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Additive effect of *Lactobacillus acidophilus* L-92 on children with atopic dermatitis concomitant with food allergy

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The authors have no financial conflicts of interest.

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ABSTRACT

Background: Atopic dermatitis (AD) in infants is often related to food allergies (FA). The beneficial effects of lactic acid bacteria towards allergic diseases have been reported, but there are few reports on their effect and preferable dosages on AD in young children with concomitant FA.
Objective: To examine additional effects of two different dose of paraprobiotic *Lactobacillus acidophilus* L-92 (L-92) on the clinical treatment in young children afflicted by AD with diagnosed or suspected FA.
Methods: Fifty-nine AD young children from 10 months to 3 years old, with FA or who had not started to ingest specific food(s) because of high specific IgE levels, were recruited and randomly allocated into L-92 group (daily intake of 20 mg L-92/day) and placebo group. Participants were given test sample with conventional treatment for AD over a 24-week period. The severity of eczema was evaluated using SCORing Atopic Dermatitis (SCORAD) index before intervention, and at 4, 12, and 24 weeks after intervention.
Results: After 24 weeks of intervention, a significant decrease in SCORAD was observed only in the L-92 group when compared with the baseline values. Significant decreases in thymus and activation-regulated chemokine (TARC) and total IgE were also detected 24 weeks after intake in the L-92 group compared with the placebo group.
Conclusion: It was suggested that intake of sufficient amounts of L-92 works as an adjunctive treatment of young children afflicted by AD with diagnosed or suspected FA.

Keywords: *Lactobacillus acidophilus*; Atopic dermatitis; Food sensitivity

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder, where patients suffer from repeated remission and exacerbation of symptoms due to seasonal variations and other factors. In infant patients, AD leads to a marked disturbance in quality of life (QOL) to both the patient and the caregivers [1-3].

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Most infants with AD suffer concomitantly with food allergies (FA) [4]; skin lesions tend to self-cure over time. A systematic review, reported by Tsakok et al. [5], confirmed that there was a strong association between AD, food sensitization and FA. Generally, AD arises before the development of food sensitization. These facts suggest that allergic factors, caused by immunological immaturity, contribute in most cases of infantile AD.

The standard treatment for AD is cutaneous application of topical steroids and moisturizing agents. After controlling the acute inflammation in the skin, appropriate avoidance of food and environmental allergens is encouraged. Supplemental treatment to promote the maturation of the immune system may also be effective in maintaining the skin condition.

Lactic acid bacteria have long used in a variety of fermented foods. They contribute not only to an improvement in the taste and preserving properties of the foods, but also to human health, in a comprehensive manner. It has been postulated that probiotics have therapeutic effects on allergic disease, however, the effects of probiotics on allergic diseases have not been proved by the systematic reviews and meta-analyses [6, 7], partly because of the large variations in Lactobacillus strains used, selection of participants, and the employed administration methods. Furthermore, the condition of eczema is affected, not only by the adherence to skin care and the usage of topical steroids, but also by seasonal variation.

Sistek et al. [8] reported that when a combination of 2 probiotics, *Lactobacillus rhamnosus* and *Bifidobacterium longum*, were given to children with AD aged 1 to 10 years old for 12 weeks, a significant effect was observed only in a subgroup of individuals associated with FA. This observation suggested that the greatest effect of probiotics can be expected in young children with immature gastrointestinal immunity and who are predisposed to allergy.

In addition to this study, a number of notable studies have suggested the efficacy of probiotics. The term probiotics is defined as "live" microorganisms that provide beneficial effects to humans by improving the constitution of their intestinal flora [9]. In contrast, a lot of research has been done on the mechanism of probiotics, it was gradually revealed that lactic acid bacteria would exert their effects even in a dead cell. Taverniti and Guglielmetti [10] proposed a new term “paraprobiotics” to indicate the use of inactivated microbial cells or cell fractions that confer a health benefit to the consumer.

