

Oral Administration of Lactic Acid Bacteria Isolated from Traditional South Asian Fermented Milk ‘Dahi’ Inhibits the Development of Atopic Dermatitis in NC/Nga Mice

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Summary We investigated the suppressive effect of lactic acid bacteria (LAB) isolated from traditional South Asian fermented milk ‘dahi’ on the development of atopic dermatitis (AD) using NC/Nga AD model mice. In the initial evaluation, we confirmed the effect of LAB on serum total IgE using ovalbumin (OVA)-induced type 1 allergy model mice. Forty-one bacterial strains isolated from dahi were evaluated for their ability to induce interleukin (IL)-12 production and suppress IL-4 production in splenocytes obtained from OVA-sensitized mice. Of the 41 strains tested, *Lactobacillus delbrueckii* subsp. *lactis* R-037 exhibited the greatest IL-12 induction, suggesting that it is a potent Th1 inducer. Oral administration of heat-treated R-037 significantly suppressed the elevation of serum total IgE in OVA-induced type 1 allergy model mice. In NC/Nga AD model mice, oral administration of heat-treated R-037 reduced inflammatory auricular thickness and alleviated the AD clinical score although the effect on serum total IgE level was unclear. Histopathological findings showed a tendency toward improvement of inflammation. Hyperkeratosis in particular showed improvement in dermatitis skin lesions. These results suggest that oral administration of R-037 may alleviate AD.

Key Words dahi, lactic acid bacteria, atopic dermatitis, NC/Nga mice

‘Dahi’ is traditional fermented milk in South Asian countries and is similar to yogurt. It is produced by a traditional method from cow milk, buffalo milk, or their mixture. Concretely, milk is heated to boiling temperature until its volume decreases to 15–20%, cooled to body temperature, inoculated with 2–3% starter (previously made dahi), poured into an earthenware vessel, wrapped with woolen cloth or a straw or jute bag to maintain warmth, and kept overnight to induce curd formation (1). Since dahi is produced by an open fermentation method, it contains various kinds of microorganisms, such as lactic acid bacteria (LAB) and yeast (2).

Oral intake of dahi has been recommended as a means of reducing the risk of chronic diseases in Asian countries and has been highly regarded in Ayurvedic medicine. Its therapeutic importance has also been previously investigated (3). There are reports that dahi can be used to cure intestinal diseases such as diarrhea (4) and that intake of dahi has anticholesteremic, anticarcinogenic, and immunostimulating effect (3). Furthermore, it is reported that fermented milk prepared with a certain LAB strain isolated from dahi has antidiabetic effect (5) and angiotensin-converting enzyme inhibi-

tory activity (6). However, the effects of dahi on atopic dermatitis (AD) have not been studied so far.

In recent years, the number of allergic patients with eczema has been increasing worldwide, and allergy is emerging as an important public-health problem (7). AD is a chronic eczematous disorder in which the skin is dry and itchy (8, 9). Most AD patients have peripheral blood eosinophilia and their serum IgE level is increased. In contrast to the increase of T helper (Th) 2 cells and Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13, in most AD patients, Th1 cytokines such as interferon gamma (IFN- γ) and IL-12 decrease, which are capable of counteracting Th2 responses (10, 11).

NC/Nga mice, an inbred strain established from Japanese fancy mice in 1957, spontaneously develop AD-like dermatitis with IgE hyperproduction under conventional housing conditions. However, under specific-pathogen free (SPF) conditions, NC/Nga mice do not develop skin lesions (12). Recently, it has also been shown that NC/Nga mice develop AD-like skin lesions after repeated application of a hapten under SPF conditions (13). In this study, we used the hapten-induced dermatitis model, since repeated hapten treatment induces apparent dermatitis in 100% of treated NC/Nga mice (14).

Interest in antiallergic immunoregulation by LAB

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has been growing over the last decade. Some of anti-allergic LAB were isolated from traditional fermented foods, such as Kyoto pickles (15), soy sauce moromi (16), and Mongolian fermented milk (17). Traditional fermented foods-derived LAB are able to exert certain immunomodulatory effects, but there are not many reports concerning their beneficial effects on AD. In this study, we examined the effect of a new LAB strain isolated from dahi on AD development as a part of our search for the beneficial functions of dahi. We selected a LAB strain derived from dahi that induced the highest IL-12 production in splenocytes of mice sensitized by ovalbumin (OVA), and confirmed the ability of LAB to suppress serum total IgE in OVA-induced type 1 allergy model mice. We then examined the effects of the LAB strain in terms of severity of skin dermatitis, histopathological findings, and serum total IgE level in NC/Nga AD model mice.

