

Dietary Supplementation with Lactic Acid Bacteria Attenuates the Development of Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice in a Strain-Dependent Manner

Hideyuki Wakabayashi Chie Nariai Fumiyo Takemura Wakako Nakao
Daisuke Fujiwara

Central Laboratories for Frontier Technology, Kirin Brewery Co., Ltd., Yokohama, Japan

Key Words

Atopic dermatitis · IgE · IL-12 · Lactic acid bacteria ·
Th1/Th2 balance

Abstract

Background: Dietary supplementation with lactic acid bacteria (LAB) is a potential approach to the prevention and manipulation of allergic diseases such as atopic dermatitis (AD). However, the influence of different bacterial strains and their immunomodulating capacities is still largely unknown. **Methods:** AD-like skin lesions were induced by sensitization to and repeated challenges with picrylchloride in the Th2-skewed NC/Nga mouse strain. The effects of LAB supplementation were assessed over time by monitoring clinical scores and plasma IgE levels. In some cases, mast cell infiltration, cutaneous hypersensitivity responses and cytokine mRNA expression in auricles were also examined. Additionally, cytokine production in vitro and cytokine mRNA accumulation in major lymphoid tissues were measured, comparing *Lactobacillus paracasei* KW3110 with *L. rhamnosus* GG (LGG). **Results:** Supplementation with KW3110 significantly reduced the development of AD-like skin lesions, accompanied by less mast cell infiltration and lower plasma IgE levels. KW3110 also suppressed immediate hypersensitivity reactions and IL-4 mRNA expression in the auricles. These preventive effects sustained when supplementation

was terminated; moreover, inhibitory effects were also observed even when supplementation was initiated after the onset of symptoms. In accordance with its effects on IL-12 and IL-4 production in vitro, KW3110 prevented the emergence of clinical symptoms more effectively than LGG in vivo. **Conclusions:** Supplementation with KW3110 significantly attenuated the onset and exacerbation of AD-like symptoms in NC/Nga mice. The effects were more prominent than those obtained with LGG, suggesting the importance of differences between LAB strains and their immunomodulating capacity.

Copyright © 2007 S. Karger AG, Basel

Introduction

Atopic dermatitis (AD) is a chronic refractory skin disease manifesting as eczema and itching with repeated exacerbations and regressions [1, 2]. Disease onset is usually during early infancy and childhood, but can also occur in adulthood. While the precise mechanisms underlying the development of AD are not yet fully elucidated, multiple factors such as immune responses, susceptibility genes, skin barrier function and the environment seem to be intricately interrelated. Aberrant regulation of cytokines and chemokines is thought to be involved in the exacerbation of AD [3, 4], and the production of Th2 cy-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2007 S. Karger AG, Basel
1018–2438/07/1452–0141\$23.50/0

Accessible online at:
www.karger.com/iaa

Correspondence: Hideyuki Wakabayashi
Central Laboratories for Frontier Technology
Kirin Brewery Co., Ltd., 1-13-5, Fukuura, Kanazawa-ku
Yokohama 236-0004 (Japan)
Tel. +81 45 330 9004, Fax +81 45 788 4047, E-Mail hwakabayashi@kirin.co.jp

tokines such as IL-4 and IL-13, which cause IgE elevation, is increased in skin lesions of AD patients [5]. Transgenic mice overproducing Th2 cytokines also develop AD-like lesions spontaneously [6].

The number of infants with AD has been increasing recently and is becoming a serious public health problem in developed countries. So far, several clinical trials have indicated that intake of lactic acid bacteria (LAB) may be beneficial to patients with AD. Kalliomaki et al. [7] first found that perinatal administration of *Lactobacillus rhamnosus* strain GG (ATCC53103; LGG) halved the development of atopic eczema in children at high risk during the first 2 years of life. In addition, Rosenfeldt et al. [8] also reported that supplementation with a combination of *L. rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460 decreased eczema in AD children. They also suggested the possibility that stabilization of the intestinal mucosal barrier by LAB may be involved in this effect. However, the benefit of LAB supplementation for the prevention of AD is still controversial because other researchers have reported that supplementation with *Lactobacillus acidophilus* (LAVR-A1) [9] and *L. rhamnosus* (LGG [10, 11]) in the form of probiotics did not reduce the risk of AD in infants. While there is a number of differences between these clinical studies, we hypothesized that one crucial variable may be the strain of LAB employed. Previously, in vitro studies had also suggested bacterial strain-dependent differences in immunomodulating capacity [12–14]. However, few in vivo studies have been reported [15], and thus the LAB strain-dependent effect on AD and host immune responses remains to be addressed.

Under conventional conditions, NC/Nga mice spontaneously develop AD-like skin lesions which resemble the clinical symptoms of human AD [16]. Additionally, repeated applications of picrylchloride (PCI) to NC/Nga mice cause a simple and reproducible hapten-induced AD-like skin disease even under specific pathogen-free conditions [15, 17, 18]. The systemic deficiency in the Th1 response in this mouse strain seems to be crucial for the Th2-dominant state and susceptibility to dermatitis, with accompanying high levels of blood IgE [19, 20]. Indeed, intraperitoneal injections of IFN- γ , IL-12 or IL-18 greatly suppress dermatitis scores, with a marked reduction in blood IgE levels [21]. Here, we examined the effect of *Lactobacillus paracasei* KW3110, which has a high capacity for IL-12 induction and IL-4 suppression [12], in this mouse model. Furthermore, KW3110 was compared with LGG to assess the influence of different strains on immunomodulating capacity.

Material and Methods

Animals and Diets Containing LAB

Five-week-old male NC/Nga mice were purchased from Charles River Japan (Yokohama, Japan) and acclimatized for 1 week with free access to water and a basic diet, AIN-76A (Oriental Yeast, Tokyo, Japan). Each mouse was individually kept under specific pathogen-free conditions throughout the experiment to avoid injury from fighting. All animal procedures and experiments were approved by the Laboratory Animal Care Committee in the Central Laboratories for Frontier Technology, Kirin Brewery Co., Ltd.

The LAB strains *L. paracasei* KW3110 and *L. rhamnosus* GG (LGG) were obtained from Kirin Brewery (Tokyo, Japan) and American Type Culture Collection (Manassas, Va., USA), respectively. After culture in MRS broth (BD Difco, Calif., USA) for 48 h at 37°C, each bacterial strain was washed twice with sterilized water, sterilized at 100°C and finally freeze-dried [22]. The heat-treated LAB preparation was added into the basic diet at concentrations of 0.03% (low dose) or 0.3% (high dose).

Induction and Clinical Evaluation of AD-Like Skin Lesions

As shown in figure 1a, 6a, and 8a, AD-like skin lesions were induced by sensitization to and repeated challenge with PCI (Tokyo Kasei Kogyo, Tokyo, Japan) in NC/Nga mice, as described [18, 23]. Briefly, 6-week-old male NC/Nga mice were evenly divided into groups based on their weights and blood levels of IgE. Animals were fed a basic diet with or without LAB for 3 weeks and then sensitized by applying 100 μ l of 5% PCI dissolved in ethanol:acetone (4:1) onto shaved bare abdominal skin and 25 μ l onto each hind paw. One week later, weekly challenge by applying 10 μ l of 0.8% PCI dissolved in olive oil (Wako Chemicals, Osaka, Japan) onto both sides of both ears was started and continued for a further 7 or 10 weeks.

