression of IgE production both in vitro and in vivo.

Materials and Methods

Reagents

The chemicals used were obtained from the following sources: bovine serum albumin and OVA from Sigma-Aldrich Japan (Tokyo, Japan); alum adjuvant from LSL Co. (Tokyo, Japan); penicillin G and streptomycin from Life Technologies (Grand Island, N.Y., USA); fetal calf serum from HyClone Laboratories (Logan, Utah, USA); mouse IL-4 from R&D Systems Inc. (Minneapolis, Minn., USA); rat antimouse CD40 antibody from AbD Serotec (Raleigh, N.C., USA), and dextran molecular size markers from GE Healthcare Bio-Sciences (Tokyo, Japan). Pulverized KGM was prepared by Shimizu Chemical (Mihara, Japan) as described before [14, 17] and provided as a gift.

Animals

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

Preparation and Characterization of Hydrolyzed KGM

Pulverized KGM was placed in distilled water in a bath at 75°C for 20 min, then partly hydrolyzed with various concentrations (0.125, 0.25, 0.5 and 1.0 N) of hydrochloric acid at 75°C for 60 min. The hydrolyzed KGM solution was neutralized to pH 7.2 with 4 N NaOH and 2 M phosphate buffer and applied to a Hi-Prep 26/60 Sephacryl S-300 HR column (2.6 × 60 cm; GE Healthcare Bio-Sciences). The column was equilibrated and eluted with distilled water. The eluates were collected as 4.5-ml fractions and sterilized by filtration with a 0.22-μm membrane (Millex-GV; Nippon Millipore Ltd., Tokyo, Japan). The amount of total sugar in each solution was determined by the phenol-sulfuric acid method [18]. The amount of KGM was expressed as that of total sugar.

Isolation and Culture of Splenic Lymphocytes, B Cells, T Cells and Monocytes

Splenic cells were isolated from 8-week-old BALB/c mice as previously described [16]. B cells, T cells and monocytes were isolated from splenic lymphocytes with the Auto-MACSTM column system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) using a B Cell Isolation kit, Pan T Cell Isolation Kit and CD11b MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) respectively. The cells were resuspended in Iscove's modified Dulbecco's minimum essential medium (Gibco BRL, Grand Island, N.Y., USA) with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin G, 100 μg/ml streptomycin, 100 ng/ml (1,000 U/ml) mouse IL-4 and 10 μg/ml antimouse CD40 antibody. Splenic cells and B cells were distributed into a 96-well tissue culture plate (Becton Dickinson, Franklin Lakes, N.J., USA) at 4 × 10⁵ cells/0.2 ml per well and maintained at 37°C in a 5% CO₂ atmosphere in the presence or absence of hydrolyzed KGM in the medium described above for 4 days to analyze the amount of εGT or 7 days to measure IgE released into culture supernatants. In

some experiments, 4×10^5 purified T cells/0.2 ml or 8×10^3 monocytes/0.2 ml were cultured with or without 4×10^5 purified B cells/0.2 ml followed by measurement of the concentration of IgE and the cytokines IFN γ , IL-10 and IL-12. Purified monocytes (1.6×10^4 cells/0.2 ml) were cultured alone in the medium for 7 days, followed by the measurement of IFN γ .

Measurement of IgE, IFN γ , IL-10 and IL-12

Mouse plasma was obtained as previously described [15]. The concentrations of IgE in plasma and/or the culture supernatants of B cells from splenic lymphocytes were determined by enzyme-linked immunosorbent assay (ELISA) kits purchased from Bethyl Laboratories (Montgomery, Tex., USA), following the manufacturers' instructions. IFN γ , IL-10 and IL-12 in the culture supernatants were measured by ELISA kits purchased from R&D Systems.

Measurement of Total and OVA-Specific IgE, IgG1 and IgG2a Titers in Plasma

Plasma OVA-specific IgE, IgG1 and IgG2a titers were analyzed by ELISA, as previously described [19, 20] with some modifications. Briefly, 96-well microtiter plates were coated with OVA solution (100 μ g/ml) at 4°C overnight. Once the wells were blocked, diluted plasma samples ($20\times$ for IgE, $25,000\times$ for IgG1, $1,000\times$ for IgG2a) were applied and incubated for at least 1 h at room temperature. Thereafter, the wells were washed and incubated with biotin-labeled rat antimouse IgE (1:250 dilution), IgG1 (1:500 dilution) or IgG2a (1:250 dilution) monoclonal antibody (BD Bioscience, San Jose, Calif., USA) and then horseradish peroxidase-streptavidin (BD Bioscience; 1:5,000 dilution). Finally, the enzyme reaction was performed using tetramethylbenzidine substrate solution (eBioscience, San Diego, Calif., USA). After the reaction was stopped by 1 M H $_3$ PO $_4$, absorbance at 492 nm was monitored by Tecan Ultra (Tecan Japan Co. Ltd., Tokyo, Japan).