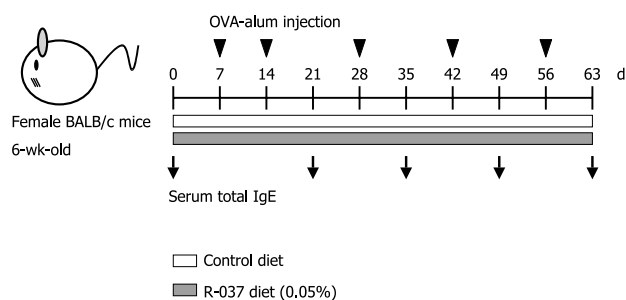
MATERIALS AND METHODS

Bacterial strains. LAB strains were prepared from dahi as described previously (2). Briefly, a diluted dahi sample was spread on plate count agar with bromocresol purple (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 0.01 g/L cycloheximide (Nacalai Tesque, Inc., Kyoto, Japan) to inhibit the growth of yeast cells. Colonies that exhibited distinct morphological features were presumed to be LAB colonies. Forty-one LAB strains isolated from dahi were inoculated in DeMan-Rogosa-Sharpe (MRS) broth (Oxoid Ltd., Basingstoke, UK), cultured at 37°C for 24 h, and collected by centrifugation at 3,000 ×g for 20 min. The cells were washed three times with saline, heat-treated at 100°C for 15 min, and lyophilized.

Animals. Female BALB/c mice (5-wk-old) and male NC/Nga mice (7-wk-old) were purchased from Charles River Japan (Kanagawa, Japan). The mice were acclimated for 1 wk before the start of experiments. They were housed in an animal room maintained on a 12-h light/12-h dark cycle at a constant temperature of 20–24°C and a relative humidity of 35–75%. They were given a standard laboratory rodent diet (CE-2; Clea Japan, Inc., Tokyo, Japan) and water ad libitum. All NC/Nga mice were housed individually throughout the experiment to prevent injury due to fighting. These studies were performed in accordance with the Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science (18).

IL-4 and IL-12 production in splenocytes derived from OVA-sensitized mice. Female BALB/c mice (6-wk-old) were given intraperitoneal injections of a mixture of 100 µg OVA (Sigma-Aldrich, St. Louis, MO, USA) and 2 mg alum (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a total volume of 200 µL on days 0 and 7. On day 14, all mice were killed and splenocytes were obtained from them. After lysis of red blood cells, the splenocytes were suspended at a concentration of 2 × 10⁶ cells/mL in RPMI-1640 medium (Invitrogen Corp., Carlsbad, CA, USA), which included 10% heat-inactivated bovine serum (Invitrogen Corp.), 50 U/mL

(a) OVA-induced allergy model



(b) AD model

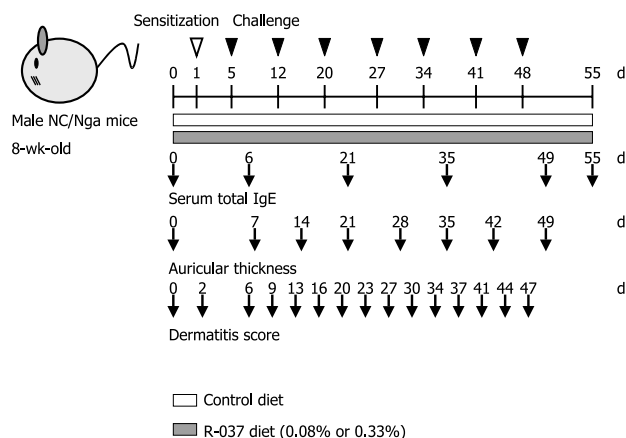


Fig. 1. Experimental procedure of (a) OVA-induced allergy model and (b) AD model.

penicillin, and 50 µg/mL streptomycin (Invitrogen Corp.). The splenocytes were cultured with 200 µg/mL OVA in the presence or absence of 1 µg/mL LAB for 7 d at 37°C in an incubator under 5% CO₂. As a positive control, 28 µg/mL of Picibanil (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), which contains 1 µg/mL *Streptococcus pyogenes* was used. The supernatant was collected for the measurement of IL-4 and IL-12 by ELISA.

