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Ingestion of heat-treated *Lactobacillus rhamnosus* GG prevents development of atopic dermatitis in NC/Nga mice

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Clinical and Experimental Allergy

Summary

Background Continuous oral administration of live *Lactobacillus rhamnosus* GG (*L. GG*) to pregnant subjects with atopic dermatitis and their children, suppressed the frequency of atopic dermatitis. The details of mechanisms and immune systems involved in this suppressive effect, however, remain speculative.

Objective We sought to clarify suppressive mechanisms of *L. GG* on atopic dermatitis by using NC/Nga mice, a model of human atopic dermatitis.

Methods Maternal mice and infant mice were fed with food containing or not containing heat-treated *L. GG* during pregnancy and breastfeeding, and after weaning.

Results Control NC/Nga mice raised under an air-uncontrolled condition spontaneously manifested typical skin lesions very similar to those in patients with atopic dermatitis. On the other hand, administration of food containing heat-treated *L. GG* inhibited the onset and development of atopic skin lesions, accompanied by smaller numbers of mast cells and eosinophils in the affected skin sites. Mice fed with *L. GG* showed a significant increase in plasma IL-10 levels compared with control mice, while there was no significant difference in the proportion of splenic CD4⁺CD25⁺ regulatory T cells between mice fed with *L. GG* and control mice. The IL-10 mRNA expression was enhanced in both Peyer's patches and mesenteric lymph nodes in mice fed with *L. GG*.

Conclusion These findings suggest that some components of heat-treated *L. GG* may have an ability to delay the onset and suppress the development of atopic dermatitis, probably through a strong induction of IL-10 in intestinal lymphoid organs and systemic levels.

Keywords atopic dermatitis, cytokines, IL-10, *lactobacillus*, rodent

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Introduction

Since the early 1900s, foods containing certain live bacteria have been believed to be good for human health [1]. Several species of biota resist the rigours of the human digestive system, compete with pathogens, and help to improve the gut flora balance. *Lactobacillus* strains are well known to benefit probiotic agents for the prevention and treatment of various diseases and infections [2–5]. Recent prospective reports have shown that the daily intake of live *Lactobacillus rhamnosus* GG (*L. GG*) from the period of gestation prevented the development of atopic eczema in their children, and this effect lasted over 4 years with no significant influence on serum IgE levels

[6, 7]. Probiotics are proposed, in general, to affect the mucosal barrier and the functional maturation of the host immune system [8]. There are several *in vitro* experiments to evaluate the effects of *Lactobacillus* strains on immune cells: *Lactobacillus* strains induce maturation of dendritic cells [9]; stimulate mononuclear cells to promote the production of IFN- γ , IL-12, and IL-18 in spite of weak secretion of IL-10 [10]; activate human monocytes [11]; inhibit the production of T-helper type 2 (Th2)-classified cytokines; and induce the production of Th1-classified cytokines [12]. These reports suggest that *Lactobacillus* strains may up-regulate the innate immunity and a shift from Th2- to Th1-classified immune reactions. In the clinical trial, however, ingestion of *L. GG* leads to high

serum IL-10 levels [13] and IFN- γ levels remains controversial [13, 14]. These discrepancies between the *in vitro* experiment and the clinical studies make it difficult to explain the possible mechanisms for this suppressive effect of *L. GG*. To evaluate and uncover this, further *in vivo* investigations are urgently required.

Although Th2 cells are dominant at skin lesions in the acute phase of atopic dermatitis, Th1 cells are subsequently increased with the chronic development of dermatitis [15]. These imbalances of Th1/Th2 responses are the consequence of a defect in the immune systems, sustaining normal balances in an unsuitable response to environmental antigens (Ag). Some mechanisms have been proposed to control and regulate the immune balances to restrain or minimize reactivity to over exuberant responses. One candidate is naturally occurring CD4⁺CD25⁺ regulatory T (Treg) cells that were originally characterized as T cells that prevent development of organ-specific autoimmune disease and allograft rejection *in vivo* and inhibit proliferation of CD4⁺CD25⁻ effector T cells *in vitro* [16]. CD4⁺CD25⁺ Treg cells also inhibit both of Th1 and Th2 reactions [17, 18]. Indeed, children who outgrew their allergy have higher frequencies of circulating CD4⁺CD25⁺ Treg cells and decreased proliferative responses to antigen in PBMC [19]. The second candidate is IL-10, which was originally recognized for its ability to inhibit activation and effector functions of T cells, monocytes, and macrophages [20, 21]. IL-10 also inhibits Ag presenting function on dendritic cells and induces a state of anergy and tolerance in allo-Ag- or peptide Ag-activated T cells [22, 23]. It is clear that this suppressive cytokine is produced by many cell populations, including T cells, B cell macrophages and dendritic cells [24]. In particular, previous studies have suggested that the T cell subset, IL-10-producing Treg cells, might be induced in the absence of Th1 and Th2 responses and were involved in establishing unresponsiveness to Ag [24, 25]. Indeed, *Lactobacillus* strains induce IL-10-producing Treg cells *in vitro* [26]. These findings lead us to speculate that *Lactobacillus* strains influence the frequencies of CD4⁺CD25⁺ Treg cells or the production of IL-10, resulting in inhibition of atopic diseases.