Measurement of ϵ GT by Real-Time RT-PCR

The levels of ϵ GT in B cells, cultured as described above, were evaluated by quantitative real-time RT-PCR with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, Calif., USA) as previously described [15, 16]. The level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA was employed as an internal standard for calibration.

OVA Immunization

The mice were immunized intraperitoneally with OVA (2 μ g/mouse) and an alum adjuvant in 0.5 ml of solution at 2-week intervals from the age of 4 weeks. Intraperitoneal injections of hydrolyzed KGM (1 mg/mouse) or distilled water (control) were started 1 week before the initial injection of OVA and were given 3 times a week thereafter. Plasma samples were collected 0, 3 and 5 weeks after the initial injection of OVA and stored at -80°C for analysis.

Statistics

Data are presented as means $\pm$ SD. The significance of differences between 2 groups was determined by Student's *t* test. The differences between control and treated groups and among the various time points within each group were determined by ANOVA. Post hoc analyses were performed using Tukey-Kramer's post hoc test. A value of $p < 0.05$ was considered significant.

Results

The Effect of Hydrolyzed KGM on in vitro IgE Production by Splenic Lymphocytes

Hydrolyzates of KGM of various molecular sizes were prepared by acid hydrolysis, followed by gel chromatography. The chromatograms of the hydrolyzates are superimposed in figure 1a. The eluates indicated with bars corresponding to each carbohydrate peak were collected, concentrated and sterilized. IgE concentrations in culture supernatants were significantly suppressed when lymphocytes were cultured with 150 μ g/ml hydrolyzed KGM obtained by hydrolysis with 0.25–0.5 N HCl (fig. 1b). However, hydrolyzed KGM obtained by hydrolysis with 0 or 1 N HCl showed no or only a marginal effect on IgE production. Therefore, we used KGM hydrolyzed with 0.25 N HCl for the following experiments (H-KGM). H-KGM, at different concentrations of 0, 15, 30 and 150 μ g/ml, inhibited IgE production in a dose-dependent manner. In contrast, no effect was observed with unhydrolyzed KGM or mannose, which constitutes KGM together with glucose (fig. 2).

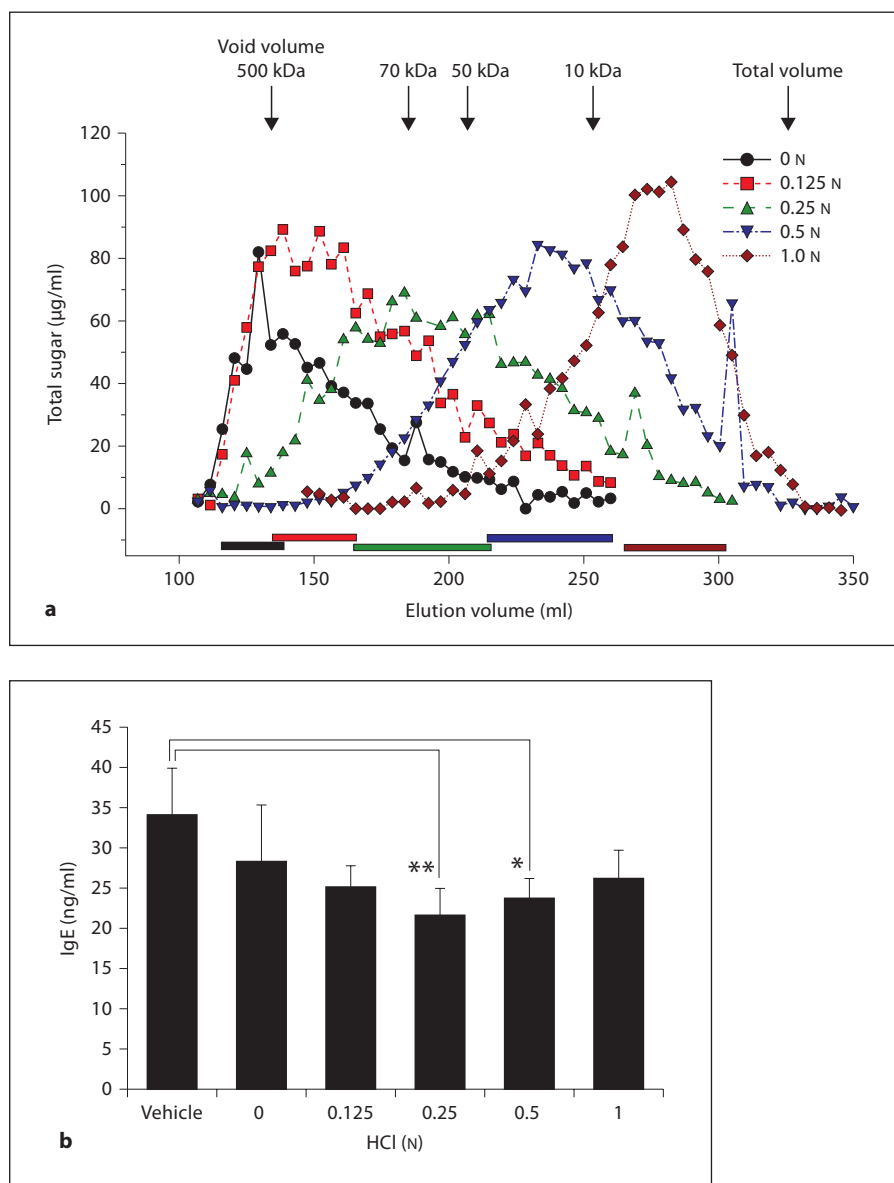
The Effect of H-KGM on IgE Production and ϵ GT in B Cells