OVA-induced allergy model mice. Female BALB/c mice (6-wk-old) were fed a diet (CE-2) with or without heat-treated LAB from day 0 to day 63 (8 mice per group). The diet contained 0.05% heat-treated LAB. Mean food intake was about 4 g/d/mouse, indicating that the daily intake of LAB was approximately 2 mg. The mice were given intraperitoneal injections of a mixture of 100 µg OVA and 2 mg alum at a total volume of 200 µL on days 7, 14, 28, 42, and 56. Sera were collected on days 0, 21, 35, 49, and 63, and the level of serum total IgE was determined by ELISA. The experimental procedure is shown in Fig. 1a.

AD model mice. Male NC/Nga mice (8-wk-old) were fed a diet (CE-2) with or without heat-treated LAB from day 0 to day 55 (8 mice per group). The diet contained 0.08% or 0.33% heat-treated LAB. Mean food intake was about 5 g/d/mouse, indicating that the daily LAB intake of the mice was approximately 4 mg (0.08% LAB in diet) or 16 mg (0.33% LAB in diet). The abdominal

skin was shaved and the mice were sensitized by application of 150 μ L of 5% picryl chloride (PiCl) in acetone/ethanol (1 : 4) to the shaved skin and sole of the foot on day 1, and challenged with 150 μ L of 0.8% PiCl in olive oil, which was applied to the dorsal skin and ear auricle once a week for 6 wk (days 5–48). Sera were collected on days 0, 6, 21, 35, 49, and 55, and the level of serum total IgE was determined by ELISA. The clinical score was defined as the sum of individual scores graded as 0 (subclinical), 1 (slight), 2 (moderate), or 3 (severe) for each of the four symptoms, edema, hemorrhage, excoriation/erosion, and scaling/dryness (total score=12) at intervals of 2–4 d. Auricular thickness was measured every week with a digital thickness gauge (Quickmini 25; Mitutoyo, Kanagawa, Japan). The experimental procedure is shown in Fig. 1b.

Histopathological analysis. Auricle and dorsal skin were sampled on day 55, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections were stained with hematoxylin and eosin (Sigma-Aldrich) for histopathological examination. The histopathological findings were evaluated as individual scores graded as follows: – (no abnormality), 1+ (slight), 2+ (mild), 3+ (moderate) for each of the seven findings—hypertrophy, hyperkeratosis, parakeratosis, erosion, inflammatory cell infiltration, extracellular edema, ulcer—of the ears and dorsal skin by the method of Hirasawa et al. (19) with modification.

ELISA. The concentrations of IL-12 and IL-4 in culture supernatants were determined using a Quantikine Immunoassay Kit (R&D Systems, Inc., Minneapolis, MN, USA). Serum level of total IgE was determined using a IgE Mouse EIA Kit (Yamasa Corp., Chiba, Japan).

Statistical analysis. Experimental data were analyzed with the SAS/STAT statistical analysis program (SAS Institute, Cary, NC, USA). The homoscedasticity and normal distribution of the data were confirmed for each group. Data comparisons between two groups were performed with the Wilcoxon test. Data comparisons among three groups were performed by one-way ANOVA or the Kruskal–Wallis test, followed by Dunnett's test, joint-ranking Dunnett's test, or Steel's test. Statistical significance of differences between groups was accepted at $p < 0.05$. Values in Figs. 2 and 3 are expressed as mean $\pm$ SD.

RESULTS

Effects of LAB on IL-4 and IL-12 production in splenocytes derived from OVA-sensitized mice

To evaluate the immunomodulatory effects of new LAB strains isolated from dahi, production levels of a Th1 type cytokine (IL-12) and a Th2 type cytokine (IL-4) were measured in the culture supernatant, in which splenocytes from OVA-sensitized BALB/c mice were cultured with OVA and heat-treated LAB. Forty-one LAB strains were tested and the results are shown in Table 1. Production of IL-12 was not stimulated in the non-LAB control. On the other hand, cultivation with various strains of LAB stimulated IL-12 production of spleno-

Table 1. Effect of LAB on IL-4 and IL-12 production in the splenocytes derived from OVA-sensitized mice.