Lactobacillus acidophilus L-92 (L-92) is a type of lactic acid bacteria with a wide range of paraprobiotic activities. It has been reported that paraprobiotic L-92 is useful in the treatment of hay fever, perennial allergic rhinitis, and AD in children and adults [11-14]. However, preferable dose of L-92 have not been well evaluated yet.

In this randomized trial, we aimed to confirm the supplemental therapeutic effects of prolonged intake of paraprobiotic L-92 in AD young children with FA or who had not started to ingest specific food(s) because of the high specific IgE levels, whose immune system were supposed to be immature. This is the first study with L-92 on AD in young children with concomitant food allergy.

MATERIALS AND METHODS

Participants

We recruited young children with clinically diagnosed AD who were at least 10 months old but less than 3 years old, and who were followed up at the pediatric allergy clinic in Aichi Children's Health and Medical Center, Japan. Inclusion criteria were: (1) patients who had undergone conventional treatment with daily topical corticosteroid, in accordance with Guidelines for the Management of Atopic Dermatitis of the Japanese Dermatological Association [15]; (2) patients with concomitant FA, or who had not started to ingest specific food(s) due to high specific IgE levels; (3) patients who did not consume foods containing lactic acid-producing bacteria or any drugs aimed at improving the intestinal bacterial flora on a daily basis; and (4) patients who had no acute skin infections such as impetigo contagiosa or cutaneous fungal disease at the time of initiation of the study.

Exclusion criteria were: (1) patients who could not ingest powdered food; (2) patients who had a history of anaphylactic shock caused by trace amounts of milk constituents; (3) patients who had severe disease, such as diabetes, gastrointestinal disease, kidney disease and cardiac disease; (4) patients who had a history of hypersensitivity against topical steroid preparations, moisturizing agents, and study foods used in this trial; (5) patients who participated or planned to participate in any other intervention study during the period of this study; or (6) patients who were determined to be ineligible by an investigator.

Study foods

Calpis Co., Ltd., which was one of the group companies of Asahi group holdings, prepared the study foods. L-92 was cultured in medium comprising food or food additives without milk-derived ingredients. The cultured medium was then pasteurized by heat and dead L-92 cell materials were collected and powdered. Two kinds of test sample were prepared: a 20-mg dose (20 mg of L-92 dry powder, approximately 2×10^{10} cells were included) and placebo. The dose was determined in reference to the study Inoue et al. [14] had demonstrated. For ethical reasons, a negligible low dose of L-92 (0.2 mg L-92 dry powder) was included in placebo. Dextrin was added to the powdered L-92 to make it up to a total of 1 g thereby ensuring that meals were indiscernible in appearance, taste, and flavor. Study foods were packaged in labeled packages as one dose unit. We determined sample size in reference to previous study [11-14]. To detect a statistically significant difference between the 2 groups with 80% power and 5% significant level, 25 participants were required in each group. Sixty children were recruited to the study, which allowed for a 15% participant withdrawal.

Protocol

This study was a double-blind randomized controlled trial. Participants were stratified based on their age i.e., those over or less than 2 years old, and randomly allocated into 2 groups with an equal probability in a 1:1 ratio; a L-92 group and a placebo group using a computer-generated random-numbers table. All participants started to take the study foods between June and August in 2011 or 2012 in order to uniform the influence of seasonal fluctuations of eczema, and they continued daily intake for 24 weeks. All pediatricians and participants were unaware of the treatment allocation during the trial.

Parents recorded the food intake and skin conditions of participants in a diary, and visited clinic before intervention (visit 1), at 4 (4–6) weeks (visit 2), 12 (11–15) weeks (visit 3), and 24 (23–27) weeks (visit 4) after the intervention of intake (with the allowable margin of error).

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On each visit, digital photographs of participants' skin lesions were taken under a unified condition. The attending pediatrician checked the participants' diary, skin condition and the amount of topical corticosteroids used. A dietitian (HU) also confirmed daily intake and absence of adverse events associated with the study food. In addition, blood and stool sampling were collected and QOL questionnaires were answered at Visits 1 and 4.

