

Dietary Pulverized Konjac Glucomannan Suppresses Scratching Behavior and Skin Inflammatory Immune Responses in NC/Nga Mice

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Key Words

Atopic dermatitis • NC/Nga mice • Scratching behavior • Mast cells • Substance P • Skin immune response • Konjac glucomannan

Abstract

Background: Feeding with pulverized konjac glucomannan (PKGM) suppresses the development of eczema and hyper-IgE production in NC/Nga mice, a model of atopic dermatitis. This study aimed to examine the effects of PKGM on scratching behavior and skin inflammatory immune responses in NC/Nga mice. **Methods:** Four-week-old NC/Nga mice were maintained for 8 or 9 weeks on diet containing PKGM. Scratching behavior and clinical symptoms were evaluated every 2 weeks. Effects of PKGM on cutaneous inflammation were evaluated by histopathological analysis. Local expression levels of substance P and proinflammatory cytokines were measured by ELISA. **Results:** An increase in scratching behavior was evident from 6 weeks of age in control mice, but this symptom was dose-dependently inhibited in PKGM-fed mice. Continuous PKGM feeding then significantly inhibited eczematous skin lesions including hyperkeratosis, dermal mastocytosis and eosinophilia. Concomitantly, cutaneous overproductions of substance P, IL-10, IL-4, and TNF- α

were all suppressed in PKGM-fed mice. **Conclusions:** PKGM feeding markedly suppressed development of scratching behavior, substance P expression with mastocytosis, and skin inflammatory immune responses in NC/Nga mice.

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Introduction

Konjac, a Japanese traditional food, is manufactured with konjac powder obtained from tubers of *Amorphophallus konjac*, and has been eaten as a relish with a firm texture for over 1,000 years in Japan. The main ingredient of konjac powder is konjac glucomannan (KGM), a highly viscous polysaccharide composed of glucose and mannose residues at a molar ratio of 2:3 with β 1-4 linkages. KGM with a higher viscosity generally exhibits more beneficial functions against diabetes and hyperlipidemia [1]. In some studies, immunomodulatory functions of highly viscous KGM were analyzed. Oral administration of KGM modulates serum IgA and IgG levels as well as immunoglobulin production by lymphocytes in rats [2, 3]. However, evidence for the ability of KGM to prevent immune disorders such as allergies has scarcely been provided so far. Moreover, physiological functions of low-

viscous KGM have not been the subject of research studies, although the high solubility of KGM is suitable for application to various food industries.

Recently, we discovered a novel and beneficial immunomodulatory function of processed KGM, namely that only low-viscous KGM with small particle size prevented the development of skin inflammation and hyper-IgE production in NC/Nga mice [4, 5], a well-known animal model of atopic dermatitis (AD) [6]. The eczema-preventive effect of pulverized KGM (PKGM) was accompanied by systemic downregulation of IFN- γ , a positive regulatory cytokine of atopic skin inflammation [7–9]. We also reported that PKGM markedly inhibits the increase in plasma IgE and IgG1 levels induced by the injection of syngeneic keratinocyte extracts in BALB/c mice [10]. However, the precise mechanisms underlying anti-inflammatory actions of PKGM are still largely unknown, and effects of PKGM on cutaneous immune response have yet to be addressed.

A major aggravating factor of chronic AD is itch-induced scratching, possibly caused by an impaired skin barrier function and/or invasion by allergens into the skin [9]. The scratch-induced mechanical injury not only allows further sensitization to allergens [11, 12], but also induces local upregulation of IL-10, a critical cytokine for Th2 skin response and eosinophil infiltration [12]. Also in NC/Nga mice, frequency of scratching behavior correlates well with severity of dermatitis [13, 14], and prevention of scratching is indeed effective in AD suppression [15]. It is also known that substance P plays an important role in both scratching behavior and skin inflammation in NC/Nga mice [16, 17]. Another hallmark of AD-like phenotype is cutaneous Th2 inflammation, which is often accompanied by simultaneous induction of the Th1 response at the chronic phase [6–9, 18]. However, the role of Th2 cells in the pathogenesis of NC/Nga mice is controversial, since those deficient in STAT6 still show AD-like skin lesions accompanied by local mastocytosis, eosinophilia and high IFN- γ expression in the skin [19]. This raises the possibility that Th2 cell-independent mechanisms are also critical for the progression of chronic inflammatory response in their skin. Collectively, these observations suggest that the mechanical injury of skin by itch-related scratching may be a crucial cue to the pathogenesis of AD and skin inflammatory immune responses in NC/Nga mice. Feeding with PKGM might also influence these inflammatory/immune pathways to prevent AD-like skin lesions and hyper-IgE syndrome.

In the present study, we examined effects of PKGM on scratching behavior, AD-like skin inflammation, and

skin immune responses in NC/Nga mice. Our data indicated that dietary PKGM indeed inhibited development of scratching behavior in a dose-dependent manner, and the inhibitory kinetics roughly correlated with subsequent eczema prevention. We also showed that PKGM feeding profoundly suppressed cutaneous mastocytosis, eosinophilia, and levels of substance P and proinflammatory cytokines in NC/Nga mice.