	IL-4 (pg/mL)	IL-12 (pg/mL)	Name of species
Control	992.6	N.D.	—
R-001	339.7	238.5	<i>Streptococcus bovis</i>
R-027	412.5	200.0	<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i>
R-032	292.8	151.6	<i>Streptococcus bovis</i>
R-033	292.8	140.8	<i>Lactococcus lactis</i> ssp. <i>lactis</i>
R-037	145.1	449.8	<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i>
R-047	221.0	398.2	<i>Streptococcus bovis</i>
R-052	271.3	218.0	<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>
R-057	219.3	197.4	<i>Lactobacillus agilis</i>
R-058	210.3	62.7	<i>Lactobacillus fermentum</i>
R-062	397.7	218.9	<i>Streptococcus bovis</i>
R-067	197.8	164.2	<i>Pediococcus pentosaceus</i>
R-072	213.8	257.4	<i>Streptococcus bovis</i>
R-074	361.5	164.8	<i>Enterococcus faecium</i>
R-075	264.1	208.5	<i>Pediococcus pentosaceus</i>
R-079	1,060.3	N.D.	<i>Lactobacillus agilis</i>
R-080	972.0	N.D.	<i>Lactobacillus fermentum</i>
R-081	253.2	176.9	<i>Streptococcus bovis</i>
R-082	341.6	157.1	<i>Enterococcus faecium</i>
R-084	476.5	208.2	<i>Lactococcus raffinolactis</i>
R-086	305.3	203.1	<i>Lactobacillus fermentum</i>
R-096	233.6	216.3	<i>Lactobacillus fermentum</i>
R-106	327.1	116.4	<i>Streptococcus bovis</i>
R-116	308.9	182.1	<i>Streptococcus bovis</i>
R-117	240.7	247.5	<i>Enterococcus faecium</i>
R-119	327.0	199.3	<i>Pediococcus species</i> group
R-126	246.0	185.2	<i>Streptococcus bovis</i>
R-136	408.5	162.8	<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>
R-137	273.0	201.6	<i>Streptococcus thermophilus</i>
R-146	260.4	187.5	<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i>
R-147	229.9	173.1	<i>Streptococcus thermophilus</i>
R-149	185.4	150.2	<i>Lactobacillus fermentum</i>
R-183	295.6	43.0	<i>Streptococcus bovis</i>
R-188	73.9	334.0	<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i>
R-191	349.8	112.2	<i>Leuconostoc mesenteroides</i> ssp. <i>dextranicum</i>
R-195	295.3	138.4	<i>Enterococcus faecium</i>
R-198	296.2	109.3	<i>Streptococcus bovis</i>
R-201	299.4	158.0	<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>
R-206	334.2	202.7	<i>Streptococcus bovis</i>
R-211	105.6	306.6	<i>Enterococcus faecium</i>
R-212	173.2	316.7	<i>Streptococcus bovis</i>
R-216	402.5	197.2	<i>Lactobacillus fermentum</i>
Picibanil	434.5	253.1	<i>Streptococcus pyogenes</i>

Forty-one LAB including various kinds of genus were selected from 266 LAB isolated from dahi. Splenocytes were cultured with or without 1 μ g/mL of heat-treated LAB, and 28 μ g/mL of Picibanil containing 1 μ g/mL of *Streptococcus pyogenes* was used as a positive control. Seven days later, supernatants were collected to measure the cytokine production by ELISA. N.D. means not detected.

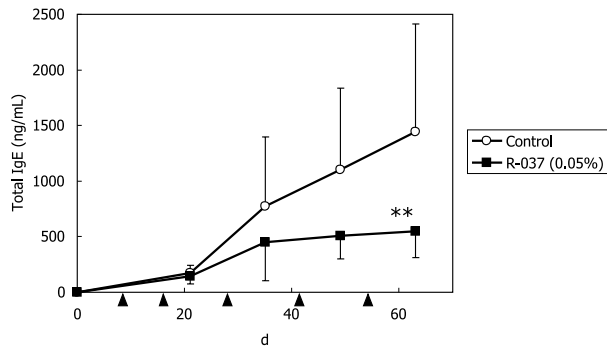


Fig. 2. Effect of R-037 on IgE production in OVA-induced allergy model mice. BALB/c mice were fed a diet with or without heat-treated LAB for 63 d, and were given intraperitoneal injection of the mixture of 100 μ g OVA and 2 mg alum on the day indicated by triangle. Serum was collected from each mouse, and total IgE was measured by ELISA. Each point and bar represents the mean $\pm$ SD of 8 mice per group. ** p < 0.01 (Wilcoxon).

cytes. The level of IL-12 production depended on the LAB strain. Several strains showed higher IL-12 induction activity than that induced by Picibanil treatment (positive control), which has been reported to induce IL-12 production and polarize the T-cell response to a Th1-dominant state. *Lactobacillus delbrueckii* subsp. *lactis* R-037 showed the highest IL-12 induction activity of all the tested LAB strains. Additionally, lower IL-4 production than that induced by Picibanil was observed in R-037-treated cells (Table 1). These results suggest that R-037 may modulate the Th1/Th2 balance toward a Th1-dominant condition.