Clinical severity of dermatitis of the auricle and scalp was scored by a modification of the methods by Matsuda et al. [16] and Hirasawa et al. [23]. This included five factors (pruritus, rubor/hemorrhage/erosion, edema, abrasion/tissue deficit and crust formation/xerosis) for the auricle and three (depilation, rubor/hemorrhage/erosion and crust formation/xerosis) for the scalp. The total clinical score was defined as the sum of the individual scores graded as 0 (subclinical), 1 (slight), 2 (moderate) and 3 (severe). Additionally, photographs of representative mice presenting average clinical symptoms in each group were taken just before sacrifice for biochemical and pathological analysis.

Biochemical and Pathological Analysis

Blood was collected from the eye socket, and plasma was immediately prepared by centrifugation and stored at -20°C until use. Plasma levels of total IgE, IgG1 and IgG2a were measured using the murine OptEIA ELISA set (BD Pharmingen, Calif., USA) or pairs of purified capture and biotinylated detection mAbs (BD Pharmingen).

At the end of the experiment, auricles were excised, fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin or toluidine blue (Sigma-Aldrich, St. Louis, Mo., USA), and histological changes were examined by light microscopy. Mast cells were counted in three different microscopic fields for each mouse, and their number per auricular surface area (n/mm²) was calculated using image processing software (Winroof; Mitani, Tokyo, Japan).

Analysis of Cutaneous Hypersensitivity Responses

Using the same method as for the induction of AD-like skin lesions, transient ear swelling reflecting cutaneous hypersensitivity responses was measured after the first or third challenge. The thickness of both ears was measured with a dial gauge (peacock G-1A type; Ozaki Seisakusyo, Tokyo, Japan) for 8 days after the challenge. Accumulation of mRNA for IFN- γ and IL-4 was examined by semiquantitative RT-PCR in a similar but independent experiment.

In vitro Analysis of Cytokine Production from Th2-Skewed Splenocytes

Six-week-old male NC/Nga mice were injected intraperitoneally with 1 mg ovalbumin (OVA; Seikagaku, Tokyo, Japan) adsorbed onto 2 mg of alum (Wako Chemicals, Osaka, Japan). One week later, they received a second identical injection. The mice were then sacrificed after a further week and the spleens excised. Splenocytes were isolated after lysis of RBC and were suspended at 2×10^6 cells/ml in RPMI-1640 medium (Sigma-Aldrich) containing 1 mg/ml OVA, 10% FCS (Roche, Basel, Switzerland) and penicillin/streptomycin, with or without 1 μ g/ml LAB. After 1 day, the culture supernatant was collected and the IL-12p70 level was measured by the murine OptEIA ELISA set (BD Pharmingen). Similarly, the levels of IL-4, IFN- γ and IL-10 were measured in the supernatant after 7 days of culture.

Semiquantitative RT-PCR

mRNA for IFN- γ and IL-4 in auricles, mesenteric lymph nodes and submandibular lymph nodes were assessed by semiquantitative RT-PCR. Total RNA was prepared with Isogen solution (Nippon Gene, Toyama, Japan), and cDNA was synthesized using the Thermo Script RT-PCR System (Invitrogen, Calif., USA). Amplification was carried out for the appropriate number of cycles with denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 60 s on a Gene Amp PCR System 9700 (Perkin-Elmer, Boston, Mass., USA). The product was separated on 2% agarose gels and quantified using Scion Image Software (Scion, Md., USA). The primer sequences for mouse IFN- γ , IL-4, β -actin and GAPDH are shown in online supplement table 1 (www.karger.com/doi/10.1159/000108139).

Statistical Analysis

Data are presented as means $\pm$ SE. The homoscedasticity and normal distribution of the data were confirmed for each group, and then comparisons between multiple groups were performed by one-way analysis of variance or nonparametric corrected Mann-Whitney's U test. Statistical significance was accepted at $p < 0.05$.

Results

Effects of KW3110 Intake on the Development of AD-Like Skin Lesions

The total clinical scores during the development of AD-like skin lesions caused by PCl in NC/Nga mice are shown in figure 1b. In control mice, continuous challenges with PCl on the auricles induced crust formation due

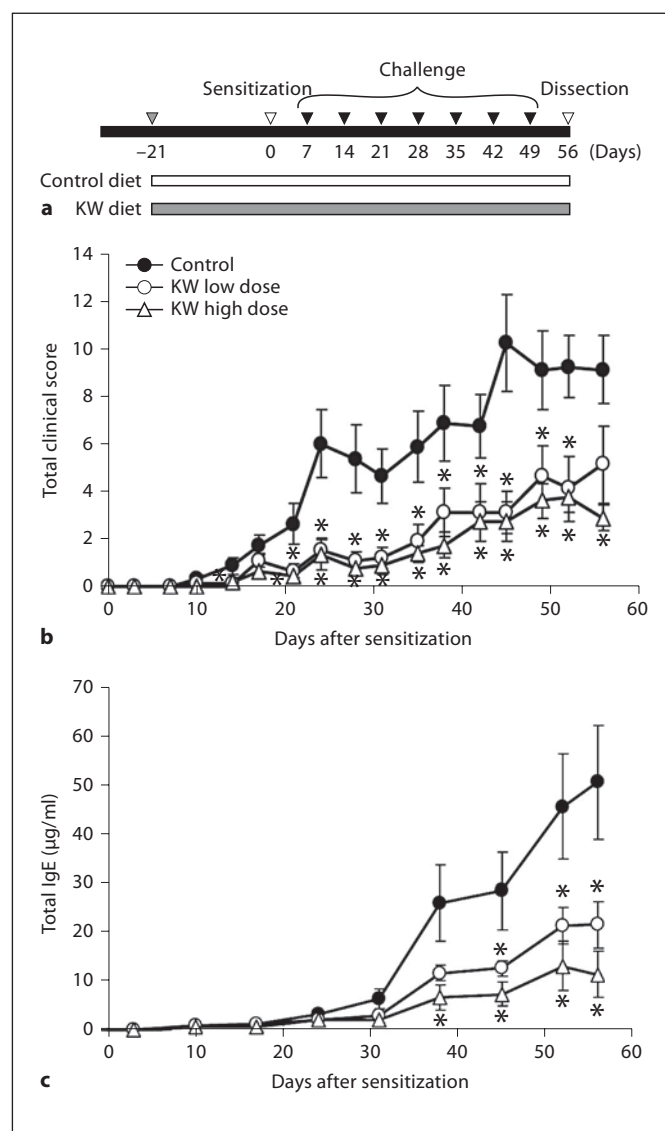


Fig. 1. Effect of KW3110 on PCl-induced AD-like symptoms in NC/Nga mice. The mice were fed either a low or high dose of KW3110 for 11 weeks. Schematic representation of the experiment (a), total clinical scores (b) and total IgE levels (c) are shown. The time points of sensitization, challenges and dissection are indicated by arrowheads. Means $\pm$ SE (n = 10). * $p < 0.05$ vs. control.

to increasing scratching behavior around day 14 and a marked increase in clinical scores around day 24 due to hemorrhage from the auricle and depilation of the scalp. Subsequently, auricles exhibited hypertrophy because of inflammation and hemorrhage around day 35, and abrasion and deficit around day 42. As a result, some of the control mice had lost most of their auricles by the end of