Materials and Methods

Pulverized Konjac Glucomannan

PKGM was prepared by Shimizu Chemical Corporation (Hiroshima, Japan) as previously described [5]. Mean particle size of PKGM was estimated to be approximately 75–100 μm , and peak viscosity of 1% solution was around 35,000 mPa·s.

Animals and Diets

Four-week-old NC/Nga male mice (conventional grade) were purchased from Japan SLC (Shizuoka, Japan). They were maintained under conventional conditions for 8 or 9 weeks on one of the following diets ad libitum: control diet (MF diet; Oriental Yeast, Tokyo, Japan) or PKGM diet [MF diet containing 0.2, 1 or 5% (w/w) PKGM powder]. Low doses of PKGM (0.2 and 1%) were only used to study dose-dependent effects. All animals were housed in a room kept at $22 \pm 2^\circ\text{C}$ with a 12-hour light/12-hour dark cycle. Twelve- and 13-week-old male NC/Nga mice kept under a specific pathogen-free (SPF) environment (Japan SLC) were used as negative controls.

Evaluation of Skin Involvement and Itching Behavior

Clinical skin features and severity of dermatitis in NC/Nga mice were scored once every 2 weeks by an observer. AD-like symptoms were evaluated by the frequency of scratching, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness on the face, ears, neck and body. Each of these 5 symptoms was graded on a scale from 0 (none) to 3 (severe). Severity of dermatitis was expressed as the sum of respective scores obtained for the 5 symptoms.

After scoring skin conditions, scratching behaviors of 5 NC/Nga mice in a cage were recorded for 30 min under unmanned conditions using a video camera. The videotape was played back to count scratchings of the face, ears, neck and body with the hind paws. Since NC/Nga mice showed various itching behaviors and short to long durations of scratching, a series of such behaviors was counted as 1 scratching incident. Numbers of scratching in NC/Nga mice per 20 min were counted.

Thickness of both ears in NC/Nga mice was measured at 13 weeks of age using an ASKER SDA-12 thickness gauge (Kobunshi Keiki Co. Ltd., Kyoto, Japan).

Histological Study

Whole ears of 13-week-old mice were fixed in 10% formalin solution, and embedded in paraffin. Deparaffinized sections (5–6 μm) were stained with hematoxylin and eosin. Numbers of mononuclear cells and eosinophils were counted in 5 fields at $\times 400$ magnification (0.0091 mm^2), in sections from 3 different

mice. To count mast cells, sections were also stained with toluidine blue. Numbers of mast cells were counted in 3–5 randomly selected serial fields at $\times 200$ magnification (0.36 mm^2), in sections from 3 different mice. Histological inspections and observations of video for scratching behavior/clinical skin symptoms were performed under blinded conditions.

Measurement of Total and Antigen-Specific Immunoglobulin Titers in Plasma

Blood samples were collected at 12 weeks of age. Total plasma IgG1, IgG2a and IgE levels were measured by sandwich ELISA using 2 kinds of anti-mouse IgG1, IgG2a and IgE monoclonal antibodies, as well as mouse IgG1, IgG2a and IgE standards (BD Pharmingen, San Diego, Calif., USA) according to the manufacturer's instructions.

Plasma-specific IgG1, IgG2a and IgE titers to mite (*Dermatophagoides farinae*) extract (LSL Co. Ltd., Tokyo, Japan) were analyzed by ELISA. An ELISA plate was coated at 4°C overnight with *D. farinae* extract ($5 \mu\text{g}/\text{well}$) in PBS. Once the wells were blocked, murine serum diluted with a blocking solution ($500\times$ for IgG1, $50\times$ for IgG2a, $5\times$ for IgE) was applied, and incubated for at least 2 h at room temperature. Thereafter, wells were washed, and incubated successively with biotin-labeled rat anti-mouse IgG1, IgG2a or IgE (BD Pharmingen, 1:250 dilution) and AP-streptavidin (BD Pharmingen, 1:1,000 dilution). Finally, the enzyme reaction was performed using the Attaphos[®] fluorescent substrate system (Promega Corporation, Madison, Mich., USA).

Analysis of Cytokines, Chemokine, Substance P and IgE Titers in the Dorsal Skin of NC/Nga Mice

Levels of cytokines, chemokine (TARC), substance P and total IgE in skin were analyzed as previously described [20], with some modifications. At 13 weeks of age, hairless dorsal skin was resected from NC/Nga mice that had been fed with control diet or PKGM, or from NC/Nga mice kept under SPF conditions. The skin was cut into 0.2-gram specimens (about $15 \times 15 \text{ mm}^2$ pieces). Once skin samples were carefully rinsed in PBS, they were homogenized with a Polytron homogenizer (PT2100; Kinematica, Switzerland) in 2 ml of mammalian tissue lysis/extraction reagent (CelLytic[™] MT; Sigma, Saint Louis, Mo., USA) containing a protease inhibitor cocktail (Sigma). The homogenate was centrifuged at $500 g$ for 10 min, and the supernatant was collected. IL-2, IL-4, IL-10 and IFN- γ levels in the supernatant were measured by a sandwich ELISA using a Mouse Th1/Th2 ELISA Ready-SET Go! (eBioscience, San Diego, Calif., USA). TNF- α levels in the supernatant were detected by a Mouse TNF- α ELISA Ready-SET Go! (eBioscience). TARC levels in the supernatant were measured by a sandwich ELISA using the mouse TARC DuoSet Kit (R&D Systems Inc., Minneapolis, Minn., USA) according to the manufacturer's instructions. Substance P levels in the supernatant were measured by a competitive ELISA using a substance P ELISA kit from R&D Systems. Total IgE titers in the supernatant were evaluated by the same methods as described above for plasma IgE titers.