Effect of oral administration of R-037 on IgE production in OVA-induced allergy model mice

Allergic symptoms are usually accompanied by an increase in the serum IgE level. To investigate the effect of oral administration of R-037 on serum total IgE level, BALB/c mice were repeatedly immunized with OVA-alum to increase the serum total IgE level. The control group, which fed only on the CE-2 diet, showed a marked increase in serum total IgE level. The experimental group, which fed on a CE-2 diet containing 0.05% heat-treated R-037, showed a significantly lower value than the control group on day 63 (Fig. 2). These results suggest that the administration of R-037 suppresses the allergen-induced elevation of serum total IgE.

Effect of oral administration of R-037 on the development of AD symptoms in PiCl-induced AD model mice

To confirm the effect of oral administration of R-037 on AD symptoms, heat-treated R-037 was administered to NC/Nga AD model mice. The control group fed a CE-2 diet without R-037 developed AD-like skin lesions, such as edema, hemorrhage, excoriation/erosion, and scaling/dryness, after the fourth challenge with PiCl (day 27). Clinical scores increased with the number of PiCl challenges. Heat-treated R-037 administration groups showed obvious alleviation of total clinical scores after day 34 (Fig. 3a), and dose-dependency was

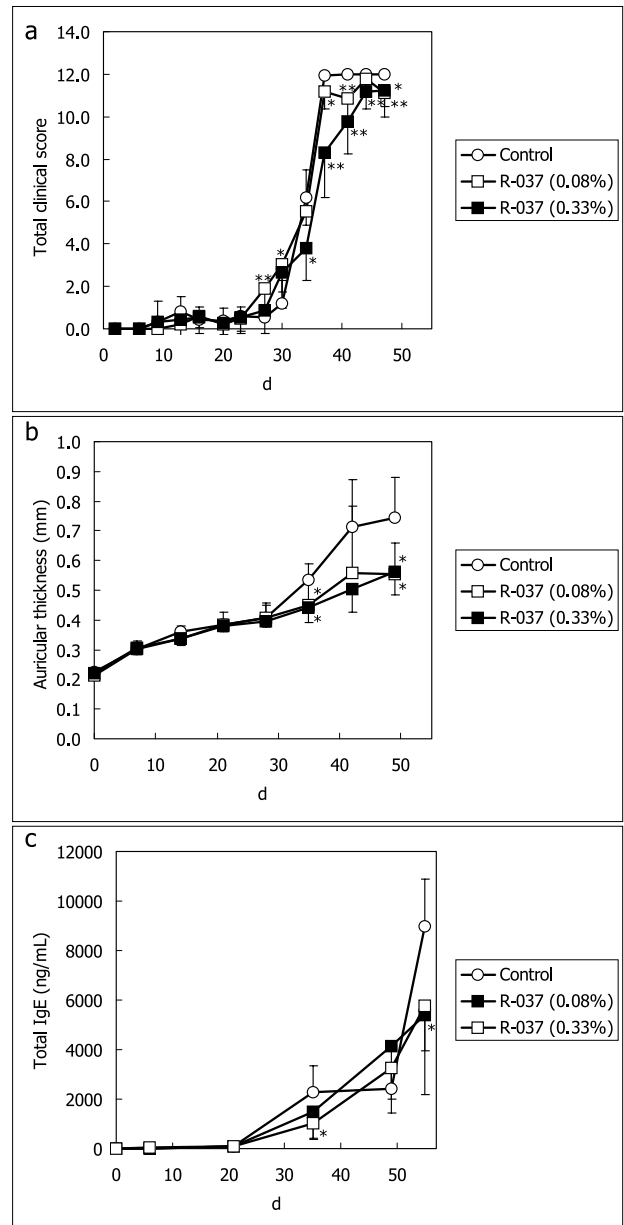


Fig. 3. Effect of R-037 on the development of dermatitis symptoms in AD model mice. NC/Nga mice were fed a diet with or without heat-treated LAB for 55 d, and were sensitized and challenged with PiCl once a week. (a) auricular thickness, (b) total clinical score, (c) serum total IgE. Each point and bar represents the mean $\pm$ SD of 8 mice per group. * p < 0.05, ** p < 0.01 vs control (a: Steel, b, c: Dunnett).

observed in the R-037 administration groups. Skin features showed a marked preventive effect of R-037 administration on the development of dermatitis, whereas the mice in the control group showed severe ear erosion and dorsal scaling, with delayed trichogenous recovery (Fig. 4a, b). Significant differences in auricular thickness were observed between the control group and the R-037 administration groups on days 35 and 49 (Fig. 3b). Serum total IgE level of the control group gradually increased with repeated PiCl challenge. The increase in serum total IgE level was significantly inhibited on day 33 in the 0.33% R-037 group. On the