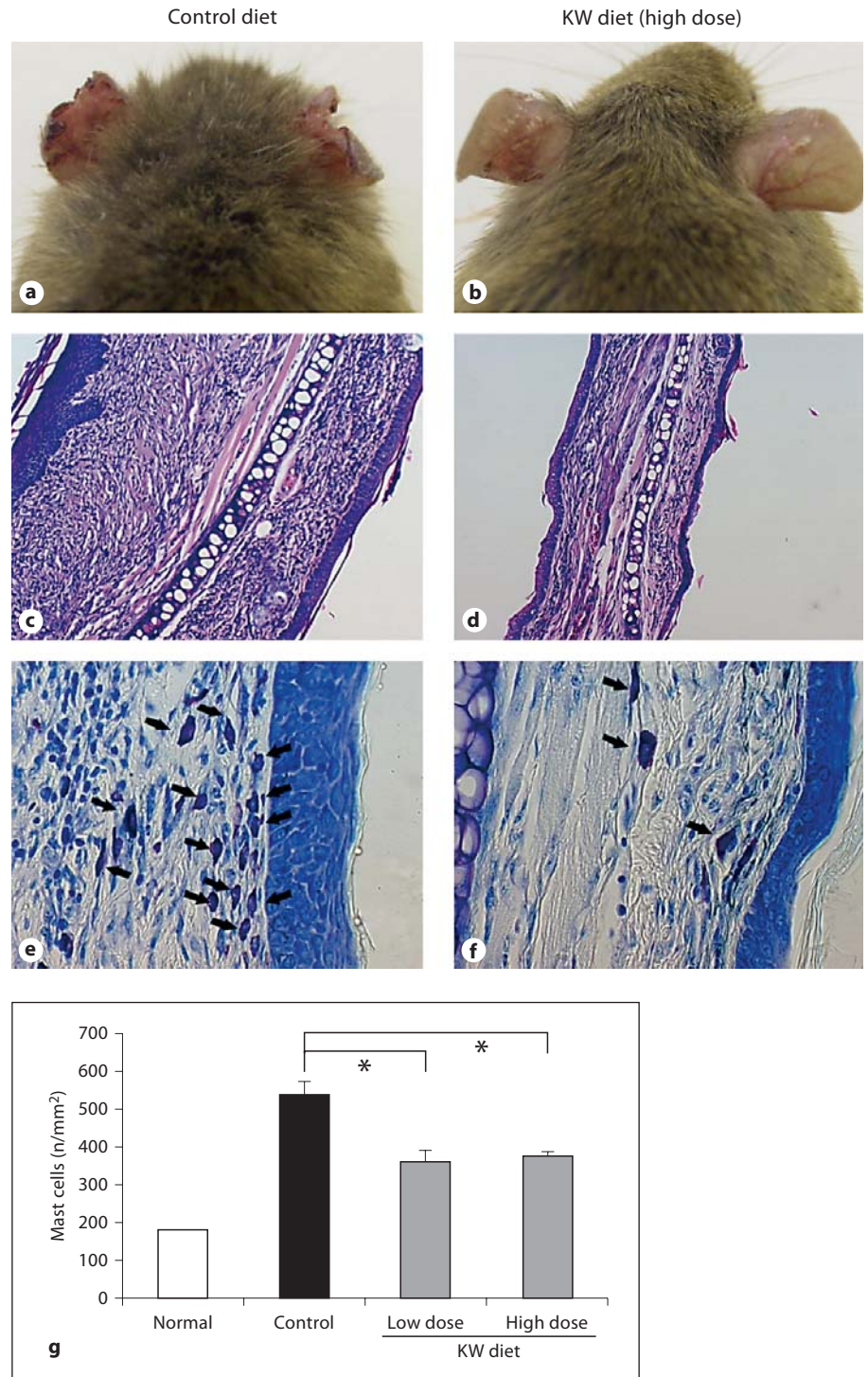
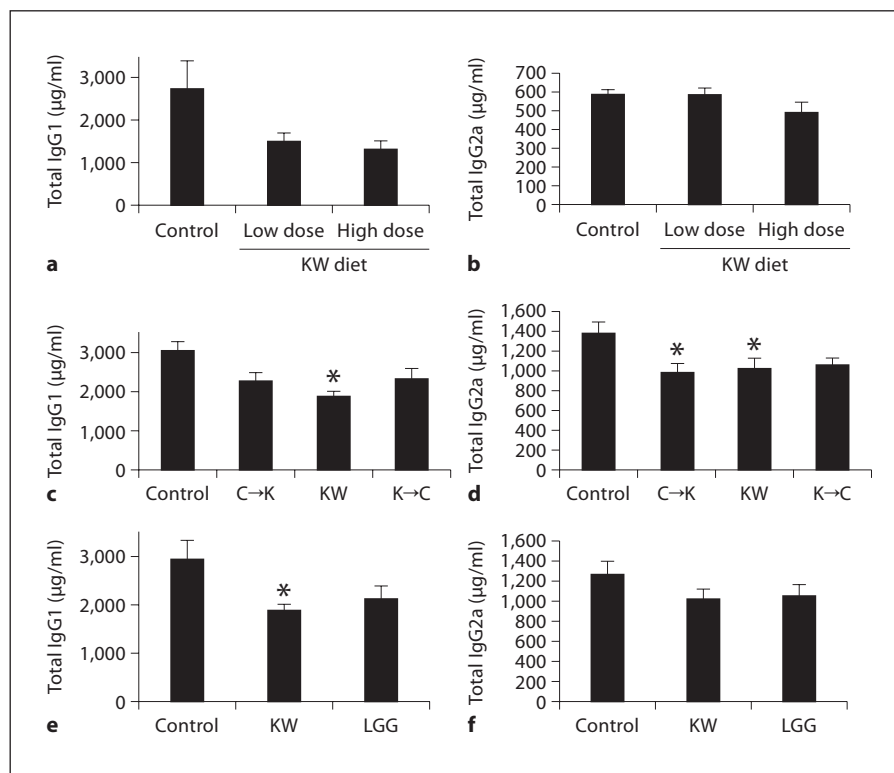


Fig. 2. Skin features and mast cell infiltration in NC/Nga mice. The mice were fed a diet with or without a high dose of KW3110 for 11 weeks. Aspects of auricles and scalp (**a**, **b**), auricular sections with HE staining (**c**, **d**; $\times 100$) or toluidine blue staining (**e**, **f**; $\times 400$) and numbers of mast cells per unit auricular surface area (**g**) are shown. Infiltrating mast cells are indicated by arrows. Means $\pm$ SE ($n = 10$). * $p < 0.05$ vs. control.

the experiment. In contrast, mice receiving KW3110 at high or low dose manifested little early-stage clinical deterioration even after 24 days. Therefore, the difference in the scores became clear from that time on, and a

marked preventive effect remained observable until the end of the experiment. Accordingly, at the end the shape and condition of the auricles were strikingly different between the group supplemented with KW3110 and non-

Fig. 3. Plasma levels of total IgG1 and IgG2a in NC/Nga mice. The levels of IgG1 (**a, c, e**) and IgG2 (**b, d, f**) at the end of the experiment were measured by ELISA. The effects of KW3110 dosage (**a, b**), feeding conditions (**c, d**) and LAB strains (**e, f**) are shown, respectively. Means $\pm$ SE (n = 8–10). * p < 0.05 vs. control.