Statistics

Statistical analysis of skin severity scores between groups was performed using the Mann-Whitney U test, and analysis of other parameters between groups was performed using Student's t test. $p < 0.05$ was accepted as significant.

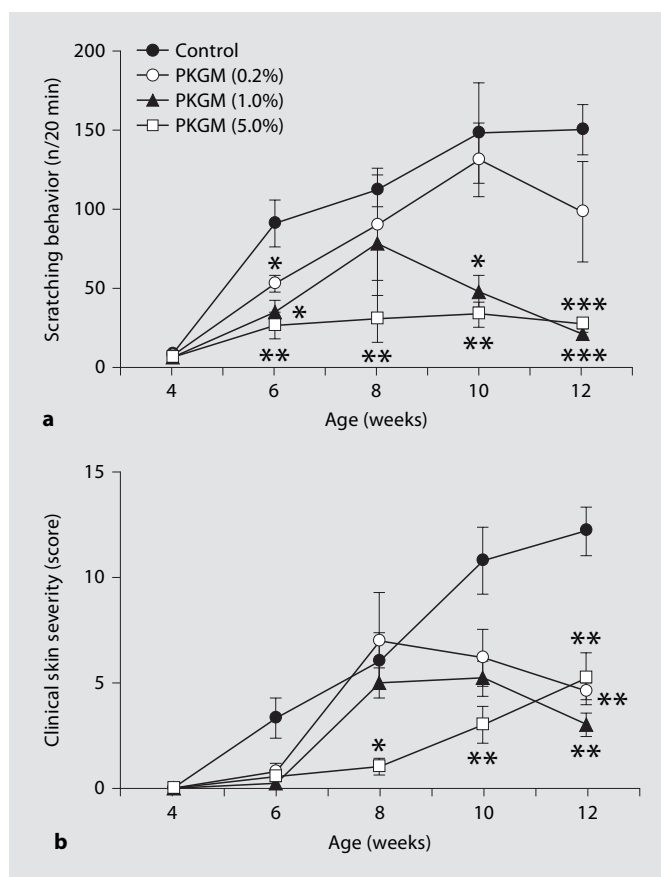


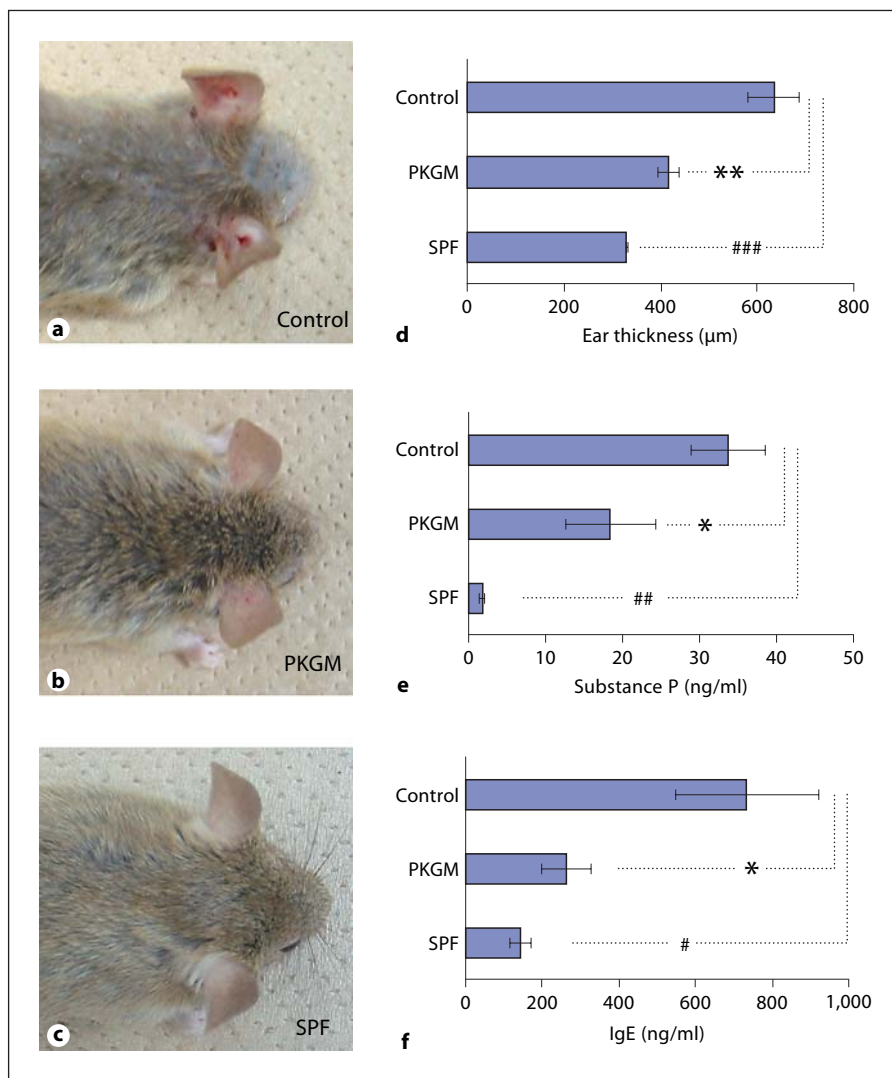
Fig. 1. Dose-dependent effects of PKGM on increasing scratching behavior and clinical skin scores in NC/Nga mice. **a** Numbers of scratching incidents per 20 min were assessed. **b** Skin severity of dermatitis was evaluated every 2 weeks using macroscopic skin severity scores. Values are expressed as means $\pm$ SE with 5 mice per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; control mice versus PKGM-fed mice.