When whole splenic lymphocytes of BALB/c mice were cultured and stimulated with IL-4 and anti-CD40 antibody, both IgE production and ϵ GT were inhibited in the presence of H-KGM (fig. 3a, b). However, neither IgE production nor ϵ GT was inhibited when purified B cells were stimulated in the presence of H-KGM (150 μ g/ml; fig. 3c, d), indicating that the effect of H-KGM on the inhibition of IgE production and ϵ GT in splenic lymphocytes is mediated by non-B cells. To elucidate the precise mechanism of action of H-KGM, purified B cells were cocultured with purified T cells or purified monocytes and then stimulated in the presence of H-KGM. IgE production was inhibited when purified B cells were cocultured with monocytes but not with T cells (fig. 4a).

Cytokine Production in Splenic Lymphocytes, B Cells, T Cells or Monocytes

Concentrations of IFN γ , IL-10 and IL-12 were also measured when splenic lymphocytes and purified B cells with or without T cells or monocytes were stimulated in the presence or absence of H-KGM. H-KGM showed no effect on IFN γ or IL-10 production from splenic lymphocytes (fig. 4b, d). IL-12 was not detectable in the culture supernatant of splenic lymphocytes (data not shown). However, H-KGM induced significant IFN γ production from purified B cells cultured with purified monocytes

Fig. 1. Fractionation of hydrolyzed KGM and its effect on IgE production by splenic lymphocytes. Pulverized KGM was hydrolyzed in various concentrations of HCl. **a** After neutralization to pH 7.2, the hydrolyzed KGM solution was applied to a Hi-Prep 26/60 Sephacryl S-300 HR column. The eluates (4.5 ml/fraction) indicated with bars corresponding to each carbohydrate peak were collected, concentrated and sterilized with a 0.22- μ m filter. Arrows indicate the estimated molecular size in the elution profiles calibrated with dextran molecular size markers. Similar results were obtained from 3 separate experiments. **b** Splenic lymphocytes were cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence of 150 μ g/ml hydrolyzed KGM for 7 days. IgE concentrations in culture supernatants were determined by ELISA. Hydrolyzed KGM prepared with HCl in a range of 0.25–0.5 N significantly inhibited IgE production. Data are means $\pm$ SD of 4 clusters of culture from a representative experiment. Similar results were obtained from 8 separate experiments with 3 different lots of hydrolyzed KGM preparations. * $p < 0.05$, ** $p < 0.01$ versus control group.



and from purified monocytes alone (fig. 4c), although the amount of IFN γ produced was quite a lot smaller than that from purified B cells with purified T cells (fig. 4b).

H-KGM Prevents the Increase in Plasma Concentrations of IgE and IgG1 but Not IgG2a Induced by OVA in BALB/c Mice

To study the effect of H-KGM on immunoglobulin production in vivo, we examined the levels of plasma IgE, IgG1 and IgG2a in BALB/c mice sensitized with OVA. H-KGM (1 mg/mouse) or distilled water (control) was injected intraperitoneally 3 times every week starting 1

week before the initiation of OVA immunization. As shown in figure 5b, c, the increase in plasma IgE was considerably suppressed in H-KGM-injected mice at 5 weeks. Levels of plasma IgG1, but not IgG2a, were also reduced at 5 weeks in mice injected with H-KGM (fig. 5c).

Discussion

We prepared hydrolyzed KGM and demonstrated that within a certain range of molecular size it exerts a suppressive effect on IgE production in vitro and in vivo.

Fig. 2. Dose-dependent effect of hydrolyzed KGM on IgE production by splenic lymphocytes. Splenic lymphocytes were cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence of 15, 30 and 150 μ g/ml H-KGM, 150 μ g/ml unhydrolyzed KGM or 150 μ g/ml mannose, a constituent of KGM, for 7 days. IgE concentrations in culture supernatants were determined by ELISA. H-KGM significantly inhibited IgE production in a dose-dependent manner. In contrast, no effect was observed with unhydrolyzed KGM or mannose. Data are means $\pm$ SD of 4 clusters of cultures from a representative experiment from 4 separate experiments. * $p < 0.05$ versus control group.