supplemented animals (fig. 2a, b). Histological analysis of auricles using HE staining showed that control mice had thickening of the dermis and epidermis, hyperkeratosis in the epidermis and increased eosinophil infiltration in the subcutaneous layer (fig. 2c). In contrast, KW3110 intake greatly suppressed these skin changes (fig. 2d). Moreover, histological analysis using toluidine blue staining also demonstrated that KW3110 significantly reduced the increased number of mast cells infiltrating subcutaneous layers (fig. 2e–g). However, no distinguishable differences between the effects of high and low doses of KW3110 were detectable either in total clinical scores or pathological symptoms (fig. 1b). Plasma total IgE is shown in figure 1c. The increase in IgE levels was slightly slower than the rise in total clinical scores, and a marked elevation was detected after day 38 in control mice. Supplementation with KW3110 resulted in a significant persistent suppression of IgE levels, with a tendency towards dose dependency, being in contrast to the results for clinical scores and mast cell numbers described above. At sacrifice, plasma IgG1 levels were decreased by KW3110 at both doses, but IgG2 levels were only slightly reduced in the high-dose group (fig. 3a, b).

Effect of KW3110 Intake on Cutaneous Hypersensitivity Responses

In NC/Nga mice, hapten challenge causes transient local inflammatory swelling in the auricles, which is a feature of cutaneous hypersensitivity responses. When the measurement was carried out after the first challenge, a triphasic reaction was observed (fig. 4a). The swelling appeared after 1 h and peaked at 48 h. Subsequently, it decreased by 96 h but then slightly increased again around 144 h. During this time, KW3110 intake significantly repressed the first- and third- but not second-phase reactions. An independent follow-up experiment was then performed to examine the involvement of IL-4 and IFN- γ expression in auricular tissue. As shown in figure 5a, supplementation with high-dose KW3110 significantly reduced IL-4 expression from the beginning of the experiment up to 4 h, suggesting suppression of the Th2 response. However, no apparent effect on IFN- γ expression was detected (fig. 5b). When auricular thickness was measured after the third challenge, the triphasic reaction changed into the monophasic pattern. In this case, the inhibitory effect of KW3110 became much clearer over the entire time course. However, no dose dependency of KW3110 was observed (fig. 4b).

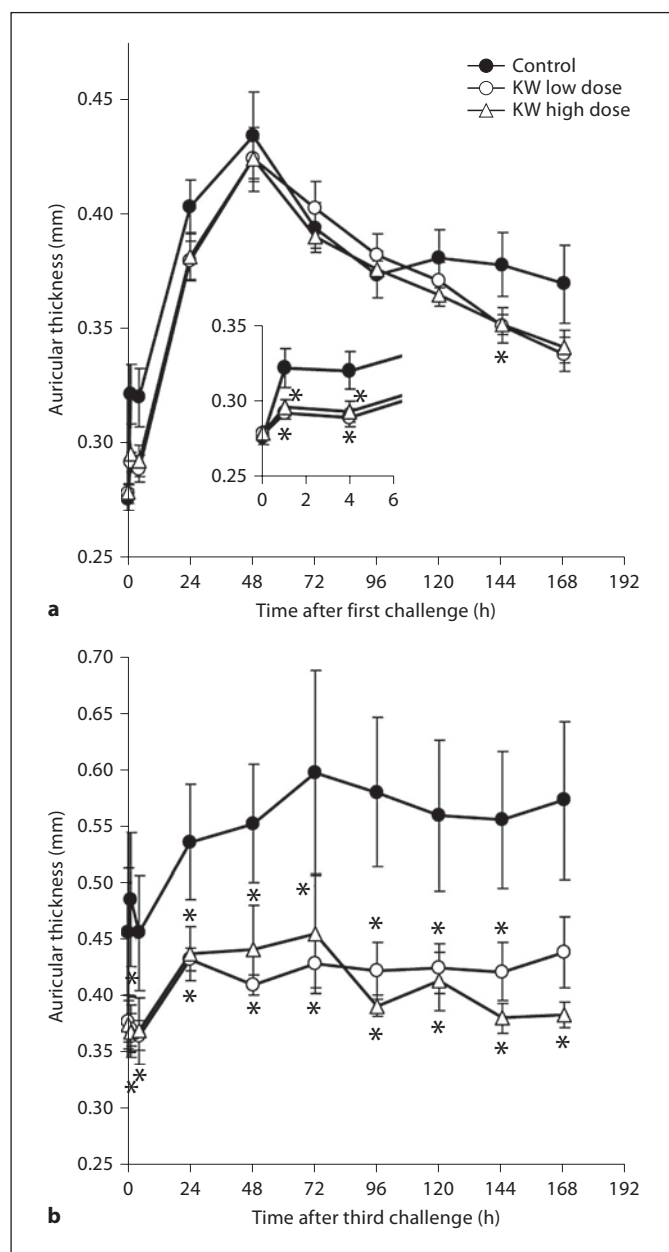


Fig. 4. Time course of cutaneous hypersensitivity responses in NC/Nga mice. The mice were fed a low or high dose of KW3110 and ear swelling in response to the first (a) and the third (b) challenge with PCl was measured. Inset is an enlarged figure of the early phase. Means $\pm$ SE (n = 10). * p < 0.05 vs. control.

Influence of Feeding Conditions on the Development of AD-Like Symptoms

As schematically presented in figure 6a, we examined the influence of feeding conditions, i.e. switching from a basic diet to the KW3110-containing diet (C→K) and vice

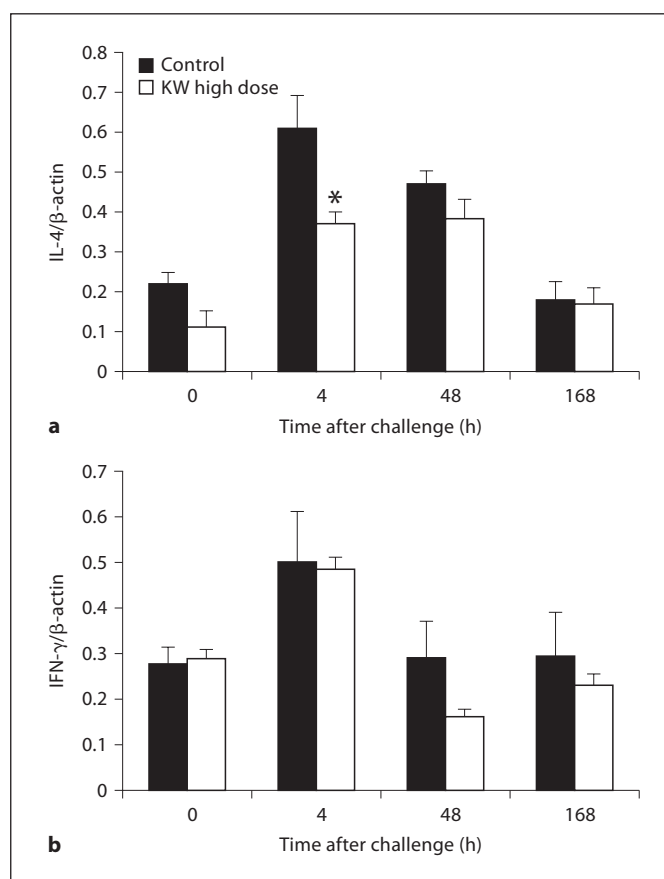


Fig. 5. Time course analysis of cytokine mRNA expression in auricles after the first PCl challenge. The mice were fed a high or low dose of KW3110 for 4 weeks. Relative mRNA expression of IL-4 (a) and IFN-γ (b) was determined by semiquantitative RT-PCR and normalization with β-actin. Means $\pm$ SE (n = 4). * p < 0.05 vs. control.