Results

Dose-Dependent Effects of PKGM on Increasing Scratching Behavior and Development of Dermatitis in NC/Nga Mice

We first investigated dose-dependent effects of PKGM on scratching behavior, eczema severity and plasma immunoglobulin levels in NC/Nga mice. As shown in figure 1a, a spontaneous increase in numbers of scratching behaviors was observed in control mice from 6 weeks of age. However, such a behavior was profoundly inhibited in PKGM-fed mice in a dose-dependent manner. Thereafter, control mice developed AD-like skin lesions on the ears, face and neck areas, and irritations spread to the back areas most likely due to the progression of scratch-

Fig. 2. Inhibitory effects of PKGM on ear swelling and cutaneous substance P and total IgE levels in 13-week-old NC/Nga mice. Representative photographs of clinical characteristics in NC/Nga mice: control mice kept under conventional conditions (**a**), PKGM-fed mice kept under conventional conditions (**b**) and control mice kept under SPF conditions (**c**). Ear thickness (**d**) in NC/Nga mice was measured by a thickness gauge. Substance P (**e**) and total IgE levels (**f**) in skin were measured by ELISA, as described in Materials and Methods. Values are expressed as means $\pm$ SE with 5 mice per group for ear thickness (**d**), 4–5 mice for substance P levels in skin (**e**) and 5 mice for total IgE levels in skin (**f**). * $p < 0.05$; ** $p < 0.01$; control mice versus PKGM-fed mice. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$; control mice versus SPF mice.



ing. Oral intake of PKGM suppressed increase in clinical skin severity scores of NC/Nga mice in a dose-dependent manner (fig. 1b). Feeding with PKGM even at a dose of 0.2% significantly suppressed the scores at 12 weeks of age compared to the control group. In control mice kept under SPF conditions, scratching behavior was scarcely observed at 12 weeks of age: 7.2 ± 6.0 counts/20 min (mean $\pm$ SE, $n = 5$).

Inhibitory Effects of PKGM on Ear Swelling and Cutaneous Substance P and IgE Levels in NC/Nga Mice

We next examined the effects of PKGM on ear swelling at 13 weeks of age. Clinical features are shown in figure 2a–c. Skin lesions in NC/Nga mice appeared in lim-

ited areas accessible to hind paws for scratching (fig. 2a), and symptoms were markedly inhibited in PKGM-fed mice (fig. 2b). Ear thickness in PKGM-fed mice was significantly lower than that in control mice with eczema (fig. 2d).

It has been reported that substance P plays crucial roles in both scratching behavior and skin inflammation in NC/Nga mice [16, 17]. We therefore analyzed cutaneous substance P levels in 13-week-old PKGM-fed mice. The levels of substance P in the skin were markedly increased in control mice, but the overproduction was significantly downregulated by PKGM feeding (fig. 2e). High amounts of total IgE were also detected in the skin of control mice (fig. 2f). However, oral intake of PKGM significantly suppressed skin IgE levels at 13 weeks of age.