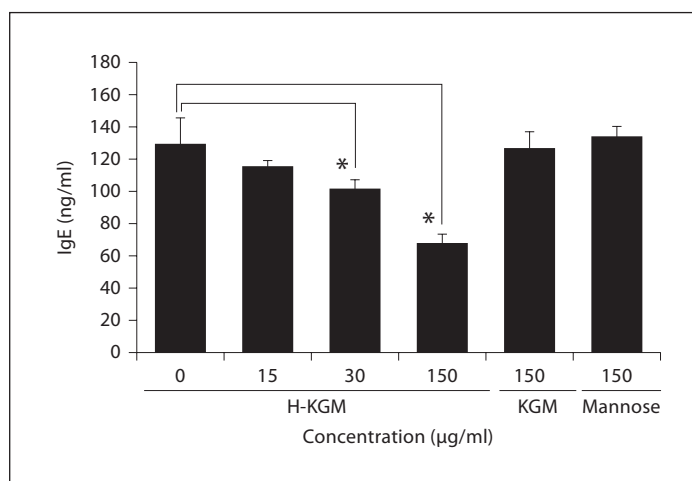
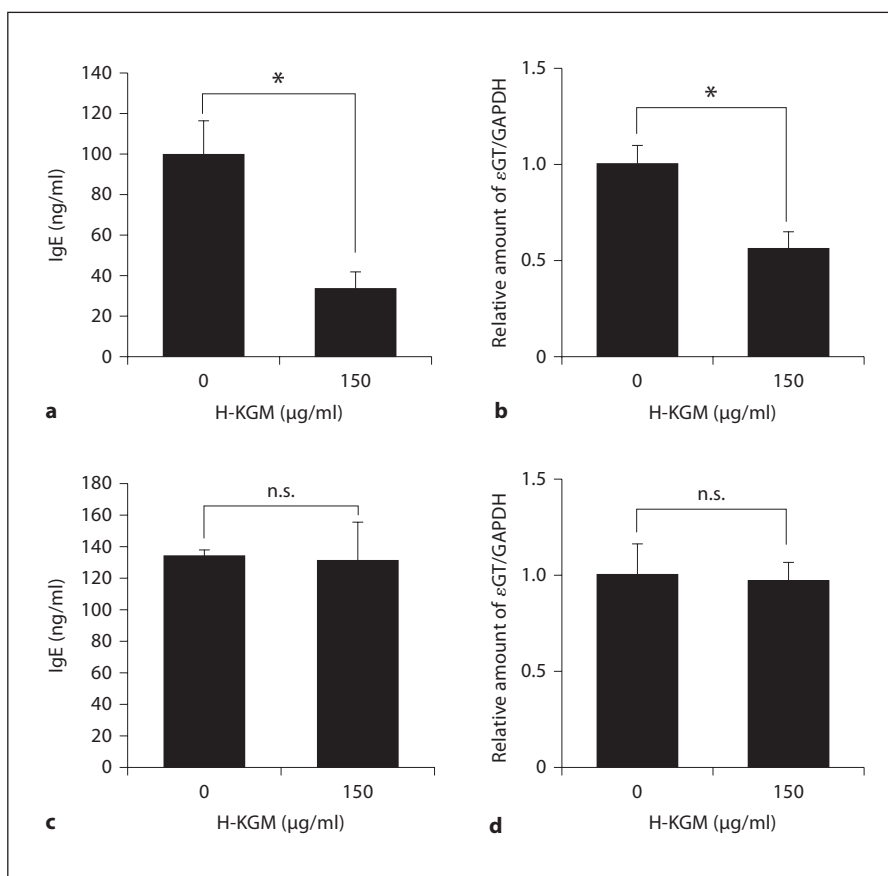


Fig. 3. The effect of H-KGM on IgE production and ϵ GT in whole splenic cells (**a**, **b**) and B cells (**c**, **d**). The amount of IgE and ϵ GT induced by IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence or absence of 150 μ g/ml H-KGM was evaluated in vitro. B cells were prepared from mouse spleen as described in Materials and Methods. Cells were cultured for 7 days to measure IgE production (**a**, **c**) and 4 days to evaluate ϵ GT (**b**, **d**). IgE concentrations in culture supernatants were determined by ELISA. The level of ϵ GT was determined by real-time RT-PCR and normalized to GAPDH expressed in the absence of H-KGM (relative value 1.0). IgE production and ϵ GT in whole splenic cells were significantly inhibited by H-KGM (**a**, **b**). However, no effect of H-KGM was observed when purified B cells were cultured and stimulated with IL-4 and anti-CD40 antibody in the presence of H-KGM (**c**, **d**). Data are means $\pm$ SD of 4 clusters of cultures from a representative experiment from 5–7 separate experiments. * $p < 0.05$ versus control group.