versa (K→C). On day 39 after sensitization, the clinical scores of control mice indicated the onset of AD-like symptoms, and a significant difference in clinical scores was also observed between KW and control groups (fig. 6b). The diets were then exchanged. Interestingly, the C→K group showed that supplementation of KW3110 after symptom onset was still effective at preventing further progression (fig. 6b). Since the effect occurred relatively early, the reductions in the clinical scores of the C→K group were comparable to those in the KW group within 5 weeks. The effects were observed for both auricle and scalp; thus similar inhibition levels were observed for their scores even when the evaluation was performed separately (data not shown). However, dietary manipulation was much less effective for the increasing plasma IgE lev-

els (fig. 6c). On the other hand, the K→C group demonstrated that cessation of KW3110 intake caused no immediate deterioration in symptoms and that a comparable suppressive effect was maintained for at least 5 weeks (fig. 6b). Similar to the clinical scores, the increase in blood IgE levels was also equally repressed (fig. 6c).

Comparison of KW3110 and LGG

Finally, we compared the effects of KW3110 and LGG. In vitro analysis of cytokine production from Th2-skewed splenocytes illustrated that KW3110 induced more IL-12 and less IL-4 than LGG, indicating its greater potential for correcting the Th1/Th2 imbalance (fig. 7a, b). However, no distinguishable difference in the production of IL-10 and IFN- γ was observed (fig. 7c, d). In accordance with these observations, KW3110 more significantly suppressed the clinical scores than LGG throughout the experimental period in this AD-like mouse model (fig. 8b). KW3110 also showed a greater suppressive activity on blood total IgE levels than LGG (fig. 8c), although reductions in IgG1 and IgG2 levels were comparable (fig. 3e, f). Further, at dissection mRNA for IFN- γ and IL-4 in the major lymph nodes showed that KW3110 induced IFN- γ expression in mesenteric and submandibular lymph nodes, and repressed IL-4 expression in submandibular lymph nodes (fig. 9a). These results were different from those with LGG, which caused essentially no alterations in cytokine expression in these tissues. Additionally, KW3110 but not LGG mediated a significant and persistent suppression of cutaneous hypersensitivity responses after the first challenge with PCl (fig. 9b).

Discussion

Here, we showed that dietary supplementation with *L. paracasei* KW3110 significantly alleviated the development of AD-like skin lesions in the Th2-skewed NC/Nga mouse strain in a reproducible fashion. The inhibition was not likely to have been due to a probiotic effect, with alterations in the intestinal flora, since it was caused by heat-killed LAB preparations. Interestingly, the preventive effects were sustained, and no rapid exacerbation was observed even after discontinuing supplementation. In addition, supplementation after disease onset also prevented further exacerbation of AD symptoms. These results suggest that dietary manipulation with KW3110 may be beneficial for the prevention and improvement in allergic disease symptoms. It is important to note that KW3110 supplementation caused no detectable side ef-

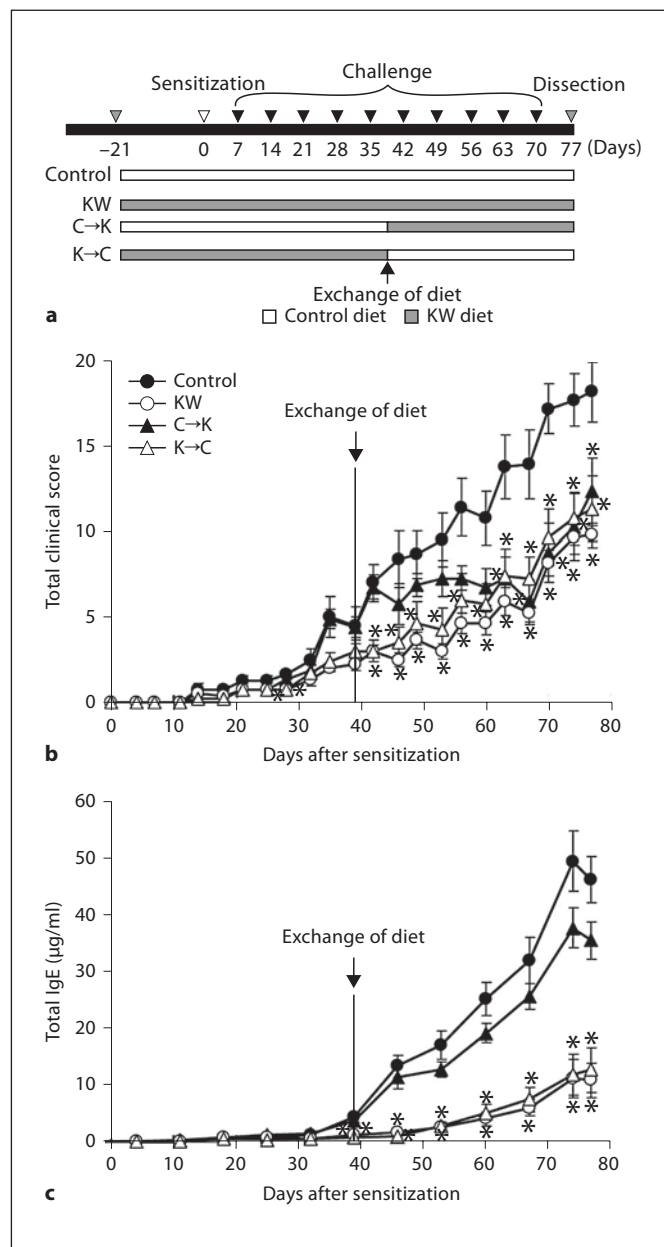
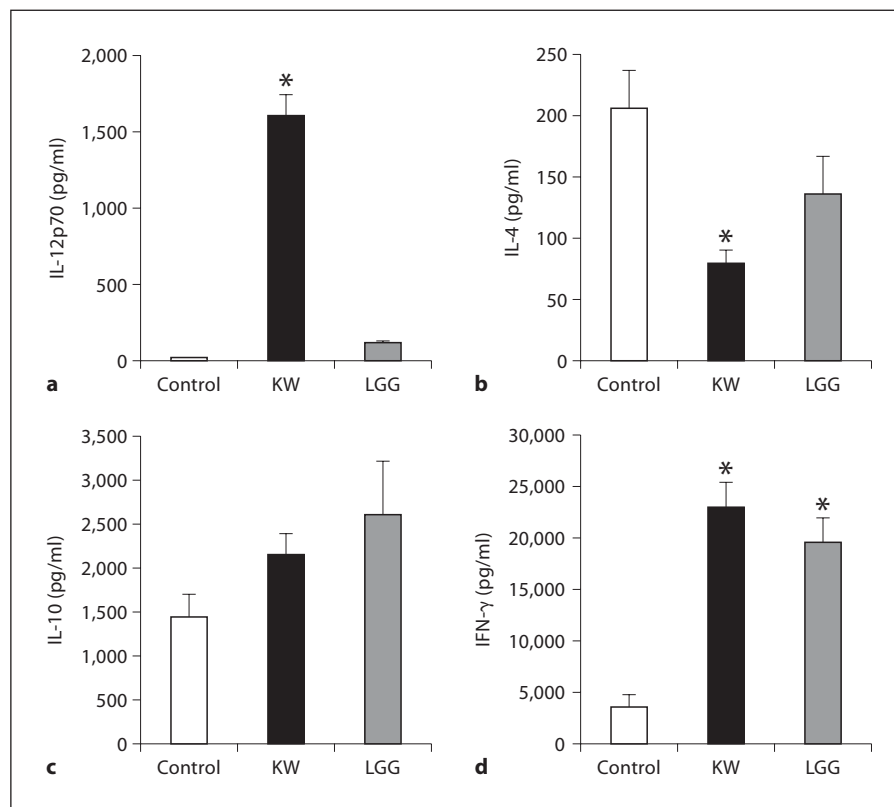