NC/Nga mice are widely accepted as a suitable model for human atopic dermatitis [27], in which mice spontaneously develop skin lesions correlating with the increase in total IgE levels when they were raised under an air-uncontrolled conventional conditions. We evaluated the suppressive effect of orally administration of heat-treated *L. GG* on development of atopic skin lesions in NC/Nga mice. Heat-treated *L. GG* significantly delayed the onset and suppressed the development of skin lesions in NC/Nga mice when they were fed with food containing *L. GG* from the period of maternal pregnancy and breastfeeding, and after ablactation.

Materials and methods

Mice

Specific pathogen-free (SPF) NC/Nga mice were obtained from Charles River Japan (Kanagawa, Japan) and raised in our laboratory. These mice were maintained in a filter-laminar flow enclosure in a bio-clean room, and were provided with γ -irradiated food and autoclaved water *ad libitum*. To obtain mice with atopic skin lesions, several mating pairs of SPF NC/Nga mice were moved to an air-uncontrolled conventional room [27, 28]. The care and handling of the animals were in accordance with the 'Tokyo University of Agriculture and Technology Animal Experiment Guidelines, April 2001.'

Preparations of *Lactobacillus rhamnosus GG*

CRF-1 (Charles River Japan) containing frozen-dried *L. GG* (ATCC 53103) (4×10^9 CFU/g) was prepared and heat treated at 90 °C for 105 min, and less than 4×10^4 CFU/g *L. GG* survived in the food pellets. The daily intake of heat-treated *L. GG* was estimated to be 30–50 mg/adult mouse.

Feeding and sampling

Female NC/Nga mice were provided with pellets containing with or without *L. GG* from day 14 of pregnancy. During the pregnancy and breastfeeding, maternal mice were fed with the pellets *ad libitum*. Four weeks after birth, their infants were weaned from the breast and continued to be fed with the pellets. The clinical severity of skin lesions was observed in mice (control, $n = 17$; *L. GG* = 16) from the age of 4–12 weeks. Mice were killed and examined for plasma IgE levels (control, $n = 17$; *L. GG* = 16), and histological analysis was conducted in 12-week-old mice ($n = 12$). Blood samples and fecal samples were collected from mice at the age of 10 weeks, and plasma cytokines and fecal bacteria were measured. To analyse CD4⁺CD25⁺ Treg cells and cytokine expressions, 10-week-old mice were killed and their spleens were collected for flow cytometric analysis. Axillary lymph nodes, Peyer's patches, and mesenteric lymph nodes were collected from 10-week-old mice for quantitative real-time PCR. These experiments were conducted separately using individual groups of mice. The number of mice used in each experiment is described in each figure.

Clinical skin severity score

The severity of dermatitis was evaluated by scoring clinical signs and symptoms on the face, ears, nose, neck, and dorsal skin as described previously [27]. A total of clinical severity scores was defined as the sum of scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe)

for each of five aspects: itch, erythema/haemorrhage, oedema, excoriation/erosion, and scaling/dryness.

Histological examination

Dorsal skin pieces were fixed with 10% phosphate-buffered formalin (pH 7.2). Serial paraffin sections (4.5- μ m thick) were made by the routine method and stained with toluidine blue (pH 4.0) for counting mast cells, Congo red for counting eosinophils, and Mayer's H&E for evaluating general pathological features.

