

ORIGINAL ARTICLE

A randomized trial of *Lactobacillus plantarum* CJLP133 for the treatment of atopic dermatitis

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Keywords

atopic dermatitis; *Lactobacillus plantarum*; probiotics

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Abstract

Background: There have been considerable disagreements regarding the therapeutic effects of probiotics in atopic dermatitis (AD). We performed this study to examine whether the oral administration of *Lactobacillus plantarum* CJLP133 improves pediatric AD.

Methods: In a randomized, double-blind, placebo-controlled study, either *L. plantarum* CJLP133 at a dosage of 0.5×10^{10} colony-forming units or placebo in children aged 12 months to 13 yr was given twice a day for 12 wk. SCORing of Atopic Dermatitis (SCORAD) scores, eosinophil counts, serum total IgE, and cytokines were evaluated.

Results: Forty-four of 58 patients in the probiotic group and 39 of 60 patients in the placebo group completed the study. The SCORAD score at week 14 was lower in the probiotic group than in the placebo group ($p = 0.044$). The mean change in the SCORAD score from weeks 2 to 14 was 9.1 in the probiotic group, which was greater than the mean change of 1.8 in the placebo group ($p = 0.004$). No statistical differences in the total use of topical corticosteroids were found between two groups ($p = 0.815$). In the probiotic group, the total eosinophil count was significantly lower at the end of the intervention compared to the baseline measurements ($p = 0.023$). Logarithmic IFN- γ and IL-4 were significantly decreased by the end of the intervention compared to baseline measurements in the probiotic group ($p < 0.001$ and 0.049).

Conclusions: Our results suggest that supplementation with probiotic *L. plantarum* CJLP133 is beneficial in the treatment of pediatric AD.

According to the hygiene hypothesis, previous studies suggest that exposure to environmental microbes including gut-colonizing microbes may be correlated with the development of allergic diseases (1–3). In addition, the intestinal tract was found to be more frequently colonized by lactobacilli in non-allergic children than in allergic children, supporting the idea that appropriate microbial colonization of the gut might lower the risk of developing atopic diseases (4–6). It is suggested that commensal bacteria, which provide the first and major source of microbial exposure, play a central role not only in Th1/Th2 polarization but also in inducing the appropriate regulatory mechanisms (1, 2). However, there has been controversy regarding the therapeutic or preventive effects of probiotics in atopic dermatitis (AD) (7–13).

Kimchi is a traditional fermented food in Korea. It is made from various vegetables and contains many beneficial health-promoting agents such as β -carotene, chlorophyll, ascorbic acid, and dietary fiber (14). Diverse bacteria are responsible for the health advantages that are produced during Kimchi fermentation, and one of the major species is *Lactobacillus* (15). *Lactobacillus plantarum* CJLP133 (KCTC 11403BP, GenBank accession nr FJ455518) is isolated from Kimchi and recently has been shown to suppress AD-like skin lesions, high serum IgE levels and epidermal thickening and also to diminish the accumulation of eosinophils and mast cells in topical inflammatory sites in NC/Nga mice model induced by house dust mites (14, 16).

In this randomized, double-blind, placebo-controlled study, we attempted to examine whether oral administration of

L. plantarum CJLP133 isolated from Kimchi improves clinical and immunological parameters in children with AD.

Methods

Study population and study design

Patients aged 1–13 yr presenting with AD were enrolled. The diagnosis of AD was based on the criteria defined by Hanifin and Rajka (17). Their SCORing of AD (SCORAD) ranged from 20 to 50. Exclusion criteria included: (i) administration of systemic corticosteroids or phototherapy in the previous 1 month, (ii) administration of systemic immunosuppressant in the previous 3 months, (iii) administration of probiotics during the past 4 weeks, (iv) concomitant skin diseases or systemic illnesses, and (v) a SCORAD score <20 after the initial washout period. Patients were randomized and double-blindly received either probiotic or placebo treatments using a computer-generated list of random numbers. The random number list was prepared and posted on the fronts of the bags containing the materials by an investigator who was not involved clinically in the trial.

The bacterial preparation contained *L. plantarum* CJLP133 manufactured by the CJ Cheiljedang Corporation (Seoul, Korea). Each dose was stored in airtight alu-bags at 4°C until administration. The first 2 wk were the wash-out period, during which placebo was administered to both groups. For the next 12 wk, patients were given either *L. plantarum* CJLP133 at a dosage of 0.5×10^{10} colony-forming units twice a day, or a placebo preparation with identical appearance and taste. The patients were examined 2 wk after discontinuation of either probiotics or placebo administration. During the 16-wk study period, the patients were instructed not to consume any fermented food products containing live microorganisms. Afterward, all participants had a 2-wk follow-up period without any study medication.

Parents were instructed to bathe the children once daily in warm water and to treat their AD lesions frequently with emollients. During the study period, topical corticosteroids (TCS) were not used unless the visual analogue scale for itch and insomnia was seven or higher. For the patients requiring TCS for rescue medicine, 0.25% prednicarbate was offered, and patients were educated to cover a body area of two hands with one fingertip unit. The TCS medication tube was weighed for quantitative measurements at each visit.