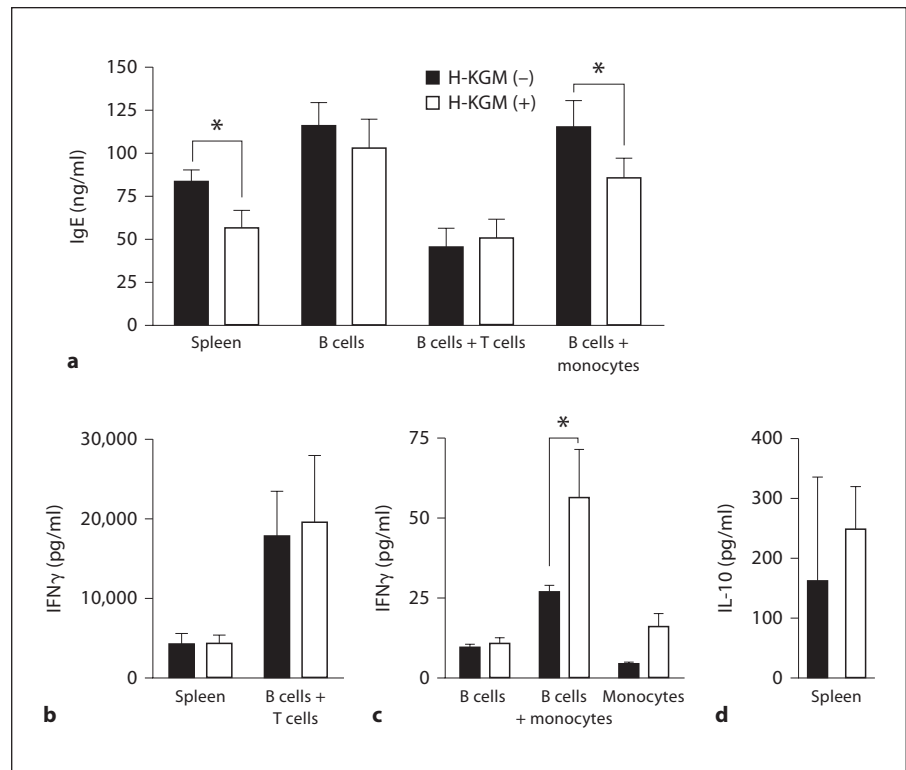


Fig. 4. The effect of H-KGM on IgE and cytokine production in B cells cocultured with T cells or monocytes. The amounts of IgE, IFN γ , IL-10 and IL-12 produced in the cultures after stimulation with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence or absence of 150 μ g/ml H-KGM were evaluated in vitro. B cells, T cells and monocytes were prepared from mouse spleen as described in Materials and Methods. **a** Splenic cells and B cells alone (4×10^5 cells/0.2 ml per well) and B cells (4×10^5 cells/0.2 ml) with T cells (4×10^5 cells/0.2 ml) or monocytes (8×10^3 cells/0.2 ml) were cultured for 7 days to determine the concentration of IgE. IgE production was inhibited in the presence of H-KGM when purified B cells were cocultured with purified mono-

cytes but not with purified T cells. However, IgE production in B cells cocultured with T cells was not inhibited in the presence of H-KGM, nor in B cells alone. **b–d** Cells were cultured under the same conditions as **a** to analyze the amounts of the cytokines IFN γ , IL-10 and IL-12. H-KGM had no significant effect on IFN γ or IL-10 production from splenic lymphocytes (data not shown). However, H-KGM induced significant IFN γ production from purified B cells cultured with purified monocytes and from purified monocytes alone. Data are means $\pm$ SD of 4 clusters of cultures from a representative experiment from 4 separate experiments. * $p < 0.05$ versus control group.

KGM has been previously described to play an important role in the prevention of diabetes and hypercholesterolemia [21]. The mechanism of such activities has been suggested to depend upon the viscosity of KGM. However, Onishi et al. [14] recently reported that oral intake of pulverized KGM restrained the progression of dermatitis, itching behavior and plasma IgE elevation in NC/Nga mice, whereas no such effect was observed with nonpulverized high-viscous KGM. These results implied that the effect of KGM on the increase in plasma IgE and the development of dermatitis depends upon the particle size rather than the viscosity of KGM. Moreover, we have demonstrated that KGM that was hydrolyzed with 0.25 N HCl