Measurement of total immunoglobulin E

IgE levels were measured by a sandwich ELISA using two kinds of rat anti-mouse IgE monoclonal antibody (mAb) (6HD5 and HMK12, Yamasa Shoyu, Chiba, Japan) according to the method described [27]. The sensitivity of this assay was 2 ng/mL.

Flow cytometric analysis

Spleen cells were stained with anti-CD25-PE mAb (7D4, Southern Biotechnology Associates, Birmingham, AL, USA) and anti-CD4-FITC mAb (RM4-5, BD Biosciences, Franklin Lakes, NJ, USA). Expressions of CD4 and CD25 were measured by flow cytometric analysis (EPICS XL, Becton Coulter, Miami, FL, USA) and analysed by an EPICS XL SYSTEM II software (Becton Coulter).

Real-time reverse transcription-polymerase chain reaction

Tissue samples were collected from mice and frozen at -80°C until use. Total RNA was extracted from frozen samples with ISOGEN (Nippon gene, Tokyo, Japan) according to the manufacturer's instructions. cDNA was synthesized with Superscript II (Invitrogen, Carlsbad, CA, USA) with an oligo(dT)₁₂₋₁₈ primer (Invitrogen). Gene expression was determined by quantitative real-time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The following sequences were used: *FoxP3* primers, 5'-CGAAAGTGGCA GAGAGGTATTGA-3' and 5'-CTGAAGGCAGAGTCAGGAG AAGTT-3'; IFN- γ primers, 5'-TGCATCTTGGCTTTGCAGC TCTTCCTCATGGC-3' and 5'-TGGACCTGTGGGTGTGTTGAC CTCAAACCTGGC-3'; IL-4 primers, 5'-CGAAAGCCCC GAAAGAGTC-3' and 5'-GGAGATGGATGTGCCAAC-3'; IL-10 primers, 5'-TGTGAAAATAAGAGCAAGGCAGTG-3' and 5'-CATTCATGGCCTTGTAGACACC-3'; TGF- β primers, 5'-TGACGTCACTGGAGTTGTACGG-3' and 5'-GGTTCAT GTCATGGATGGTGC-3'; and actin primers, 5'-TGGTCGT ACCACAGGCATTG-3' and 5'-TGATGTCACGCACGATT CC-3'. Target gene mRNA expression was quantified using SYBR Green (Applied Biosystems) and normalized to the actin mRNA levels.

Measurement of plasma cytokine levels

Blood samples were collected from the abdominal aorta of anaesthetized mice. Plasma concentrations of cytokines were assayed by a ProteoPlex™ 16-well murine cytokine array kit (EMD Biosciences, Darmstadt, Germany) according to the manufacturer's instructions.

Fecal fluorescence in situ hybridization

Fecal samples ($n=6$) were analysed for fluorescence *in situ* hybridization (FISH) as described previously [29]. Briefly, samples were suspended in phosphate-buffered saline and homogenized. Bacteria were fixed with paraformaldehyde and hybridized with a Cy3 indocarbocyanine-labelled oligonucleotide probe. Probes included Bif164 for bifidobacteria, Bac303 for bacteroides, Lab158 for lactobacilli and enterococci, and His150 for clostridia. Total bacterial counts were determined by staining with 4',6-diamino-2-phenylindole (DAPI). The bacteria were washed and filtered on a 0.2- μ m polycarbonate filter. The filters were mounted on a slide and counted visually under an epifluorescence microscope using Cy3- and DAPI-specific filters.

Statistical analysis

A two-tailed Student's *t*-test was performed for statistical analysis of the data, and $P < 0.05$ was considered as the level of significance.

Results

Suppression of dermatitis in NC/Nga mice by feeding with heat-treated Lactobacillus rhamnosus GG

Various grades of dermatitis were observed clinically on the face, ears, nose, neck, and dorsal skin in mice fed with control food, starting from the age of 4 weeks, and the severity scores of skin lesions increased drastically with age (Figs 1a and b). On the other hand, NC/Nga mice fed with *L. GG* manifested only lower grades of dermatitis until the age of 12 weeks: less than half the levels in skin severity scores as compared with control mice (Fig. 1b). Moreover, the ingestion of food with *L. GG* delayed the onset of dermatitis. Thus, we concluded that continuous feeding of food with *L. GG* significantly suppressed the onset and development of atopic lesions in NC/Nga mice raised under an air-uncontrolled conventional conditions.