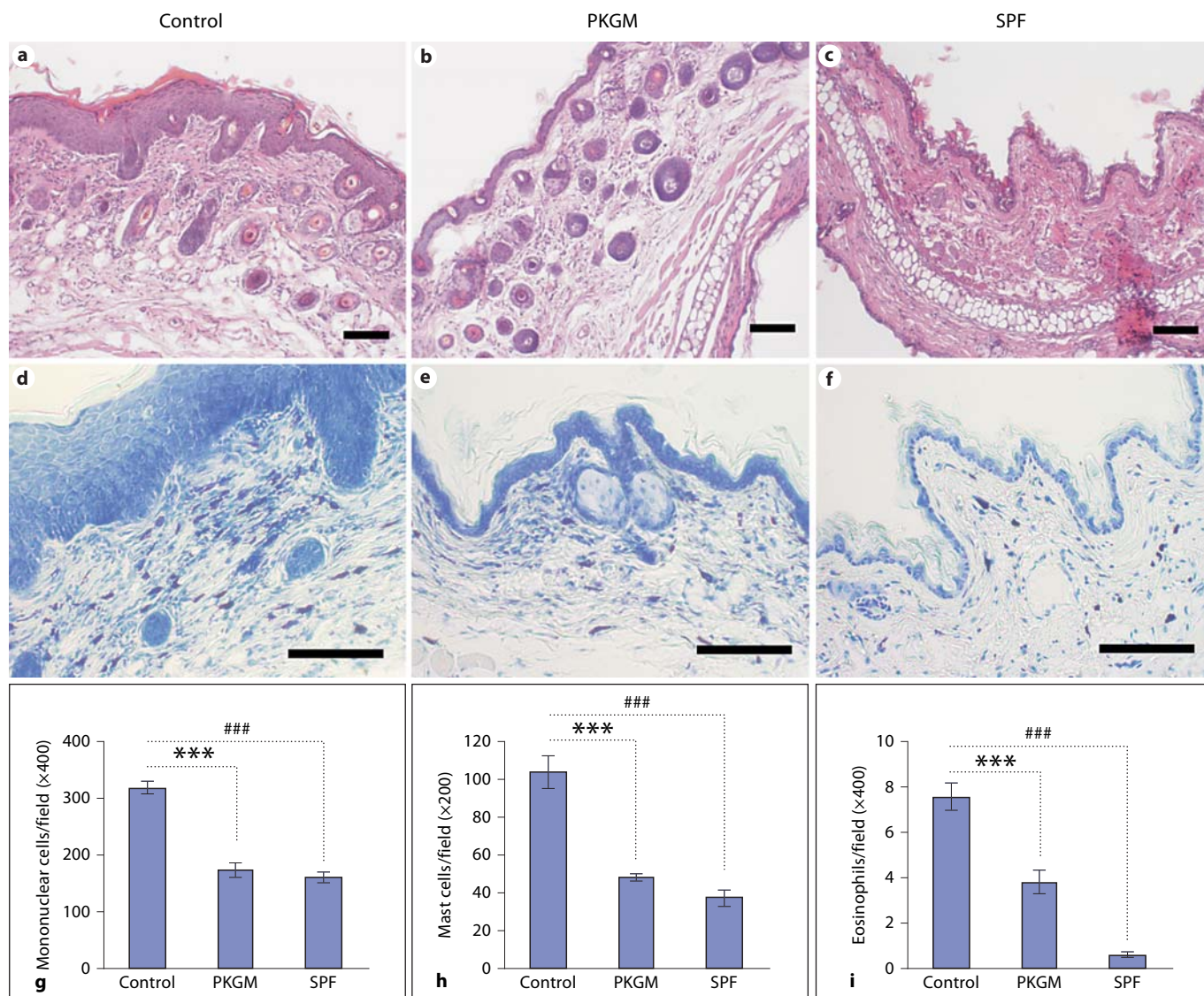


Fig. 3. Inhibitory effects of PKGM on hyperkeratosis and on mast cell and eosinophil accumulation in the skin of 13-week-old NC/Nga mice. Representative photographs of histological [hematoxylin and eosin (a–c); toluidine blue (d–f)] characteristics in NC/Nga mice: control mice kept under conventional conditions (a, d); PKGM-fed mice kept under conventional conditions (b, e); control mice kept under SPF conditions (c, f). Histological sections

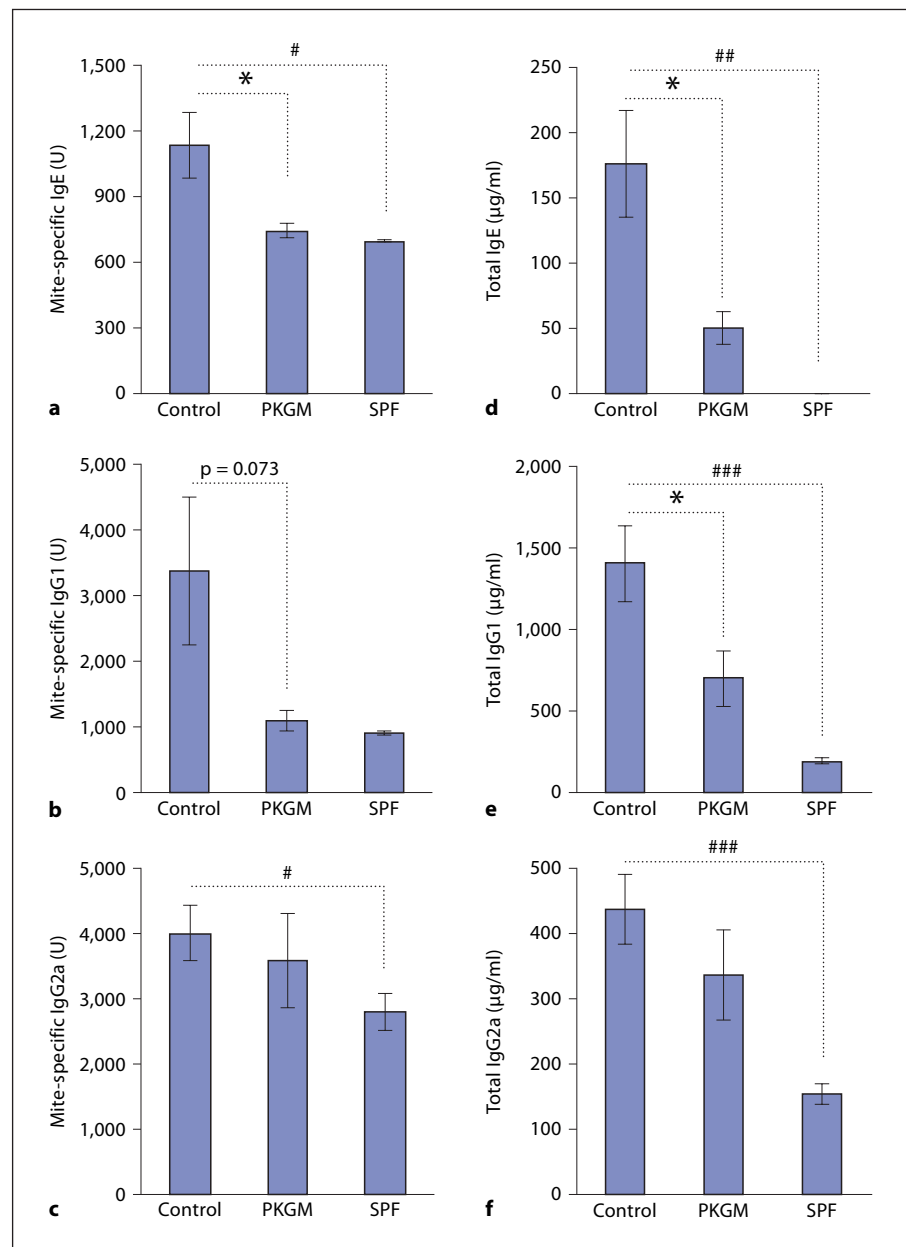
were observed using a ×100, ×200 or ×400 magnification lens. Scale bar = 100 μm. Numbers of mononuclear cells (g), mast cells (h) and eosinophils (i) in ear sections were counted as described in Materials and Methods. Values are expressed as means ± SE with 3 mice per group. *** $p < 0.001$; control mice versus PKGM-fed mice. ### $p < 0.001$; control mice versus SPF mice.