Clinical outcomes

The disease severity of AD was evaluated with the SCORing Atopic Dermatitis (SCORAD) [16] using digital photographs. Scores were assessed by an independent allergy specialist (MF) who was not part of the clinic, and was blinded to the allocation and order of visit, upon completion of the intervention of all participants. The method for details of scoring has been published elsewhere [17].

Pediatricians prescribed new topical corticosteroids at visit 1. The quantities of corticosteroid usage were calculated by subtracting the amounts of remaining tubes at each visit. Medication score was calculated by multiplying the used drug quantities with a coefficient number depending on the strength of corticosteroid (very strong 0.3; strong 0.2; mild 0.1; weak 0.05). The strength grading of topical corticosteroids has been previously described in the Guidelines for the Management of Atopic Dermatitis of the Japanese Dermatological Association [15].

Laboratory studies

Blood biochemical tests, including lactic dehydrogenase (LDH), thymus and activation-regulated chemokine (TARC), total IgE and eosinophil count, were measured at visits 1 and 4. IgE was measured by ImmunoCAP (Thermo Fisher Diagnostics, Tokyo, Japan). TARC was measured in Bio Medical Laboratories, Inc. (Tokyo, Japan) using HISCL TARC assay kit (Shionogi Pharma, Osaka, Japan) Intestinal flora was analyzed after visit 1 before initiating intake, and immediately before visit 4. Fresh stools from participants were immediately cooled at their home for use in generating quantitative isolation cultures at the Intestinal Flora Laboratory, Calpis Co., Ltd. performed within 48 hours, in accordance with Mitsuoka's method: a culture-based technique used for comprehensive investigation of intestinal [18]. The analyzed intestinal bacterial species were: *Enterobacteriaceae*, *Streptococcus*, *Staphylococcus*, Yeasts, *Candida* sp., *Corynebacterium*, *Bacillus*, *Pseudomonas aeruginosa*, *Lactobacillus*, *Bifidobacterium*, *Eubacterium*, *Bacteroidaceae* (*B. fragilis* group, *Fusobacterium*, *Megamonas hypermegas*, *Mitsuokella*), Anaerobic GPC, lecithinase (-) *Clostridium,* lecithinase (+) *Clostridium*, *Veillonella*, and *Megasphaera*. The results of the viable bacteria count were indicated as the number of colonies per g of stool.

Quality of life

Evaluation of QOL was implemented at Visits 1 and 4 through a questionnaire completed by parents. The Infants' Dermatitis Quality of Life index (IDQOL), developed by Lewis-Jones et al. [19], was translated into Japanese and used as a questionnaire for QOL evaluation in infant patients with AD by permission (Professor Yukihiro Ohya, National Center for Child Health and Development, Japan). The questionnaire consisted of 10 items, and the questions were evaluated on 4- or 5-level rating scales. For comparisons of data, the results of the answers for each question were scored. The highest score was given when a favorable answer was indicated for a question (the highest total score being 41 points).

Statistical analysis

The primary outcome was a decrease in the SCORAD index at visit 4 and the secondary outcomes were changes in IDQOL score, dose of topical corticosteroid, intestinal flora, eosinophils, serum total IgE, LDH, aspartate aminotransferase, alanine aminotransferase, and TARC.

In the per-protocol population, to compare SCORAD and blood indices, Wilcoxon signed-rank test was used for a before-and-after comparative analysis, Mann-Whitney test and Student *t* test was used for between groups. The paired *t* test was used for a before-and-after comparative analysis of enteric bacteria. A *p* value of <0.05 was considered statistically significant. Statistical analysis was conducted using SPSS ver. 20.0J (IBM, Tokyo, Japan).

Ethical statement

Written informed consent was obtained from the parents of participants. The ethics committee of the Aichi Children's Health and Medical Center, which complied with the Declaration of Helsinki, approved this trial on the 5/27/2011. The trial was registered as UMIN000006209.