Histological features of skin lesions in NC/Nga mice

In control NC/Nga mice fed with control food alone, typical histological features of atopic dermatitis were

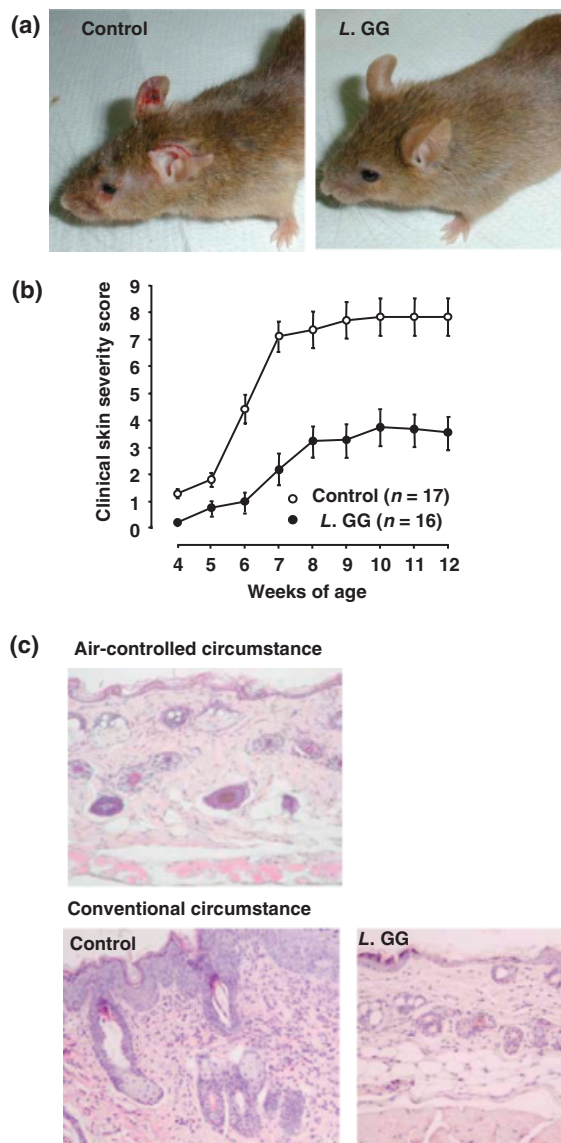


Fig. 1. Skin aspects in 12-week-old NC/Nga mice fed with or without food containing *Lactobacillus rhamnosus* GG (*L. GG*). (a) Male NC/Nga mice. (b) Clinical skin severity scores. (c) H&E-stained skin sections of mice.

observed in dorsal skins at the age of 12 weeks: a thickened epidermis with moderate hyperplasia with elongation of the rete ridges and prominent hyperkeratosis, slight intercellular oedema, an increased number of mast cells with moderate or marked degranulation, and infiltration of numerous eosinophils and mononuclear cells (Fig. 1c and Table 1). In contrast, mice fed with *L. GG* showed histological features similar to those of mice without dermatitis grown under an air-controlled conditions. Only slight changes were observed in the dorsal skin of mice fed with *L. GG*: a small increase in the number of mast cells with mild degranulation and mild infiltration of eosinophils and mononuclear cells (Fig. 1c). The numbers

Table 1. The numbers of mast cells and eosinophils in dorsal skins of NC/Nga mice fed with or without *Lactobacillus rhamnosus* GG (*L. GG*)[‡]

<i>L. GG</i>	Granulated	Mildly degranulated	Severely degranulated	Eosinophils
No	568.2 ± 76.1	30.9 ± 7.9	8.4 ± 2.5	10.9 ± 1.7
Yes	234.0 ± 24.1*	8.7 ± 1.8 [‡]	0.8 ± 0.3*	2.8 ± 0.7*

* $P < 0.001$,

[‡] $P < 0.05$, when compared with control mice.

[‡]Cells between the epithelium and *panniculus carnosus* were counted under a microscope at a magnification of $\times 100$ for mast cells and $\times 400$ for eosinophils, and were expressed as the number of the cells per centimetre and in five fields, respectively. Each value represents the mean $\pm$ SE ($n = 12$).

of mast cells and eosinophils in mice fed with *L. GG* were significantly lower than in control mice (Table 1).