(H-KGM), i.e. completely water-soluble and nonviscous KGM, inhibits the production of IgE and IgE class-switching in vitro, whereas pulverized KGM, which is still highly viscous in culture medium, does not show such activities in vitro. Peristalsis with gastric acid in vivo should be insufficient to digest KGM even in a pulverized form, since the gastric temperature (37°C) is much lower than that employed for in vitro digestion in this study (75°C). However, it is conceivable that pulverized KGM is further processed in the gastrointestinal tract into a smaller size, thereby leading to its immunomodulatory activities. Although neither humans nor mice have enzymes that digest glucomannan, bacteria in the intestinal tract may de-

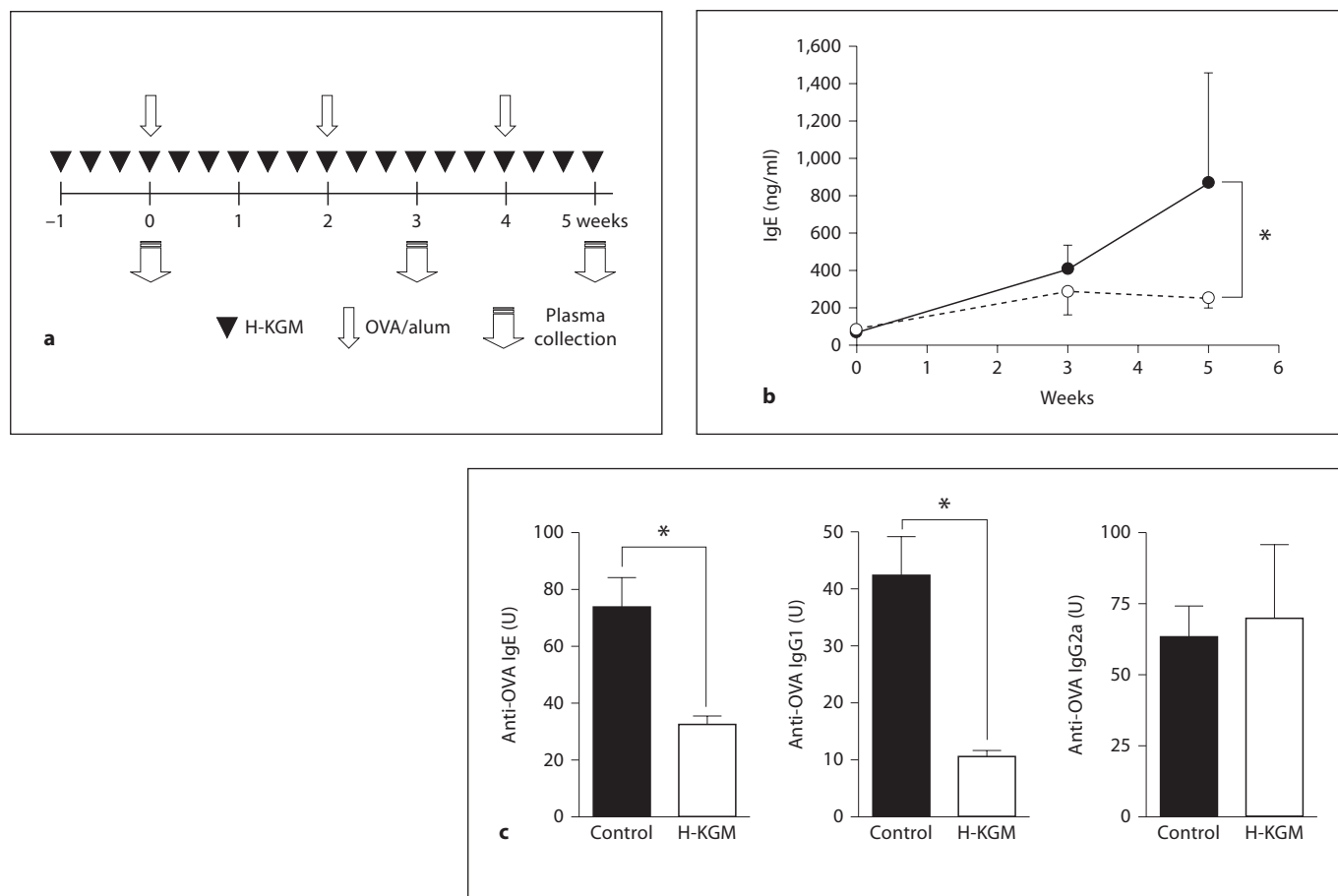


Fig. 5. H-KGM prevents the increase in plasma IgE and IgG concentration induced by OVA in BALB/c mice. **a** Mice were immunized with OVA (2 µg/mouse) and an alum adjuvant from the age of 5 weeks at 2-week intervals and injected with either H-KGM (1 mg/mouse) or distilled water (control) 3 times a week starting 1 week before the initial injection of OVA. Blood samples were collected by retro-orbital bleeding, and levels of total IgE and OVA-specific IgE, IgG1 and IgG2a were determined by ELISA.