Fig. 6. Influence of feeding conditions on the development of AD-like symptoms in NC/Nga mice. Schematic representation of the experiment (a), total clinical scores (b) and total IgE levels (c) are shown. The diet was switched from basic to a KW3110-containing one (C→K) and vice versa (K→C) on day 39 as indicated by an arrow. Means $\pm$ SE (n = 8). * p < 0.05 vs. control.

fects such as food avoidance and weight loss. Whereas plasma total IgE levels showed a tendency towards dose-dependent inhibition, we could not detect dose dependency of KW3110 intake for the prevention of clinical

Fig. 7. Effects of KW3110 and LGG on cytokine production from Th2-skewed splenocytes in vitro. Splenocytes were prepared from OVA-sensitized NC/Nga mice and secretion of IL-12p70 (a), IL-4 (b), IL-10 (c) and IFN- γ (d) was measured by ELISA. Means $\pm$ SE (n = 4). * p < 0.05 vs. control.



symptoms. While the exact reason for this is still unclear, it is possible that the inhibitory effects may reach a plateau at a relatively low dose of LAB. Accordingly, further clinical and epidemiological evaluations will be required to elucidate the efficacy of KW3110 in patients at risk for AD.

In addition to the thickening of the dermis and epidermis in auricles, KW3110 intake clearly suppressed the population of mast cells, which may contribute – via histamine, LTC₄ and IL-13 secretion – to essential features of AD, pruritus and subsequent infiltration by eosinophils [24–26]. Indeed, clear suppression of eosinophil infiltration into the skin was observed histologically. Since granulocyte infiltration is frequently observed in AD patients [24, 27], these effects are likely to be of importance. Supplementation with KW3110 also inhibited the elevated plasma IgE levels in NC/Nga mice, in agreement with previous results in OVA-sensitized BALB/c mice [12]. Although the role of IgE in allergic diseases is still debated, the inhibitory effect may be beneficial since most AD patients do have increased blood IgE levels [28–30]. However, KW3110 also decreased both IgG1 and IgG2a levels in the plasma. These results are different from recent

observations that CpG-oligodeoxynucleotides [31] and preparations from *Actinidia arguta* [32], which decreased IgE and IgG1 but conversely increased IgG2a. It is known that IgE and IgG1 are commonly induced by IL-4 but IgG2a is differently regulated [33, 34]. While KW3110 revealed a less significant effect on IgG2a than on IgG1, it may not only modulate Th1/Th2 imbalance.

After the first PCI challenge during the development of skin lesions, NC/Nga mice transiently exhibited a triphasic cutaneous response, which included (1) an immediate hypersensitivity reaction; (2) a delayed-type hypersensitivity reaction, and (3) a very-late-phase reaction [35–37]. KW3110 almost completely abrogated the first and third phases but not the second phase. Since the transient transcriptional induction of IL-4 but not IFN- γ was significantly inhibited during the first phase, the suppression of Th2 activity appeared to contribute to the inhibition of the immediate hypersensitivity response. While the reason for the inhibition of the very-late-phase reaction is currently unclear, it may be associated with an inhibitory effect on granulocyte infiltration, a major pathological feature in this phase, which encompasses inflammatory reactions commonly mediated by IgE [37–39]. After the

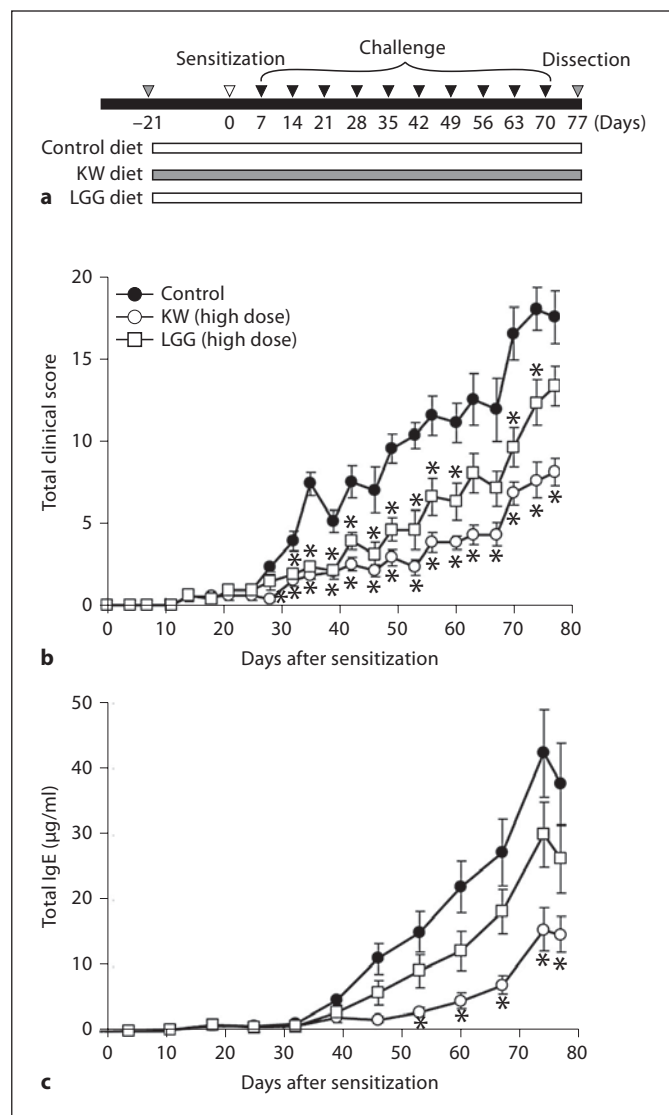


Fig. 8. Effects of KW3110 and LGG on AD-like symptoms in NC/Nga mice. Schematic representation of the experiment (a), total clinical scores (b) and total IgE levels (c) are shown. Means $\pm$ SE (n = 8). * p < 0.05 vs. control.

third challenge, the inhibitory effect of KW3110 became much clearer over the entire time course. Since previous reports indicated that consecutive hapten applications induce Th2 cytokine production and granulocyte infiltration in cutaneous lesions [40, 41], the inhibitory effect of KW3110 on these pathophysiological changes may contribute to the suppressed auricular swelling.