RESULTS

Fifty-nine recruited participants were allocated into two groups (29 participants for the L-92 group, 30 participants for the placebo group). Of these participants, 5 patients in the placebo group discontinued the study without specific reasons, 4 patients were removed from the analysis due to noncompliance, 4 patients could not continue the study because they were affected by other diseases (2 patients with rotavirus infection, 1 patient with norovirus infection and 1 patient hospitalized with asthma), and 1 patient could not continue as they did not have definitive atopic eczema at the start of the study. Consequently, 45 participants (25 participants in the L-92 group, 20 participants in the placebo group) were included in the analyses (**Fig. 1**).

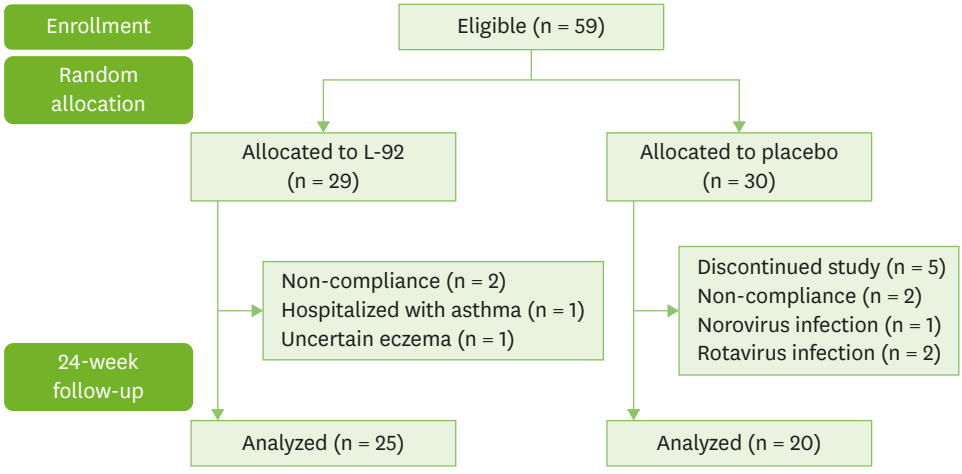

Fig. 1. Enrollment of participants.

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Characteristics at baseline

The characteristics of the participants included in this study are shown in **Table 1**. The median age of the L-92 group and placebo group was 1.7 (range, 0.9–3.0) and 1.8 (range, 0.9–2.7), respectively. The number of subjects diagnosed with a food allergy was as follows: hen's egg (n = 11), milk (n = 15), wheat (n = 10), and others (n = 11). The number of subjects who were sensitized to but had not started to ingest specific foods was as follows: hen's egg (n = 26), milk (n = 15), wheat (n = 11), and others (n = 22). There were no statistically significant differences between each group observed for each item at the baseline of the trial. At the end of the study, all subjects were confirmed as having FA to at least one specific food by a convincing clinical history or an oral food challenge test.

Changes in dermatitis severity

The data from 2011 and 2012 were virtually identical, therefore we analyzed the integrated data obtained from both years. The median SCORAD value at visit 1 was 39.1 (range, 12.2–83.8) and 34.0 (11.9–66.5) in the L-92 group and placebo group, respectively. The decreases in the SCORAD index at visit 4 from baseline were 8.1 ± 18.5 in the L-92 group ($p = 0.040$) and 2.5 ± 25.6 in the placebo group ($p = 0.55$) (**Fig. 2A**).

No significant difference was observed between the groups at visit 4, however, in the earlier visits the median SCORAD value showed a decreasing tendency in the L-92 dose group, and a significant between-group difference was observed at visit 3 (**Fig. 2B**).

The median value of medication scores between visits 1 and 2, visits 2 and 3, and visits 3 and 4 were 0.3 (range, 0.0–2.1), 0.6, (range, 0.0–2.3), and 1.0 (range, 0.0–2.9) in the L-92 group, respectively. The medication scores in the placebo group were 0.4 (range, 0.2–3.3), 0.8 (range, 0.1–3.1), and 1.4 (range, 0.0–3.7), respectively. No significant between-group difference was observed but the medication score remained at a low level in the L-92 group.