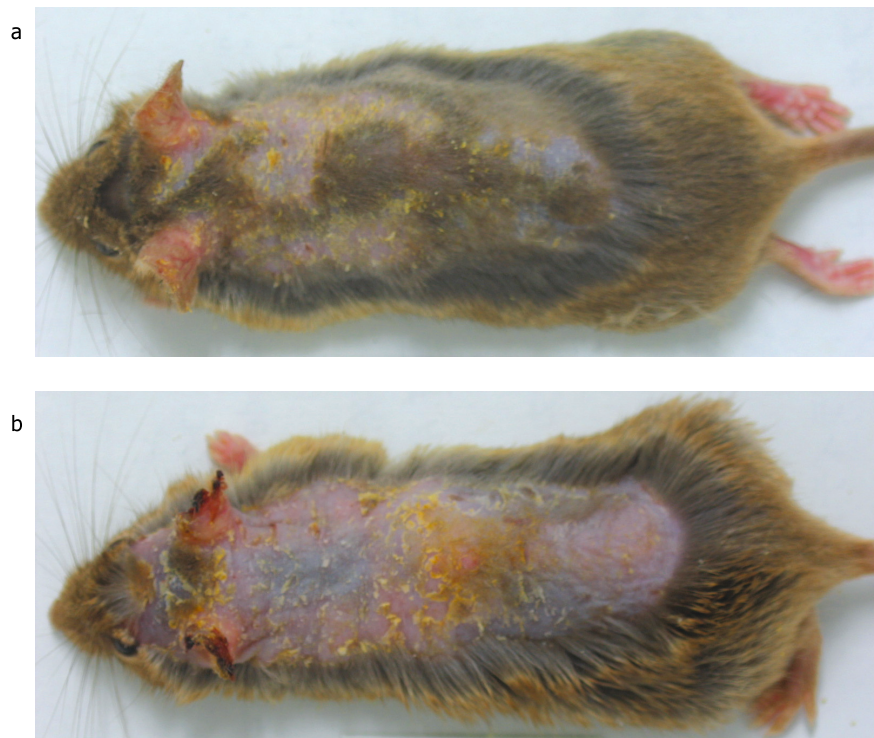


Fig. 4. Comparison of dermatitis in R-037 administration mouse and control mouse. Aspects of dorsal skin in 0.33% R-037 administration mouse (a) and control mouse (b) on day 42.