Inhibitory Effects of PKGM on Hyperkeratosis, Mastocytosis and Eosinophilia in the Skin

We next examined effects of PKGM on histological changes, including eosinophil and mast cell accumulation in the skin of NC/Nga mice at 13 weeks of age. Histological features are shown in figure 3a–f. Hypertrophy of the epidermis and increase in mononuclear cell infiltration in

the dermis were observed in control mice, while these symptoms were strikingly inhibited in mice fed with PKGM (fig. 3a, b, g). Toluidine blue staining showed an increase in mast cells in the skin of control mice (fig. 3d). However, the mast cell accumulation was significantly inhibited in mice that were fed with PKGM (fig. 3e and h). Increased numbers of eosinophils in the dermis were also

Fig. 4. Effects of PKGM on total and mite-specific IgE, IgG1 and IgG2a levels in plasma of 12-week-old NC/Nga mice. Plasma IgE (**a**), IgG1 (**b**) and IgG2a (**c**) levels specific to mite (*D. farinae*) extracts were measured by ELISA, as described in Materials and Methods. Total IgE (**d**), IgG1 (**e**) and IgG2a (**f**) levels in plasma were quantified by sandwich ELISA. Values are expressed as means $\pm$ SE with 5–6 mice per group. * $p < 0.05$; control mice versus PKGM-fed mice. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$; control mice versus SPF mice.



observed in control mice, but the eosinophilia was significantly suppressed in PKGM-fed mice (fig. 3i). In control mice kept under SPF conditions, neither hyperkeratosis nor infiltration of mononuclear cells, eosinophils or mast cells was observed at all (fig. 3c, f–i).

Analysis of Mite-Specific IgE, IgG1 and IgG2a Levels in Plasma of NC/Nga Mice

It has been reported that fur mite infestation plays an important role in causing hyper-IgE production in NC

mice [21]. To assess whether disruption of epidermal tissues by scratching causes local sensitization to exogenous mite allergens, we measured mite-specific (*D. farinae*) IgE, IgG1 and IgG2a titers in plasma of mice fed with PKGM (12 weeks old) were significantly lower than those of control mice (fig. 4a). A similar trend toward decrease in *D. farinae*-specific IgG1 titers was also seen in PKGM-fed mice (fig. 4b), although the difference was not statistically significant compared to control mice ($p = 0.073$). By