Table 1. Characteristics of participants

Characteristic	L-92 (n = 25)	Placebo (n = 20)
Age (yr)	1.7 (0.9–3.0)	1.8 (0.9–2.7)
Sex, male:female	16/9	12/8
SCORAD	39.1 (12.2–83.8)	34.0 (11.9–66.5)
WBC (/μL)	9,990 (6,090–25,000)	10,805 (5,050–18,230)
Eosinophil (%)	6.1 (1.6–23.4)	4.65 (0.8–12.1)
TARC (pg/mL)	1,236.5 (455–20,470)	1,722 (0–33,066)
LDH (IU/L)	330 (232–478)	312.5 (246–573)
AST (IU/L)	34 (22–49)	36.5 (23–53)
ALT (IU/L)	16 (10–49)	16.5 (10–33)
Total IgE (IU/mL)	632 (66–23,063)	358 (17–2,620)
No. of subjects diagnosed with food allergy		
Egg	7	4
Milk	9	6
Wheat	7	3
Other	6	5
No. of subjects who had not started to ingest specific food		
Egg	17	9
Milk	12	3
Wheat	9	2
Other	16	6

Values are presented as median (range) or number.
SCORAD, SCORing Atopic Dermatitis; WBC, white blood cell; TARC, thymus and activation-regulated chemokine; LDH, lactic dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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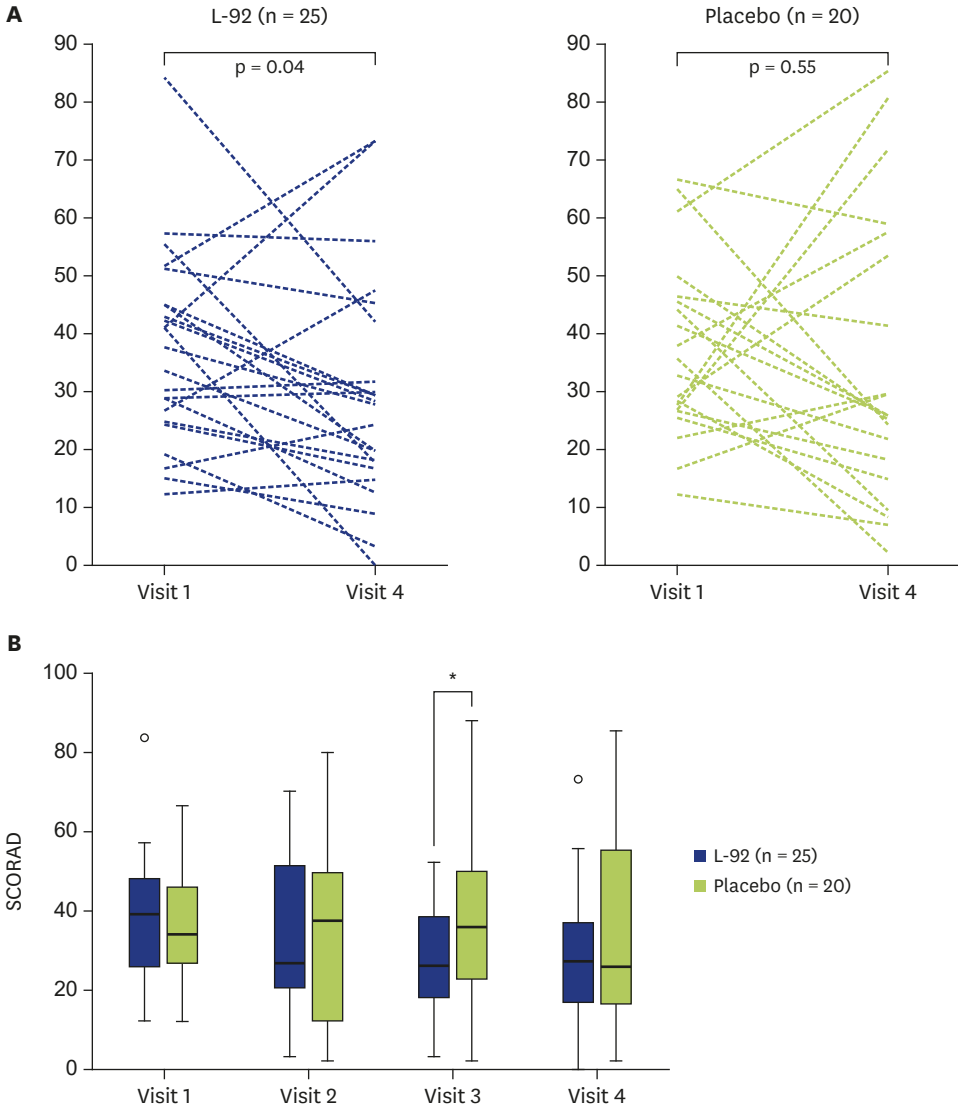