Since LGG is one of the best-studied LAB strains for the prevention of AD in clinical trials [7, 8, 42, 43], we also compared the anti-allergic properties of KW3110 with LGG. In assays of in vitro cytokine production from Th2-

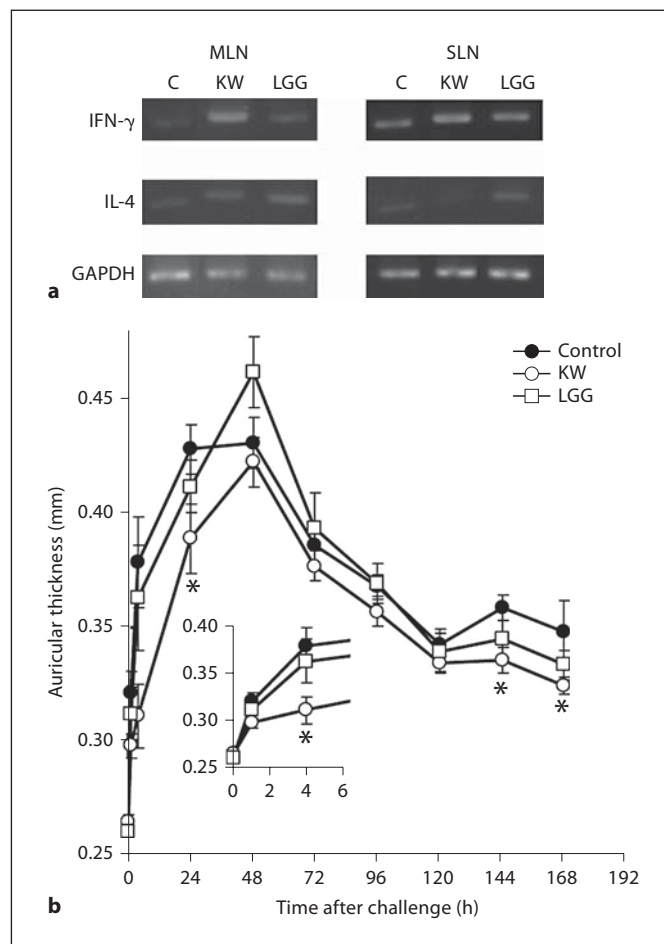


Fig. 9. Effects of KW3110 and LGG on cytokine mRNA expression in lymph nodes and cutaneous hypersensitivity reactions in auricles. a Gene expression of IFN- γ and IL-4 in mesenteric (MLN) and submandibular lymph nodes (SLN) of NC/Nga mice measured by semiquantitative RT-PCR in a representative mouse evaluated by the total clinical scores in each group (i.e. control 18; KW 9; LGG 13). C = Control. b Auricular thickness after the first PCI challenge. Inset is an enlarged figure of the early phase. Means $\pm$ SE (n = 8). * p < 0.05 vs. control.

skewed splenocytes of NC/Nga mice, KW3110 induced much more IL-12 and less IL-4 than LGG, coincident with previous results in splenocytes of BALB/c mice [12]. Together, these findings suggest a greater potential of KW3110 for improving the Th1/Th2 imbalance. The capacity for IL-12 activation may be particularly important because previous reports indicated that it correlated well with the improvement in AD symptoms by known pharmaceutical agents [31, 44]. In accordance with these differences in immunomodulating capacity, KW3110 mediated more pronounced effects than LGG on all the pa-

rameters tested in NC/Nga mice in vivo, including total clinical scores, blood IgE levels and immediate hypersensitivity responses. In addition, the distribution of cytokine mRNA in major lymphoid tissues in these mice also supported the possibility that strain-dependent differences in IL-4 and IFN- γ induction might reflect these improvements. Since NC/Nga mice fail to suppress Th2 cell differentiation due to their low production of Th1 cytokines [20], the stronger induction of host innate immune responses by KW3110 might preferably contribute to the attenuation in the symptoms in vivo. However,

STAT6-deficient NC/Nga mice, which fail to produce IgE and Th2 cytokines but express high IFN- γ levels in skin lesions, show equal clinical symptoms in AD [45]. Therefore, we cannot exclude the involvement of unknown factors other than IgE and Th2 cells. A possible target is CD4+CD25+ T cells (T_{reg}), because they suppress Th2-cell activation, and their functional failure was reported in patients with AD [46, 47]. Future studies on these different factors will also be necessary to address the anti-allergic effects of KW3110.

References

- 1 Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA: New insights into atopic dermatitis. *J Clin Invest* 2004;113:651–657.
- 2 Cooper KD: Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;102:128–137.
- 3 Yoshizawa Y, Nomaguchi H, Izaki S, Kitamura K: Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol* 2002;27:225–229.
- 4 Pivarcsi A, Homey B: Chemokine networks in atopic dermatitis: traffic signals of disease. *Curr Allergy Asthma Rep* 2005;5:284–290.
- 5 Tazawa T, Sugiura H, Sugiura Y, Uehara M: Relative importance of IL-4 and IL-13 in lesional skin of atopic dermatitis. *Arch Dermatol Res* 2004;295:459–464.
- 6 Lee GR, Flavell RA: Transgenic mice which overproduce Th2 cytokines develop spontaneous atopic dermatitis and asthma. *Int Immunol* 2004;16:1155–1160.
- 7 Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E: Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. *Lancet* 2001;357:1076–1079.
- 8 Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A: Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 2003;111:389–395.
- 9 Taylor AL, Dunstan JA, Prescott SL: Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007;119:184–191.
- 10 Brouwer ML, Wolt-Plompen SA, Dubois AE, van der Heide S, Jansen DF, Hoijsen MA, Kauffman HF, Duiverman EJ: No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. *Clin Exp Allergy* 2006;36:899–906.
- 11 Folster-Holst R, Muller F, Schnopp N, Abeck D, Kreiselmaier I, Lenz T, von Ruden U, Schrezenmeier J, Christophers E, Weichen-enthal M: Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. *Br J Dermatol* 2006;155:1256–1261.
- 12 Fujiwara D, Inoue S, Wakabayashi H, Fujii T: The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. *Int Arch Allergy Immunol* 2004;135:205–215.
- 13 Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML: Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005;115:1260–1267.
- 14 Christensen HR, Frokiaer H, Pestka JJ: Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. *J Immunol* 2002;168:171–178.
- 15 Ogawa T, Hashikawa S, Asai Y, Sakamoto H, Yasuda K, Makimura Y: A new synbiotic, *Lactobacillus casei* subsp. *casei* together with dextran, reduces murine and human allergic reaction. *FEMS Immunol Med Microbiol* 2006;46:400–409.
- 16 Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, Matsumoto M, Ushio H, Saito S, Askenase PW, Ra C: Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997;9:461–466.
- 17 Shiohara T, Hayakawa J, Mizukawa Y: Animal models for atopic dermatitis: are they relevant to human disease? *J Dermatol Sci* 2004;36:1–9.
- 18 Ohmura T, Tsunenari I, Hayashi T, Satoh Y, Konomi A, Nanri H, Kawachi M, Morikawa M, Kadota T, Satoh H: Role of substance P in an NC/Nga mouse model of atopic dermatitis-like disease. *Int Arch Allergy Immunol* 2004;133:389–397.
- 19 Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, Imai T, Yoshie O, Irimura T, Mizutani H, Matsushima K: Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 1999;104:1097–1105.
- 20 Matsumoto M, Itakura A, Tanaka A, Fujisawa C, Matsuda H: Inability of IL-12 to down-regulate IgE synthesis due to defective production of IFN- γ in atopic NC/Nga mice. *J Immunol* 2001;167:5955–5962.
- 21 Habu Y, Seki S, Takayama E, Ohkawa T, Koike Y, Ami K, Majima T, Hiraide H: The mechanism of a defective IFN- γ response to bacterial toxins in an atopic dermatitis model, NC/Nga mice, and the therapeutic effect of IFN- γ , IL-12, or IL-18 on dermatitis. *J Immunol* 2001;166:5439–5447.
- 22 Makino T, Nomoto K, Kato I, Yokokura T: Effect of growth conditions on the cellular composition and antitumor activity of *Lactobacillus casei*. *Annu Rep Yakult Central Inst Microbiol Res* 2001;21:11–17.
- 23 Hirasawa Y, Ohtsu S, Matsui Y, Nagase T, Shimizu M, Masaaki O, Kyuki K, Takahashi T, Takahashi K: Assessing effects of a product containing crude drugs including Scutellaria root, Phellodendron bark, Coptis rhizome and a product containing crude drugs including Lithospermum root, Japanese Angelica root, sesame oil in atopic dermatitis NC/Nga mice (in Japanese). *Pharmacometrics* 2000;59:123–134.
- 24 Stander S, Steinhoff M: Pathophysiology of pruritus in atopic dermatitis: an overview. *Exp Dermatol* 2002;11:12–24.