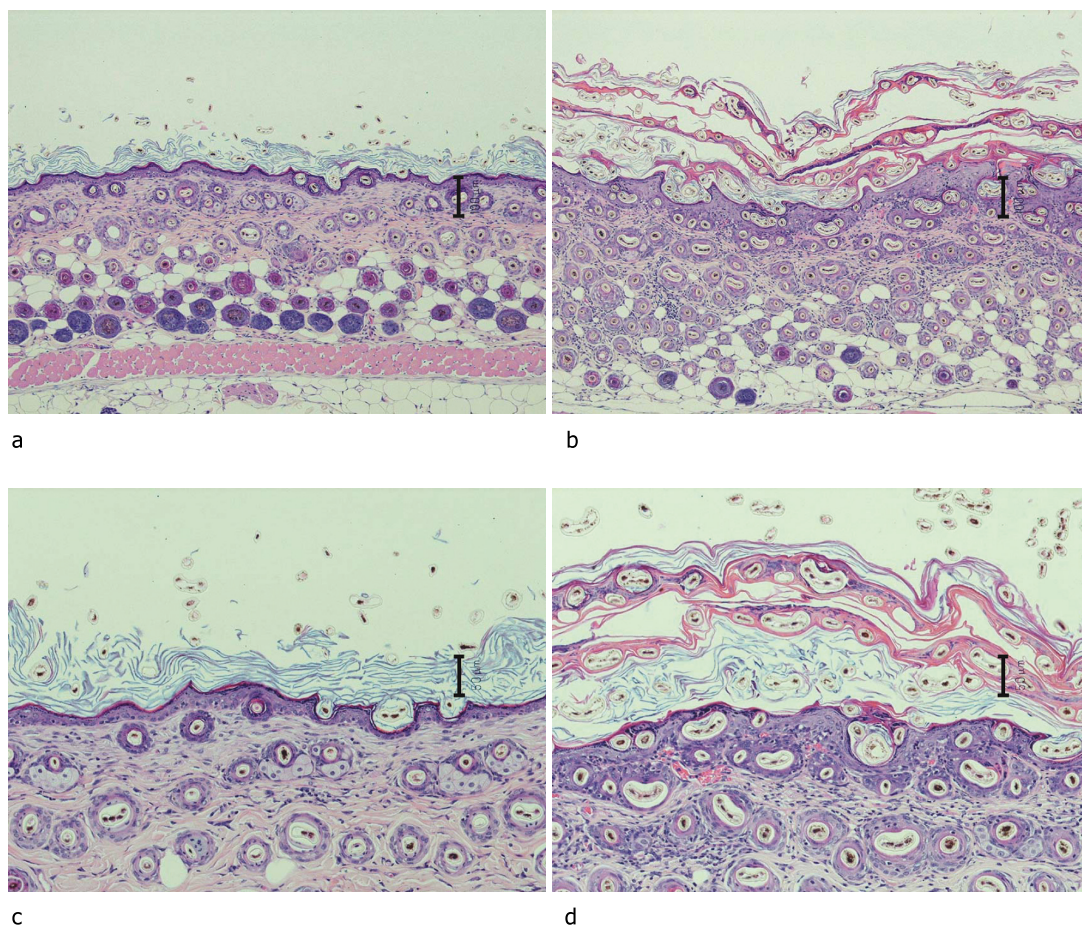


Fig. 5. Comparison of dermatitis in R-037 administration mouse and control mouse. Bright-field image of HE stained dorsal skins in 0.33% R-037 administration mouse (a, c) and control mouse (b, d) on day 55. (a, b) low magnification (bar=100 μm), (c, d) high magnification (bar=50 μm).

Table 2. Histopathological findings in dorsal skin and ears of AD model mice on day 55.

Group			Control				R-037 (0.33%)				R-037 (0.08%)			
Number			5				5				5			
Grade			–	1+	2+	3+	–	1+	2+	3+	–	1+	2+	3+
Skin	Epidermis	Hypertrophy	0	0	1	4	0	0	3	2	0	0	2	3
		Hyperkeratosis	0	0	0	5	0	0	4	1*	0	0	2	3
		Parakeratosis	0	1	3	1	0	3	2	0	0	1	3	1
		Erosion	3	1	1	0	4	1	0	0	2	2	1	0
		Inflammatory cell infiltration	0	2	3	0	0	3	2	0	0	0	5	0
		Extracellular edema	0	4	1	0	0	5	0	0	0	3	2	0
	Corium	Ulcer	4	0	1	0	5	0	0	0	5	0	0	0
		Inflammatory cell infiltration	0	0	2	3	0	0	4	1	0	0	0	5
	Subcutis	Inflammatory cell infiltration	0	3	2	0	0	4	1	0	0	2	3	0
	Ears (Outer)	Hypertrophy	0	0	1	4	0	0	2	3	0	0	3	2
		Hyperkeratosis	0	2	2	1	0	2	3	0	0	1	4	0
		Parakeratosis	1	3	1	0	0	3	2	0	1	2	2	0
		Erosion	2	0	2	1	0	1	3	1	1	2	2	0
		Inflammatory cell infiltration	0	2	3	0	0	1	4	0	0	2	3	0
		Extracellular edema	0	5	0	0	0	5	0	0	0	3	2	0
	Corium	Ulcer	5	0	0	0	5	0	0	0	5	0	0	0
		Inflammatory cell infiltration	0	0	1	4	0	0	3	2	0	0	2	3

Grade of histological finding; –: no abnormality, 1+: slight, 2+: mild, 3+: moderate.

* $p < 0.05$ vs Control (Joint-ranking Dunnett).

day the mice were killed (day 55), there was a significant difference in the serum total IgE level between the 0.08% R-037 group and the control group in NC/Nga mice. However, no significant difference was observed on day 49 (Fig. 3c).

Histopathological analysis

Histopathological examination of dorsal skin and ear was performed on day 55. The 0.33% R-037 group showed a significant improvement in hyperkeratosis of the dorsal skin compared with the control group (Table 2). All findings in the dorsal skin showed improvement on administration of R-037. Specifically, mild hypertrophy, mild hyperkeratosis, and slight or mild infiltration of inflammatory cells were observed in the epidermis and corium of the 0.33% R-037 group. In contrast, moderate hypertrophy, moderate hyperkeratosis, and mild or moderate infiltration of inflammatory cells were observed in the epidermis and corium of the control group (Fig. 5a–d). However, there were no appreciable differences in the histological findings of the ear between the control group and the R-037 group.