Fig. 2. Changes in SCORing Atopic Dermatitis (SCORAD). (A) Changes in SCORAD between visits 1 and 4. Broken lines represent individual data. Wilcoxon signed-rank test was used for a comparison between visits 1 and 4. (B) Boxplots of SCORAD. Open circles (○) represent outliers. Mann-Whitney test was used for a comparison between groups. $^{*}p < 0.05$. $^{**}p < 0.01$.

Blood analysis

Changes in laboratory data from visit 1 to visit 4 are shown in **Table 2**. The median value of the decrease in total IgE was 144 IU/mL in the L-92 group, which was significantly apparent ($p = 0.02$) compared with that observed in the placebo group (12 IU/mL). There was a significant decrease in serum TARC levels between the L-92 group and the placebo group ($p = 0.03$).

Table 2. Changes in laboratory data

Variable	L-92 group	Placebo group	*p* value*
Eosinophil (/μL)	−184.9 (−2,704 to 193)	−158 (−1,129 to 1,023)	0.28
Log Total IgE (IU/mL)	−0.14 (−0.34 to 0.39)	−0.01 (−0.28 to 0.53)	0.03
TARC (pg/mL)	−504 (−19,279 to 1,068)	86 (−29,661 to 805)	0.03
LDH (IU/L)	−26 (−139 to 82)	−18 (−279 to 108)	0.48

Changes between before (visit 1) and after intake (visit 4) are shown as the median (range) values.
TARC, thymus and activation-regulated chemokine; LDH, lactic dehydrogenase
*Student *t* test (log total IgE) and Mann-Whitney test (others).

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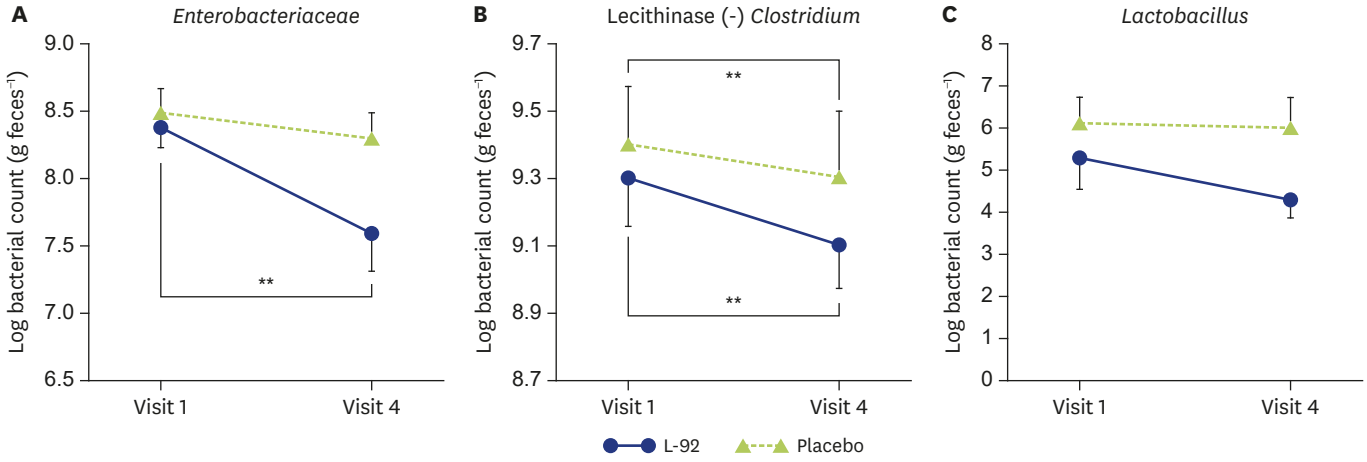