b The levels of total IgE in H-KGM-injected mice (○) were significantly lower than those in mice injected with distilled water (control; ●). **c** Levels of OVA-specific IgE and IgG1, but not IgG2a, in H-KGM-injected mice were lower than those in control mice. Data are means $\pm$ SD of 5–7 mice from a representative experiment. Similar results were obtained from 5 separate experiments. * $p < 0.05$ versus control.

grade KGM [22]. Pulverization of KGM may help KGM to be digested into the optimal size for antiallergic effects in vivo. Neither unhydrolyzed KGM, hydrolyzed KGM smaller than 10 kDa, hydrolyzed KGM larger than 500 kDa nor their constituent, mannose, exhibited even marginal activity on the production of IgE in vitro. Moreover, H-KGM also inhibited the increase in both total and OVA-specific IgE and IgG1, but not IgG2a, induced by OVA sensitization in BALB/c mice. It would be interesting to study whether H-KGM shares molecular characteristics with natural KGM, which is absorbed by the intestinal tract presumably after digestion by intestinal bacteria.

The direct target of KGM is a subject for further studies. We previously reported that oral administration of KGM inhibits IgE class-switching in B cells of the BALB/c mouse in vivo. In this study, we have demonstrated that both IgE production and class-switching were inhibited when spleen cells, but not purified B cells, were stimulated in the presence of H-KGM in vitro (fig. 3). We have also demonstrated that H-KGM inhibited the production of IgE when purified B cells were cocultured with purified monocytes but not with purified T cells (fig. 4). H-KGM has no effect on the production of IL-10 or IL-12 from splenic cells. However, H-KGM induced small but

substantial enhancement of IFN γ production in purified B cells cocultured with purified monocytes (fig. 4c) and in purified monocytes alone. These results suggest that H-KGM may first act on monocytes rather than B cells to suppress IgE production. However, the amount of IFN γ produced by monocytes was much smaller than that produced by B cells cocultured with T cells or whole splenic cells. Therefore, the mechanism of action of H-KGM to suppress IgE production may be different from the modulation of the Th1/Th2 balance by IFN γ itself. We have confirmed that there is no effect of H-KGM on living cell proliferation of splenocytes, using a CellTiter 96[®] Aqueous One Solution Cell Proliferation Assay (Promega, Madison, Wisc., USA; data not shown). Further study would be necessary to fully elucidate the mechanism by which H-KGM acts on monocytes and suppresses IgE production.

We have recently demonstrated that fucoidan, a polysaccharide isolated from sea weed, inhibits IgE production by both spleen cells and B cells purified from spleen in vitro [17]. This effect is mediated by the suppression of NF κ B p52, which is activated by the ligation of CD40 on B cells. Since H-KGM did not show a direct effect on B cells purified from a BALB/c mouse, the mechanism of action of H-KGM may be different from that of fucoidan. The preparation of H-KGM should be useful to reveal the mechanism by which KGM inhibits the increase in plasma IgE, class-switching and the development of atopic dermatitis.

Another advantage of H-KGM is its hydrosolubility. To prevent the increase in IgE by B cell class-switching,

the administration of KGM before full exposure to antigens, presumably in childhood, should be most beneficial. However, pulverized KGM is rather viscous in water and difficult to swallow. On the other hand, H-KGM is easy to drink and can even be administered by injection. The optimal methods for H-KGM administration, involving timing, amounts and route, in order to achieve its maximal effect in preventing an IgE increase and other atopic symptoms in vivo, such as dermatitis, is the next subject to be studied for its eventual use for human atopic diseases. For such purposes, further studies of intrabody distribution and measurements of plasma H-KGM concentrations will be essential. The detection of H-KGM with an enzyme assay is under way in our laboratory.

In summary, we prepared solubilized KGM that preserves its inhibitory activity on IgE production via the suppression of IgE class-switching in vitro and in vivo. Further studies of the mechanism of action of KGM and methods for administration may allow us to use H-KGM to prevent IgE-mediated diseases.

Acknowledgements

We thank Shimizu Chemical Co. for kindly supplying pulverized KGM. We wish to thank the Analysis Center of Life Science, Hiroshima University, for the use of their facilities and Dr. Faiz Kermani for reviewing the manuscript. This work was supported by the Cooperative Link of Unique Science and Technology for Economy Revitalization of the Hiroshima Prefectural Institute of Industrial Science and Technology, Japan.