Plasma total immunoglobulin E levels

As total IgE levels in the peripheral blood are increased, correlating with the clinical severity of dermatitis in NC/Nga mice [27] and patients with atopic diseases have a hereditary pre-disposition to produce IgE antibody against common environmental allergens [30], we assessed plasma levels of total IgE in NC/Nga mice at the age of 12 weeks. Although IgE levels tended to be decreased in mice fed with *L. GG*, there was no significant difference between mice fed with and without *L. GG* (the concentration of plasma total IgE in mice fed with *L. GG* was 9.3 ± 0.8 and 11.0 ± 1.0 $\mu\text{g/mL}$ in control mice).

Regulatory T cells in spleens

To assess whether Treg cells were expanded in mice fed with *L. GG*, we quantified the proportion of $\text{CD4}^+\text{CD25}^+$ T cells in spleens of mice at the age of 10 weeks (Figs 2a and b). There was no significant increase in the proportion of $\text{CD4}^+\text{CD25}^+$ T cells in mice fed with *L. GG*, when compared with control mice. Rather, the proportion of $\text{CD4}^+\text{CD25}^+$ T cells was increased in relation to the severity of dermatitis, and a significant correlation ($r = 0.59$; $P = 0.016$) was observed between clinical severity scores and the proportion of $\text{CD4}^+\text{CD25}^+$ T cells (Fig. 2c). We also assessed mRNA expressions of *Foxp3*, a specific transcription factor for $\text{CD4}^+\text{CD25}^+$ Treg cells (Fig. 2d), in spleens; however, there was no significant difference between mice fed with *L. GG* and control mice.

Plasma cytokine levels

To assess another candidate inhibiting atopic dermatitis, we measured plasma levels of cytokines in mice fed with or without *L. GG* (Fig. 3). Mice fed with *L. GG* showed significantly higher levels of IL-10 than those in control mice.

Plasma levels of IL-1, IL-2, IL-4, and IFN- γ tended to increase in mice fed with *L. GG* as compared with those in control mice, although no significant differences were observed.

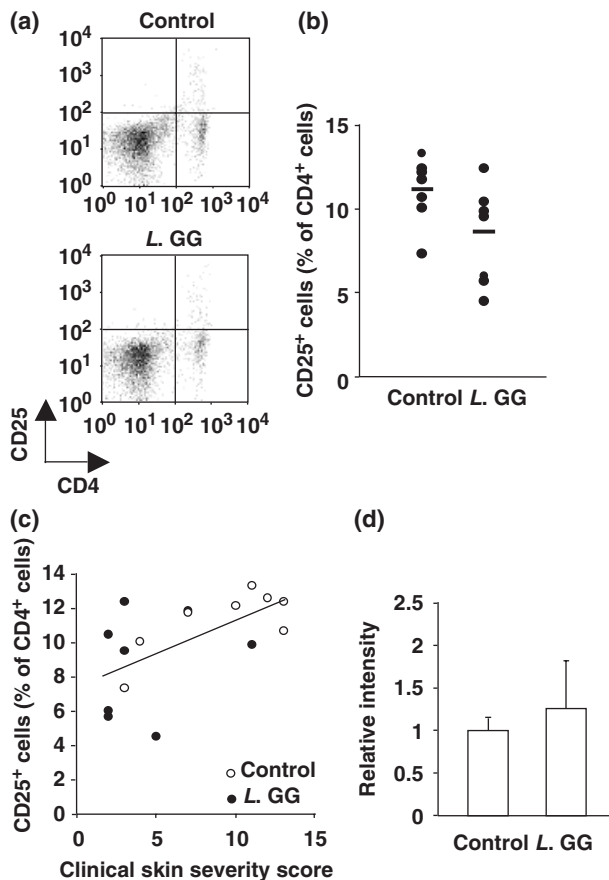


Fig. 2. Proportion of CD4⁺CD25⁺ T cells in spleens in mice at the age of 10 weeks. (a) Dual plots represent CD4⁺CD25⁺ T cell pools. (b) Individual percentages of CD25⁺ cells within CD4⁺ T cells. (c) Dual plots of clinical skin severity scores and percentages of CD25⁺ cells. A calculated linear regression line is shown. (d) Relative gene expression of *Foxp3* in spleens. Each value represents the mean $\pm$ SE ($n = 4-5$).