Fig. 3. Changes in the intestinal bacterial flora. The viable cell counts of *Enterobacteriaceae* (A), lecithinase (-) *Clostridium* (B), and *Lactobacillus* (C) in each group at visits 1 and 4 are shown. Data are expressed as the mean ± standard error. $^{**}p < 0.01$ using the paired *t* test.

Intestinal flora

Of a total of 22 types of isolated cultures, significant changes were observed in 2 types in the L-92 group and 3 types in the placebo group from visit 1 to visit 4. Of these isolated cultures, a significant decrease was observed in *Enterobacteriaceae* (**Fig. 3A**) in the L-92 group. A significant decrease was observed in lecithinase (-) *Clostridium* (**Fig. 3B**) in each group. There was no significant change in *Lactobacillus* (**Fig. 3C**) in either group.

IDQOL

Both groups showed a tendency towards improvement in IDQOL scores at visit 4. The QOL scores at visit 4 indicated significantly higher values in the L-92 group compared with the placebo group (**Fig. 4**).

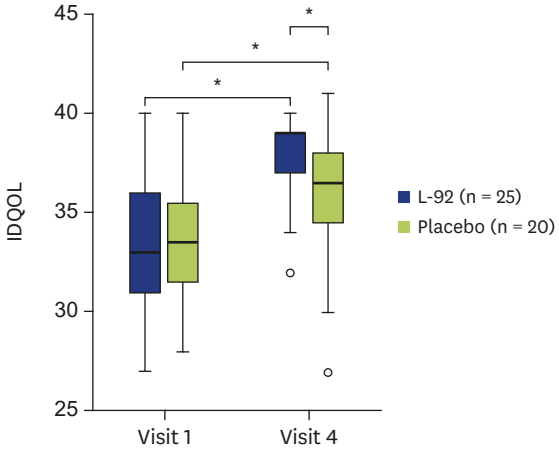

Fig. 4. Infants' Dermatitis Quality of Life index (IDQOL). IDQOL scores for each group at visits 1 and 4 are shown. $^{*}p < 0.05$ using the Mann-Whitney test for between groups, and Wilcoxon signed-rank test for a before-and-after comparative analysis.

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DISCUSSION

In this study, the primary outcome of 24 weeks of L-92 intake was a decrease in the SCORAD index in young children having conventional treatment for AD. Compared to the baseline value, a significant decrease in SCORAD was observed in the L-92 group but not in the placebo group. A significant difference was not observed between the 2 groups. Symptoms of AD fluctuate depending on the season [20]. In summer, the symptoms are prone to exacerbation due to sweat and bacterial infections. In winter, a decrease in the skin barrier function develops due to the dryness of skin. This study was conducted with unifying seasonal cycles, starting from summer and ending in winter in both years. In addition, both group allocation and the timing of visits (visits 1 to 4) was unknown to an independent third person who determined the severity of dermatitis.

Regardless of this perfect blinded-evaluation, we demonstrated an effect of L-92 by selecting AD children with concomitant FA as participants, who was expected to have allergic disposition and be responsive to probiotics, because probiotics were thought to modulate immune system and unified therapeutic conditions.

A significant between-group difference in the severity of dermatitis was observed at visit 3, but not at visit 4. This may be influenced by exacerbation of dry skin during the winter season. As previously reported, deterioration of skin symptoms was observed in Japanese winter season [21]. One of the major reasons why skin symptoms were deteriorated in winter, cool and dry weather causes itchy skin. Generally, the hypothesized mechanisms of action of L-92 on ameliorating allergic symptoms is to exert anti-inflammatory effects through immunological modulation. Therefore, it was supposed that L-92 itself has lower ability to ameliorate deterioration of skin symptoms caused by dry skin than skin moisturizer. From this point of view, exacerbation of eczema during the dry season could be controlled by not only L-92 but appropriate application of skin moisturizer. Assumed other reasons why disappeared difference of SCORAD between two groups was that L-92 used in this study was pasteurized and could not be colonized in intestine. Therefore, accumulative effects of L-92 is not expected.