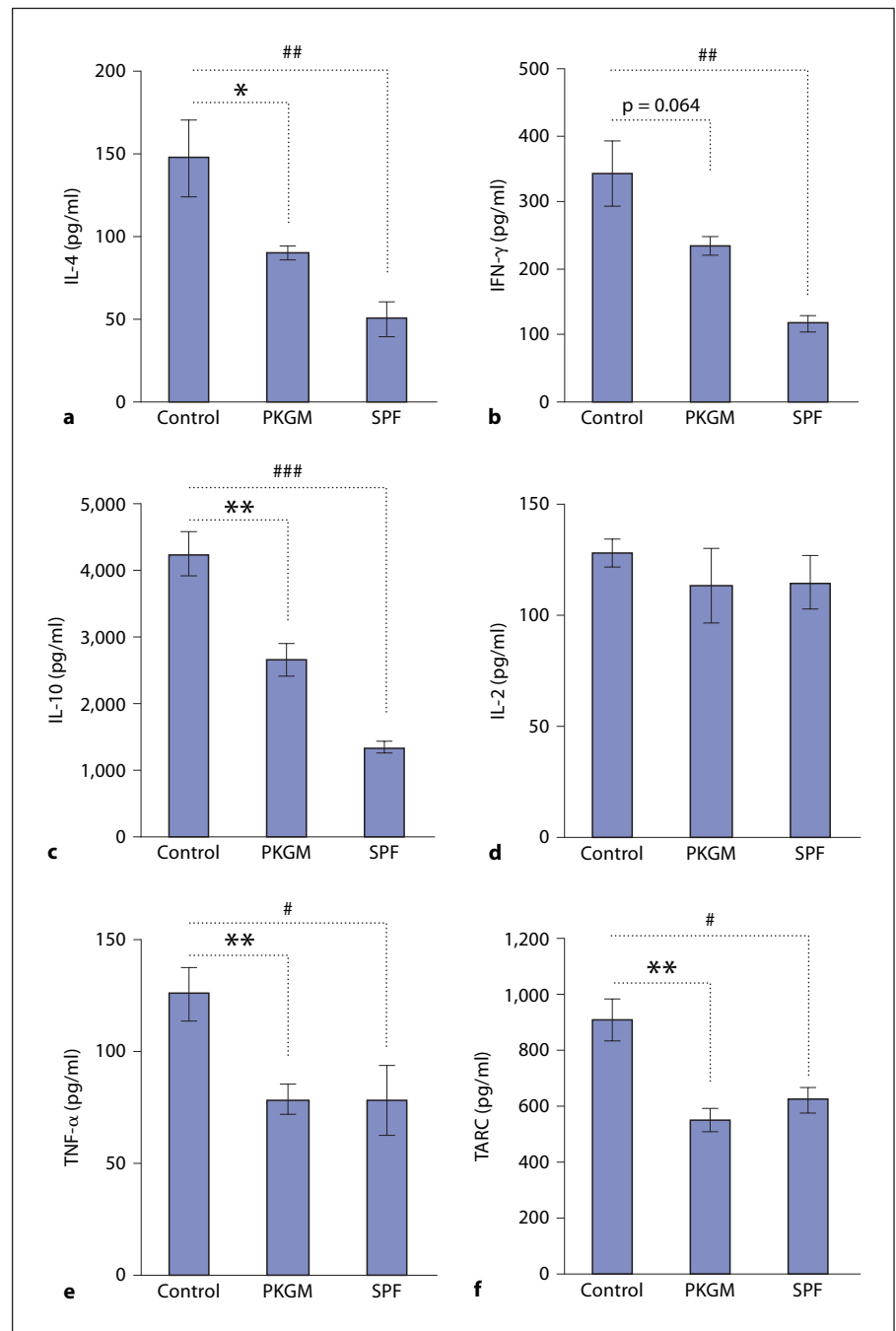


Fig. 5. Effects of PKGM on cytokines and TARC levels in the dorsal skin of 13-week-old NC/Nga mice. Levels of IL-4 (a), IFN-γ (b), IL-10 (c), IL-2 (d), TNF-α (e) and TARC (f) in the back skin were quantified by sandwich ELISA. Values are expressed as means $\pm$ SE with 5 mice per group. * $p < 0.05$; ** $p < 0.01$; control mice versus PKGM-fed mice. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$; control mice versus SPF NC/Nga mice.

contrast, *D. farinae*-specific IgG2a titers were unaffected by PKGM feeding (fig. 4c). As we previously reported [5], plasma total IgE and IgG1 levels were also downregulated by PKGM feeding, while IgG2a levels were comparable to those seen in control mice (fig. 4d-f).

Effects of PKGM on Cytokine and Chemokine Levels in the Dorsal Skin of NC/Nga Mice

To further examine effects of PKGM on skin immune responses, we finally analyzed cytokine (IL-4, IFN-γ, IL-10, IL-2 and TNF-α) and chemokine (TARC/CCL17) levels in the dorsal skin of 13-week-old NC/Nga mice. Overproductions of IL-4, IFN-γ, IL-10, TNF-α and TARC

were evident in the back skin of control mice with eczema (fig. 5). However, these responses were strikingly suppressed in NC/Nga mice fed with PKGM, although differences in IFN- γ levels between PKGM and control mice were not statistically significant ($p = 0.064$; fig. 5b). Cutaneous IL-2 level was unaffected by PKGM feeding (fig. 5d).

Thus, oral intake of PKGM profoundly prevented the development of scratching behavior and dermatitis with mast cell infiltration, and markedly inhibited skin-inflammatory immune responses in NC/Nga mice.

Discussion

In the present study, we first confirmed that oral administration of PKGM suppressed the increases in scratching behavior and skin severity scores in a dose-dependent manner at an early stage (approximately 8 weeks old) of skin irritation in NC/Nga mice (fig. 1a and b). In accordance with a previous study [5], plasma IgE levels were not different among the groups at 8 weeks of age (data not shown). Our observations supported the idea that the increased IgE levels are not a prerequisite for the development of scratching behavior and eczema in NC/Nga mice [19, 22]. Although the suppressive effects on scratching behavior in mice fed with PKGM at low doses (0.2 and 1.0%) were not so apparent at an early age, continuous feeding with PKGM increased this activity slowly (fig. 1a). Low doses of PKGM eventually inhibited eczema (fig. 1b), followed by downregulation of IgE (data not shown) in 12-week-old NC/Nga mice. Thus, continuous ingestion of PKGM may be critical for the suppression of scratching behavior and later skin inflammation in NC/Nga mice.