DISCUSSION

Allergy is thought to be provoked mainly by a Th2-predominant condition, which causes production of IgE antibody (20, 21). The Th1/Th2 balance plays a crucial role in the maintenance of immune homeostasis. IL-12 is a potent differentiation factor for Th1 cells, whereas IL-4 promotes the development of Th2 cells (22). Some

studies have reported that LAB exert antiallergic activity by modulating the Th1/Th2 balance toward a Th1-predominant condition and suppressing the serum total IgE level when administered orally to BALB/c mice (23, 24). We assessed 41 strains of LAB isolated from dahi on the basis of IL-12 production in cultured splenocytes and selected *Lactobacillus delbrueckii* subsp. *lactis* R-037, which exhibited the highest IL-12 induction and potent IL-4 suppression. The IL-12 induction activity of R-037 in vitro was greater than that of Picibanil, a known inducer of IL-12 (25). Besides this activity, oral administration of heat-treated R-037 suppressed the serum total IgE level in OVA-induced BALB/c mice. It would appear that oral administration of R-037 induced Th1 via IL-12 and suppressed Th2, thereby modulating the Th1/Th2 balance.

In this study, we investigated the suppressive effect of heat-treated R-037 on the development of AD symptoms. We used induced AD-like skin lesions in NC/Nga mice because AD induced by repeated application of Picl has higher reproducibility than spontaneous AD in NC/Nga mice. The repetitive application of haptens such as Picl and DNFB induces a Th2 immune response (26, 27). The skin lesions, with increased numbers of eosinophils, mast cells, CD4-positive T cells, and macrophages, are histopathologically similar to those in human AD and develop with elevation of serum total IgE level in NC/Nga mice (28). In this study, we demonstrated that the oral administration of heat-treated R-

037 alleviated the development of AD-like skin lesions in NC/Nga mice. In R-037 administered mice, the clinical score showed significant improvement in a dose-dependent manner. In the 0.33% R-037 administration group, histopathological analysis revealed that hyperkeratosis showed significant improvement and that the other findings showed a tendency to improve as well, such as a reduction in the population of inflammatory cells. It is likely that these preventive effects do not depend on a probiotic effect because they are caused by heat-treated R-037 preparations. In NC/Nga mice, it has been known that dietary restriction delays the onset and progression of spontaneous AD (29) and suppresses serum IgE production (30, 31). In the present study, no differences in body weight gain or food intake were observed between the control and R-037-treated groups. We therefore concluded that the suppressive effects of R-037 on AD-like skin lesions were not caused by food restriction.

In the present study, the effect of R-037 administration on serum total IgE level in NC/Nga mice was unclear because a dose-response relationship was not observed except on day 35, in contrast to the result observed in BALB/c mice. In NC/Nga mice, serum IgE suppression is reported to be comparatively difficult because they are insensitive to IL-12 compared with BALB/c mice (32, 33). Hence it is possible that the R-037 administration could not suppress IgE elevation sufficiently in NC/Nga mice.

In association with the improvement effect of LAB administration on AD in NC/Nga mice, the involvement of IgE suppression is still controversial. Sawada et al. reported that oral administration of heat-treated LAB (*Lactobacillus rhamnosus* GG) prevented the development of AD in NC/Nga mice, although there was no significant difference in the plasma total IgE level between the LAB administration group and the control group. They speculated that dermatitis in skin lesion and IgE level might not be regulated coincidentally and the LAB might drive dermatitis to normalize without regulating the IgE production in NC/Nga mice (34). On the other hand, Segawa et al. reported that there was a high correlation between clinical score and serum total IgE level while oral administration of heat-treated LAB (*Lactobacillus brevis* SBC8803) significantly prevented the development of AD and serum total IgE elevation in NC/Nga mice (35). Wakabayashi et al. reported that the increase in total IgE levels was slower than the rise in clinical score while oral administration of heat-treated LAB (*Lactobacillus paracasei* KW3110) significantly prevented the development of AD and serum total IgE elevation in NC/Nga mice (36). In our study, it would appear that the clinical score did not correlate well with serum total IgE level. We therefore speculated that appearance of AD and the serum total IgE level might not be regulated coincidentally by R-037 in NC/Nga mice.

We showed the effect of R-037 isolated from dahi on AD development. Many other LAB that have the ability to induce IL-12 production are contained in dahi. Fur-

ther studies are required on aspects such as the component ratio of these LAB in dahi, the mechanism involved in the alleviation of AD, and the effectiveness of dahi itself. Moreover, it is necessary to conduct further research with regard to the contribution of dahi to human health.

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