Significant decreases in serum TARC and total IgE levels were observed in the L-92 group compared with the placebo group, which reflects the suppression of skin inflammation. TARC is a T helper cell 2 (Th2) chemokine and an indicator of the degree of inflammation in AD [22]. The decreased total IgE suggests that L-92 suppressed continuous production of IgE antibody by suppressing Th2 cytokine function.

A significant improvement in IDQOL was observed in the L-92 group compared with the placebo group. The contents of the questionnaire were evaluated from the standpoint of a caregiver who observes the child's activity on a daily basis, and it was suggested that the parents experienced the therapeutic effects of L-92 intake.

In recent years, the relationship between gut microbiota and health has attracted a lot of attention. The importance of intestinal bacterial flora has been successively reported with the development of "-omics" analysis techniques [23]. It has been postulated that microbiome composition or diversity are strongly associated with the development of allergic disease. Children who have a less diverse gut microbial flora in their early life may be more susceptible to development of asthma or allergic disease [24, 25]. The microbiota present

in early life seems to be closely related to the development of allergic diseases. Fujimura et al. [26] reported that the data obtained from a United States birth cohort with a high risk of multisensitized atopy at 2 years of age and asthma at 4 years age was predictable on the basis of the composition of microbiota in the neonates (median age 35 days).

L-92 used in this study was dead bacterial cell material. Unsurprisingly, no significant changes were observed in *Lactobacillus* organisms in the intestinal bacterial flora of participants before and after intake. Significant decreases were observed in *Enterobacteriaceae* and lecithinase (-) *Clostridium* in the L-92 group only. Some reports have suggested that *Enterobacteriaceae* and *Clostridium* are involved in the development of allergic disease. Gut microbiota profiles of 166 young children from the CHILD (Canadian Healthy Infant Longitudinal Development) study revealed that a higher ratio of Enterobacteriaceae to Bacteridaceae in early infancy was associated with subsequent food sensitization [27]. Hong et al. [28] have reported a similar association between Enterobacteriaceae to Bacteridaceae ratios and AD in caesarean-delivered young children. A recent report revealed that over-representation of unclassified Enterobacteriaceae and 2 *Clostridium* species were found in the first year of life among young children affected with FA or other allergies during their first 3 years [29]. Atarashi et al. [30] attempted to isolate regulatory T cell inducible bacterial strains from human indigenous microbiota: they found 17 strains, many of which were classified into the genus *Clostridium*. The relationship between gut microbiota and development of allergic disease is still unclear; further detailed and larger studies are required.

Kawamoto et al. [31] reported on the interaction between microbiome and immune homeostasis: they found that the regulatory T cell mediates the balance of microbiota via production of immunoglobulin A. It has been reported that L-92 induced CD4+CD25+Foxp3+ regulatory T cell in mice [32]. Thus, L-92 might influence composition microbiota through modulation of the mucosal immune system. The detailed mechanism needs to be elucidated.

Study limitations should be noted exist in this study. First, it was small sample size. At the beginning of the study, we calculated 25 participants in each group were needed to detect significant difference, and estimated dropout rate was about 15%. However, dropout rate was 33% (10 of 30) in placebo group, which would bias the result of our statistical analysis. Second, it was appropriate dosage of L-92. We have used 20 mg of L-92 in this study, which was almost same dose of L-92 reported in adult. It was needed to determine appropriate dose of L-92 in children.

In conclusion, it was suggested that intake of a specific amount of L-92 works as an adjunctive treatment in young children afflicted by AD with diagnosed or suspected FA. However, a significant difference was not observed between the placebo group and L-92 group at 24 weeks. Further studies are needed to clarify the ameliorating effect of L-92 on AD.

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