- 25 Chari S, Clark-Loeser L, Shupack J, Washe-
nik K: A role for leukotriene antagonists in
atopic dermatitis? *Am J Clin Dermatol* 2001;
2:1–6.
- 26 Akdis CA, Akdis M, Trautmann A, Blaser K:
Immune regulation in atopic dermatitis.
Curr Opin Immunol 2000;12:641–646.
- 27 Leung DY: Atopic dermatitis: the skin as a
window into the pathogenesis of chronic al-
lergic diseases. *J Allergy Clin Immunol* 1995;
96:302–318; quiz 319.
- 28 Bruijnzeel-Koomen C: The role of IgE in the
pathogenesis of atopic dermatitis. *Allergy*
1998;53:29–30.
- 29 Leung DY: Role of IgE in atopic dermatitis.
Curr Opin Immunol 1993;5:956–962.
- 30 Abramovits W: Atopic dermatitis. *J Am*
Acad Dermatol 2005;53:S86–S93.
- 31 Inoue J, Aramaki Y: Suppression of skin le-
sions by transdermal application of CpG-ol-
igodeoxynucleotides in NC/Nga mice, a
model of human atopic dermatitis. *J Immu-
nol* 2007;178:584–591.
- 32 Park EJ, Park KC, Eo H, Seo J, Son M, Kim
KH, Chang YS, Cho SH, Min KU, Jin M, Kim
S: Suppression of spontaneous dermatitis in
NC/Nga murine model by PG102 isolated
from *Actinidia arguta*. *J Invest Dermatol*
2007;127:1154–1160.
- 33 Snapper CM, Finkelman FD, Paul WE: Dif-
ferential regulation of IgG1 and IgE synthe-
sis by interleukin 4. *J Exp Med* 1988;167:183–
196.
- 34 Hasbold J, Hong JS, Kehry MR, Hodgkin PD:
Integrating signals from IFN-gamma and
IL-4 by B cells: positive and negative effects
on CD40 ligand-induced proliferation, sur-
vival, and division-linked isotype switching
to IgG1, IgE, and IgG2a. *J Immunol* 1999;
163:4175–4181.
- 35 Tahara E, Satoh T, Toriizuka K, Nagai H, Nu-
nome S, Shimada Y, Itoh T, Terasawa K, Saiki
I: Effect of Shimotsu-to (a Kampo medicine,
Si-Wu-Tang) and its constituents on triphas-
ic skin reaction in passively sensitized mice.
J Ethnopharmacol 1999;68:219–228.
- 36 Ray MC, Sharp MD, Sullivan TJ, Tigelaar
RE: Contact hypersensitivity reactions to di-
nitrofluorobenzene mediated by monoclonal
IgE anti-DNP antibodies. *J Immunol*
1983;131:1096–1102.
- 37 Satoh T, Tahara E, Yamada T, Watanabe C,
Itoh T, Terasawa K, Nagai H, Saiki I: Differ-
ential effect of antiallergic drugs on IgE-me-
diated cutaneous reaction in passively sensi-
tized mice. *Pharmacology* 2000;60:97–104.
- 38 Katayama I, Igawa K, Minatohara K, Nishi-
oka K: Topical glucocorticoid augments IgE-
mediated passive cutaneous anaphylaxis in
Balb/C mice and mast cell deficient WBB6F1
v/v mice. *Clin Exp Allergy* 1997;27:1477–
1483.
- 39 Mukai K, Matsuoka K, Taya C, Suzuki H, Yo-
kozeki H, Nishioka K, Hirokawa K, Etori M,
Yamashita M, Kubota T, Minegishi Y, Yo-
nekawa H, Karasuyama H: Basophils play a
critical role in the development of IgE-me-
diated chronic allergic inflammation indepen-
dently of T cells and mast cells. *Immunity*
2005;23:191–202.
- 40 Kitagaki H, Fujisawa S, Watanabe K, Hay-
akawa K, Shiohara T: Immediate-type hy-
persensitivity response followed by a late re-
action is induced by repeated epicutaneous
application of contact sensitizing agents in
mice. *J Invest Dermatol* 1995;105:749–755.
- 41 Kitagaki H, Ono N, Hayakawa K, Kitazawa
T, Watanabe K, Shiohara T: Repeated elicit-
ation of contact hypersensitivity induces a
shift in cutaneous cytokine milieu from a T
helper cell type 1 to a T helper cell type 2 pro-
file. *J Immunol* 1997;159:2484–2491.
- 42 Isolauri E, Arvola T, Sutas Y, Moilanen E,
Salminen S: Probiotics in the management of
atopic eczema. *Clin Exp Allergy* 2000;30:
1604–1610.
- 43 Kalliomaki M, Salminen S, Poussa T, Ar-
vilommi H, Isolauri E: Probiotics and pre-
vention of atopic disease: 4-year follow-up of
a randomised placebo-controlled trial. *Lancet*
2003;361:1869–1871.
- 44 Horiuchi Y, Bae S, Katayama I, Oshikawa T,
Okamoto M, Sato M: Lipoteichoic acid-re-
lated molecule derived from the streptococ-
cal preparation, OK-432, which suppresses
atopic dermatitis-like lesions in NC/Nga
mice. *Arch Dermatol Res* 2006;298:163–
173.
- 45 Yagi R, Nagai H, Iigo Y, Akimoto T, Arai T,
Kubo M: Development of atopic dermatitis-
like skin lesions in STAT6-deficient NC/Nga
mice. *J Immunol* 2002;168:2020–2027.
- 46 Ling EM, Smith T, Nguyen XD, Pridgeon C,
Dallman M, Arbery J, Carr VA, Robinson
DS: Relation of CD4+CD25+ regulatory T-
cell suppression of allergen-driven T-cell ac-
tivation to atopic status and expression of al-
lergic disease. *Lancet* 2004;363:608–615.
- 47 Robinson DS, Larche M, Durham SR: Tregs
and allergic disease. *J Clin Invest* 2004;114:
1389–1397.