The areas of skin lesions in NC/Nga mice were limited to those accessible to their hind paws for scratching (fig. 2a), while the symptoms were markedly inhibited in PKGM-fed mice (fig. 2b). Our observations strongly support the previous idea [8, 9] that mechanical injury of the skin by scratching may be an important factor inducing dermatitis in NC/Nga mice.

Stimulation of high-affinity receptors for IgE on mast cells induces secretion of a variety of inflammatory mediators such as histamine, substance P and chymase [23, 24]. Based on the fact that these mediators induce itching and an intense desire to scratch [25], mast cells are thought to play a part in the pathogenesis of AD. Recent studies showed that substance P-positive mast cells and nerve fibers were observed in the dermis of hapten-treated NC/

Nga mice with eczema, and a substance P antagonist (BIIF 1149 CL) profoundly inhibited scratching behavior [16, 17]. In the present study, increased numbers of mast cells and substance P levels in the skin were significantly reduced in NC/Nga mice fed with PKGM (fig. 2e, 3h). These evidences suggest that inhibitory effects of PKGM on scratching behavior and skin inflammation are closely associated with downregulation of the local mastocytosis and substance P in NC/Nga mice.

Since fur mite infestation plays an important role in causing hyper-IgE production in NC mice [21], the skin barrier disrupted by scratching is considered to cause local sensitization to mite allergens. In fact, increased mite-specific (*D. farinae*) IgE and IgG1 levels in plasma and total IgE levels in dorsal skin were detected in NC/Nga mice with dermatitis (fig. 2f, 4a and b). However, these responses were markedly reduced in PKGM-fed mice. Our findings imply that the suppressive effect of PKGM on scratching behavior might contribute to the prevention of local sensitization to foreign mite allergens in NC/Nga mice.

It has been reported that hypertrophy of the epidermis was induced in the skin of NC/Nga mice with eczema [6, 24], and keratinocyte apoptosis induced by IFN- γ played a critical role on hypertrophy of the skin in AD [26]. In this study, the striking ear swelling and hypertrophy of the epidermis were observed in control mice, while these symptoms were markedly inhibited in mice fed with PKGM (fig. 2d, 3a and b). Skin IFN- γ production tended to decrease in PKGM-fed mice (fig. 5b), although no statistically significant difference was seen compared to the control group. These evidences together with our previous findings that PKGM inhibits systemic IFN- γ production [5] suggested that the inhibitory effect of PKGM on hyperkeratosis might be associated with downregulation of IFN- γ in NC/Nga mice.

IL-10 is overproduced in the skin of NC/Nga mice with eczema as well as in AD patients [20, 27]. It has been reported that physical or chemical stimulation of the skin induces overexpression of IL-10 in epidermal keratinocytes [12, 28]. Accordingly, inhibitory effects of PKGM on scratching behavior might contribute to downregulation of cutaneous IL-10 production in NC/Nga mice. IL-10 production by APCs has recently been demonstrated to play a key role in the Th2 response to antigens and in skin infiltration with eosinophils in murine models of AD [12]. Moreover, IL-10 augments production of TARC, a Th2 chemokine overexpressed not only in AD skin [29, 30], but also in the keratinocyte cell line HaCaT stimulated by TNF- α and IFN- γ [31]. In fact, injection of anti-

sense oligonucleotides against IL-10 to local skin ameliorated skin lesions in NC/Nga mice [20]. In the present study, cutaneous eosinophilia with massive IL-10, IL-4, IFN- γ , TNF- α and TARC productions were elicited in eczematous control mice. However, all these responses were profoundly suppressed in mice fed with PKGM (fig. 3i, 5). Our data together with previous findings [12, 20] strongly suggest that the inhibitory effect of PKGM on cutaneous IL-10 production might be involved in the prevention of dermatitis with eosinophilia and Th2 skin responses in NC/Nga mice.

Importantly, increased Th2 cells are not necessary for the development of eczema in NC/Nga mice, although mastocytosis, eosinophilia and IFN- γ expression in skin are tightly correlated with the pathogenesis [19]. It has also been reported that numbers of mast cells producing IL-4 and IL-5 are remarkably increased in the eczematous skin of NC/Nga mice [6, 32] and the downregulation of mastocytosis is particularly important for the prevention of AD [32]. In our preliminary experiment, numbers of CD3+ T cells in skin of eczematous control mice were rather lower than those of PKGM-fed mice or SPF-maintained mice (data not shown). On the other hand, dietary PKGM significantly inhibited mastocytosis as well as overproductions of IL-4 and TNF- α in the skin (fig. 3h,

5a and e). Mast cells are also known to be the most important sources of TNF- α during IgE-dependent responses [33]. Very recently, it has been reported that mast cells, activated by skin-derived thymic stromal lymphopoietin, in synergy with IL-1/TNF, produce high levels of Th2 cytokines, and induce T cell-independent inflammation [34]. Thus, the inhibitory effects of PKGM on the skin inflammatory responses in NC/Nga mice may be closely associated with the prevention of mastocytosis rather than with Th2 cell regulation.

In conclusion, our study demonstrated that oral intake of PKGM inhibited local mastocytosis, substance P expression and skin inflammatory immune responses in combination with a suppressive effect on the scratching behavior in NC/Nga mice. Further studies are needed to understand the puzzling mechanisms of eczema prevention by PKGM.

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