Cytokine expressions in spleens, axillary lymph nodes, Peyer's patches, and mesenteric lymph nodes

To address the source of cytokines that influenced systemic cytokine levels, we evaluated cytokine expressions in several lymphoid organs. In spleens, no significant difference was observed in mRNA expressions of IFN- γ , IL-4, TGF- β , and IL-10 between the two groups (Fig. 4a). On the other hand, in axillary lymph nodes, expressions of IFN- γ and IL-4 in mice fed with *L. GG* tended to be lower than that in control mice, and there was a significant difference in the IL-10 expressions between the two groups (Fig. 4b). The discrepancy between systematic cytokine levels and cytokine expressions in axillary lymph nodes prompted us to further explore the source of the high levels of IL-10 in plasma. We assayed the expressions of cytokine mRNA in Peyer's patches and mesenteric lymph nodes (Figs 4c and d). It was noted that the high expressions of IL-10 mRNA were detected in both Peyer's patches and mesenteric lymph nodes in mice fed with *L. GG*. In addition, the expressions of IFN- γ and IL-4 tended to be increased. Thus, the mRNA expressions of IL-10 in Peyer's patches and mesenteric lymph nodes were similar to the profile of these concentrations in plasma, but different from that in axillary lymph nodes. Although we evaluated microbes in fecal samples by FISH analysis to investigate the possibility that heat-treated *L. GG* influenced the intestinal microbiota, there was no significant difference in the number of each bacterium between mice fed with and without *L. GG* (Table 2).

Discussion

Continuous ingestion of regular food containing heat-treated *L. GG* in pregnant NC/Nga mice raised under an air-uncontrolled condition not only delayed the onset of dermatitis but also markedly suppressed its development in all the progeny. The improvement in clinical

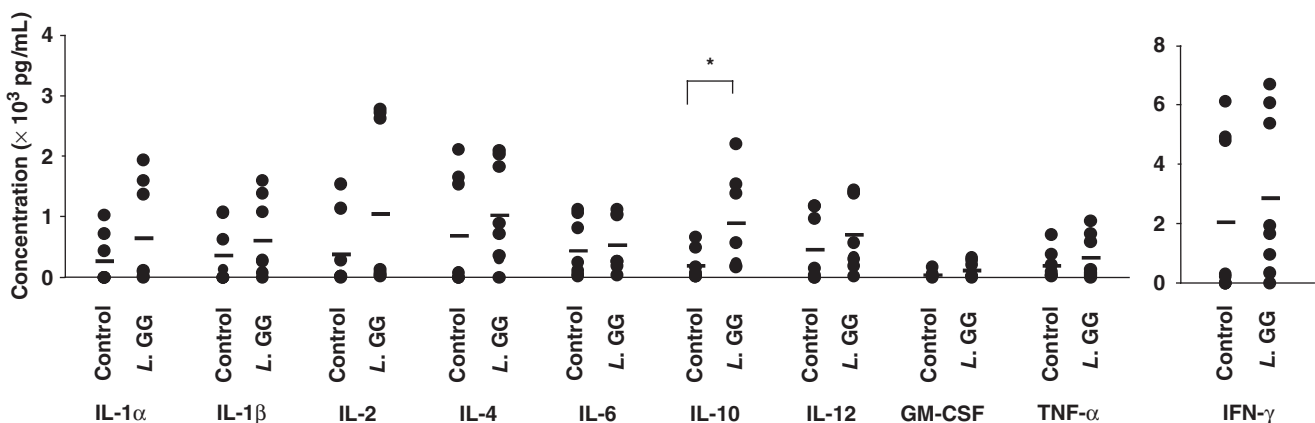


Fig. 3. Plasma cytokine levels in mice fed with or without *Lactobacillus rhamnosus* GG (*L. GG*) at the age of 10 weeks. Levels of IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, TNF- α , and IFN- γ were measured using a cytokine array kit. Each point represents individual concentrations, and each line represents the mean value of each group ($n = 8$). $*P < 0.05$