References

- 1 Jones HE, Inouye JC, McGerity JL, Lewis CW: Atopic disease and serum immunoglobulin-E. *Br J Dermatol* 1975;92:17–25.
- 2 Casolaro V, Georas SN, Song Z, Ono SJ: Biology and genetics of atopic disease. *Curr Opin Immunol* 1996;8:796–803.
- 3 Gurevitch AW, Heiner DC, Reisner RM: IgE in atopic dermatitis and other common dermatoses. *Arch Dermatol* 1973;107:712–715.
- 4 Stone SP, Muller SA, Gleich GJ: IgE levels in atopic dermatitis. *Arch Dermatol* 1973;108:806–811.
- 5 Clendenning WE, Clack WE, Ogawa M, Ishizaka K: Serum IgE studies in atopic dermatitis. *J Invest Dermatol* 1973;61:233–236.
- 6 Werfel S, Massey W, Lichtenstein LM, Bochner BS: Preferential recruitment of activated, memory T lymphocytes into skin chamber fluids during human cutaneous late-phase allergic reactions. *J Allergy Clin Immunol* 1995;96:57–65.
- 7 Corren J, Casale TB, Lanier B, Buhl R, Holgate S, Jimenez P: Safety and tolerability of omalizumab. *Clin Exp Allergy* 2009;39:788–797.
- 8 Verbruggen K, Van Cauwenberge P, Bachert C: Anti-IgE for the treatment of allergic rhinitis – and eventually nasal polyps? *Int Arch Allergy Immunol* 2009;148:87–98.
- 9 Belloni B, Ziai M, Lim A, Lemerrier B, Sbornik M, Weidinger S, Andres C, Schnopp C, Ring J, Hein R, Ollert M, Mempel M: Low-dose anti-IgE therapy in patients with atopic eczema with high serum IgE levels. *J Allergy Clin Immunol* 2007;120:1223–1225.
- 10 Bacharier LB, Geha RS: Molecular mechanisms of IgE regulation. *J Allergy Clin Immunol* 2000;105:S547–S558.
- 11 Grogan JL, Locksley RM: T helper cell differentiation: on again, off again. *Curr Opin Immunol* 2002;14:366–372.
- 12 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH: A novel transcription factor, T bet, directs Th1 lineage commitment. *Cell* 2000;100:655–669.
- 13 Kato K, Matsuda K: Studies on the chemical structure of konjac mannan. I. Isolation and characterization of oligosaccharides from the partial acid hydrolyzate of the mannan. *Agric Biol Chem* 1969;33:446–453.

- 14 Onishi N, Kawamoto S, Nishimura M, Nakanao T, Aki T, Shigeta S, Shimizu H, Hashimoto K, Ono K: A new immunomodulatory function of low-viscous konjac glucomannan with a small particle size: its oral intake suppresses spontaneously occurring dermatitis in NC/Nga mice. *Int Arch Allergy Immunol* 2005;136:258–265.
- 15 Oomizu S, Onishi N, Suzuki H, Ueda K, Mochizuki M, Morimoto K, Kawamoto S, Ono K, Kameyoshi Y, Hide M: 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. *Clin Exp Allergy* 2006;36:102–110.
- 16 Morimoto K, Nabeshima Y, Mochizuki M, Oomizu S, Yamamoto S, Hide M: The extract of syngeneic keratinocytes enhances IgE production from BALB/c mouse splenic lymphocytes in vitro. *Arch Dermatol Res* 2006;297:358–366.
- 17 Oomizu S, Yanase Y, Suzuki H, Kameyoshi Y, Hide M: Fucoidan prevents C ϵ germline transcription and NF κ B p52 translocation for IgE production in B cells. *Biochem Biophys Res Commun* 2006;350:501–507.
- 18 Cuesta G, Suarez N, Bessio MI, Ferreira F, Massaldi H: Quantitative determination of pneumococcal capsular polysaccharide serotype 14 using a modification of phenol-sulfuric acid method. *J Microbiol Methods* 2003;52:69–73.
- 19 Onishi N, Kawamoto S, Suzuki H, Santo H, Aki T, Shigeta S, Hashimoto K, Hide M, Ono K: Dietary pulverized konjac glucomannan suppresses scratching behavior and skin inflammatory immune responses in NC/Nga mice. *Int Arch Allergy Immunol* 2007;144:95–104.
- 20 Onishi N, Kawamoto S, Ueda K, Yamanaka Y, Katayama A, Suzuki H, Aki T, Hashimoto K, Hide M, Ono K: Dietary pulverized konjac glucomannan prevents the development of allergic rhinitis-like symptoms and IgE response in mice. *Biosci Biotechnol Biochem* 2007;71:2551–2556.
- 21 Chen HL, Sheu WH, Tai TS, Liaw YP, Chen YC: Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects – a randomized double-blind trial. *J Am Coll Nutr* 2003;22:36–42.
- 22 Matsuura Y: Degradation of konjac glucomannan by enzymes in human feces and formation of short-chain fatty acids by intestinal anaerobic bacteria. *J Nutr Sci Vitaminol (Tokyo)* 1998;44:423–436.