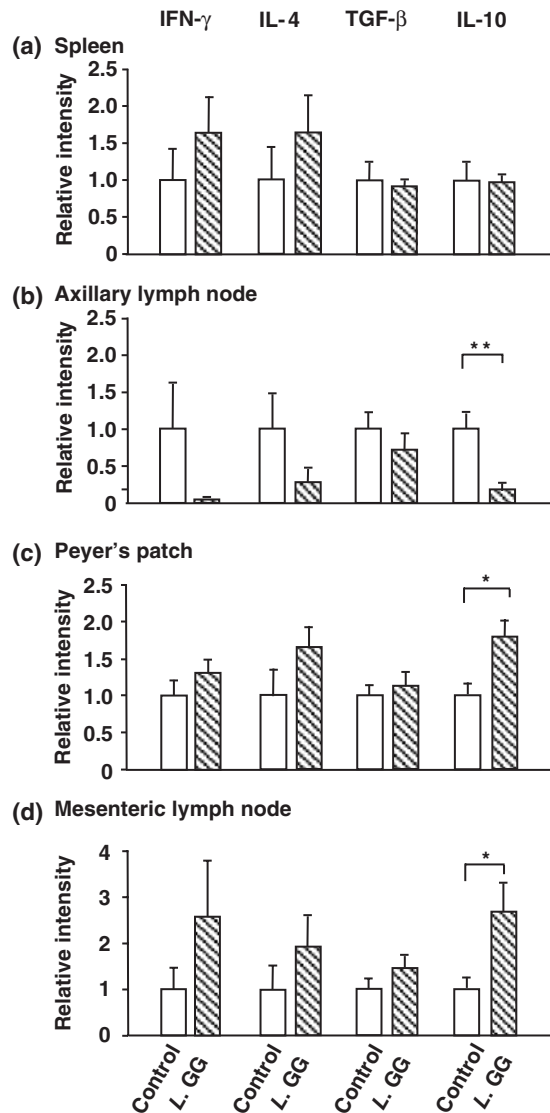


Fig. 4. Expressions of cytokine genes in spleens, axillary lymph nodes, Peyer's patches, and mesenteric lymph nodes. Total RNA was extracted from (a) spleens, (b) axillary lymph nodes, (c) Peyer's patches, and (d) mesenteric lymph nodes from 10-week old mice fed with or without *Lactobacillus rhamnosus* GG (L. GG). mRNA expressions of IFN- γ , IL-4, TGF- β , and IL-10 were evaluated by using quantitative real-time PCR. Each value represents the mean $\pm$ SE (a and b, $n = 4-5$; c and d, $n = 6$). * $P < 0.05$, ** $P < 0.01$.

aspects was accompanied by slight epidermal thickness and decreased number of inflammatory cells in the skin lesions. These results were similar to previous prospective reports that showed the preventive effect of probiotics on atopic diseases in humans [6, 7]. Therefore, the effect of L. GG on atopic dermatitis seemed to be retained in both human and mouse even if L. GG was heat treated. As the food was frozen, dried, and heated to kill L. GG, there was less possibility that live L. GG influenced the intestinal microbiota, contributing to the improvement of dermatitis. Indeed, the fecal bacteria present in the gut of NC/Nga

Table 2. Bacterial counts in fecal samples of NC/Nga mice fed with or without *Lactobacillus rhamnosus* GG (L. GG) at the age of 10 weeks*

Bacteria	Control	L. GG	P
Bifidobacteria	0.00 $\pm$ 0.00	1.05 $\pm$ 1.05	0.39
Bacteroides	9.00 $\pm$ 0.06	9.08 $\pm$ 0.09	0.46
Lactobacilli/Enterococci	8.61 $\pm$ 0.15	8.59 $\pm$ 0.12	0.92
Clostridia	8.54 $\pm$ 0.14	8.14 $\pm$ 0.23	0.16

*Each value represents the mean log₁₀ cells/g $\pm$ SE ($n = 6$).

mice fed with L. GG were not different from those in the gut of control mice. Heat-treated *Lactobacillus* strains and the cell wall of *Lactobacillus casei* are effective in protecting against intestinal inflammation [31] and reducing contact hyper-sensitivity reaction [32]. Several nutrients have been proposed to have the ability to modulate immune responses and counter inflammatory processes. These immunonutrients show a pharmacologic effect on immune and inflammatory parameters [33]. Hence, as immunonutrition, certain constitutional components of heat-treated L. GG are speculated to lead to an improvement in atopic dermatitis without having an influence on the gut microbiota.

There was no significant difference in plasma levels of total IgE between mice fed with L. GG and control mice as reported in the human prospective study [6]. We speculated that dermatitis in skin lesions and IgE levels might not be regulated coincidentally and that L. GG might drive dermatitis to normalize, not IgE production.

We attempted to investigate whether ingestion of L. GG influenced the expansion of CD4⁺CD25⁺ Treg cells. However, no proportion of CD4⁺CD25⁺ Treg cells was changed in spleens of mice fed with L. GG, suggesting that other candidates might be responsible for improving dermatitis by ingestion of L. GG. Moreover, an increase in the proportion of CD4⁺CD25⁺ Treg cells was positively correlated with the severity of dermatitis. There is convincing evidence that the proportion of CD4⁺CD25⁺ Treg cells is increased in patients with atopic dermatitis and atopic asthma [34, 35]. Therefore, inflammation at the affected skin sites may eventually lead to the expansion of naturally occurring CD4⁺CD25⁺ Treg cells without having any correlation with the administration of L. GG.

It is noteworthy that high levels of IL-10 were detected in the plasma of mice fed with L. GG. Oral ingestion of L. GG has an effect on the induction of plasma IL-10 in atopic children [13, 36]. Moreover, IL-10, an anti-inflammatory cytokine that inhibits expressions of MHCII and CD80, and CD86 on monocytes [37, 38], contributes to the state of anergy to Ag [22]. Therefore, there is a possibility that IL-10 produced by heat-treated L. GG reduces allergic sensitization and attenuates induction of atopic dermatitis. We embarked on more detailed examinations to explore the source of IL-10 because expressions of

cytokines in axillary lymph nodes did not reflect systemic cytokine levels. In contrast to axillary lymph nodes, the expressions of IL-10 in Peyer's patches and mesenteric lymph nodes were markedly enhanced in mice fed with *L. GG*. Thus, continuous ingestion of heat-treated *L. GG* may strongly induce the production of IL-10 in intestinal lymphoid organs and enhance systemic levels of IL-10, resulting in suppression of sensitization and symptoms of dermatitis at the affected skin sites. The fact that *Lactobacillus* strains induced IL-10-producing Treg cells by modulating dendritic cell functions [26] supports the possibility that heat-treated *L. GG* may induce IL-10-producing Treg cells in Peyer's patches and mesenteric lymph nodes.

Intestinal dendritic cells up-take commensal bacteria in mucosal tissue and migrate into mesenteric lymph nodes, where in lymph nodes function as a barrier to prevent the microbiota from reaching the systemic compartment of the host immune system [39, 40]. One subset of dendritic cells, CD11b⁺CD8a⁻CCR6⁺ myeloid-type dendritic cells isolated from Peyer's patches, prime naïve T cells to secrete a high level of IL-10 in response to microbial stimulation [41]. This ability is not attributable to myeloid dendritic cells from axillary and inguinal lymph nodes. Therefore, it has been assumed that myeloid dendritic cells up-taking *L. GG* migrate into Peyer's patches or mesenteric lymph nodes, where they stimulate T cells to secrete IL-10 strongly. Considering no induction of IL-10 by *L. GG* in spleens and axillary lymph nodes, dendritic cells might not directly influence systemic immune systems because of the barrier function in these intestinal lymphoid organs. However, highly secreted IL-10 in Peyer's patches and mesenteric lymph nodes might influence systemic IL-10 levels and indirectly affect local immune systems, resulting in the prevention of dermatitis.

Peripheral blood mononuclear cells derived from children with atopic dermatitis had impaired IL-10 secretions in response to not only allergens but also heat-treated *Lactobacillus* species when cells were assayed *in vitro* [42]. Taking *L. GG* orally as food, on the other hand, infants with atopic dermatitis showed significant increases in the serum IL-10 levels [36]. These reports support the fact that *L. GG* provokes IL-10 production not from peripheral blood cells but from intestinal immune organs. For compensating weak secretion of IL-10 from peripheral blood cells in atopic subjects, administration of *L. GG* in food appears to be an effective treatment to prevent atopic dermatitis.

Viljanen et al. [43] reported the curative effect of *L. GG*, which reduced dermatitis in IgE-sensitized infants. Although we have clearly shown the preventative effect of *L. GG*, further evaluation might be required with respect to the curative effect of *L. GG*.

Taken together, *L. GG* might possess a cushioning effect, which suppresses excessive immunoreactions, resulting in

the prevention of atopic dermatitis and the retention of normal immune balances.

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