The study was approved by the institutional review board at Chung-Ang University Hospital in Seoul, and a written informed consent was obtained from parents prior to participation in the study. Our study protocol was registered in the WHO International Clinical Trials Registry Platform (ICTRP). The registration number is KCT0000358.

Clinical evaluation and laboratory tests

The patients were examined at 0, 2, 8, 14, and 16 wk after the beginning of the study. At consecutive visits, the same pediatric allergist (JK) assessed the SCORAD, ranging from 0 to 103 (18). This severity grading is composed of objective and

subjective scores including evaluation of pruritus and insomnia on scales ranging from 0 to 10.

At week 2, measurements were taken of eosinophil count, serum total IgE levels, and specific IgE to *Dermatophagoides pteronyssinus*, *D. farinae*, egg white, cow's milk, soy, wheat, and peanut. Serum IgE levels were determined by ImmunoCAP (Pharmacia, Uppsala, Sweden) system, and the cutoff value for sensitization was 0.35 IU/ml. At week 14, the eosinophil count and serum total IgE levels were measured again.

Real-time PCR for cytokine measurements

These are described in detail in the Appendix S1 (Supporting information).

Microbial composition of *L. plantarum* in fecal samples

These are described in the Appendix S1 (Supporting information).

Statistical analysis

Sample size was determined on the estimate of a 30% and 10% response rate in probiotic and placebo groups, respectively, based on a previous clinical trial (10). We calculated that 58 patients in each group (80% power and 5% significance) would be needed. To allow for a 30% dropout rate, we aimed to enroll 75 patients per group. Two interim analyses of primary outcomes were performed when 50% and 75% of the expected patients were enrolled, and the study was discontinued after the second interim analysis showed statistically significant differences between the two groups.

Statistical analysis was performed using the spss 13.0 software (SPSS Inc., Chicago, IL, USA) for per-protocol (PP) and intention-to-treat (ITT) analyses. Differences in gender, allergic sensitization and use of TCS between the two groups were analyzed using a chi-square test. Baseline ages, SCORAD scores, and laboratory tests between two groups were compared using *t*-tests. The changes in SCORAD scores, laboratory data, and cytokine levels between each visit were analyzed using repeated measures ANOVA, paired *t*-test, and Wilcoxon's signed-rank test. Mean amounts of TCS between two groups and the prevalence of *L. plantarum* among fecal samples were analyzed by Mann–Whitney *t*-tests. The levels of total IgE and the cytokine measurements were analyzed on a logarithmic scale. Because the use of separate univariate *t*-tests leads to an inflated type 1 error, Bonferroni's correction was applied to the subgroup analysis of patients who had not used TCS by adjusting the *p* value. A *p* value < 0.05 was considered to be significant.

Results

Study population

Out of 118 subjects who met the eligibility criteria, 44 of 58 patients in the probiotic group and 39 of 60 patients in the

placebo group completed the study (Fig. 1). At the beginning of the study, no statistical differences were noted between the two groups in age, sex, SCORAD scores, eosinophil counts, total IgE levels, and proportions of allergen sensitization (Table 1).

Clinical effects of probiotics on AD

In PP analysis, the SCORAD score at week 14 was lower in the probiotic group than in the placebo group ($p = 0.022$), and the mean change of SCORAD score from the baseline in the probiotic group was greater than that in the placebo group ($p = 0.002$). ITT analysis also showed that the SCORAD score at week 14 was lower in the probiotic group than in the placebo group ($p = 0.044$; Table 2). During the period from weeks 2 to 14, the mean SCORAD was significantly decreased over time in the probiotic group compared to the placebo group in ITT analysis ($p = 0.004$; Fig. 2).

During the study period, 13 patients (22.4%) in the probiotic group and 14 patients (23.3%) in the placebo group applied TCS ($p = 0.905$). The total amount of TCS through the trial in the probiotic and placebo groups was 1.6 ± 6.5 g (median, 0; range, 0–48.3) and 1.5 ± 4.0 g (median, 0; range, 0–21.7; $p = 0.815$). The use of TCS from weeks 2 to 14 in the probiotic and placebo groups were 1.6 ± 5.3 g (median, 0; range, 0–34.0) and 2.0 ± 4.2 g (median, 0; range, 0–18.6; $p = 0.705$), respectively.

The data analyzed in the patients who had not used TCS showed that the mean SCORAD was significantly decreased over time during the period from weeks 2 to 14 in the probiotic group ($p = 0.016$; Fig. 2). The mean change of SCORAD score from the baseline in the probiotic group was greater than that in the placebo group ($p = 0.017$; Table 3).

Laboratory data

In the probiotic group, the total eosinophil counts were significantly lower at week 14 compared to baseline measure-

Table 1 Baseline clinical characteristics of study participants

Characteristics	Probiotic (n = 58)	Placebo (n = 60)	p Value
Sex (M/F)	34/24	35/25	0.975
Age (yr)*	4.6 ± 3.3	5.1 ± 3.3	0.431
Onset of AD (yr)*	0.7 ± 1.2	1.1 ± 1.7	0.169
Duration of AD (yr)*	3.9 ± 3.1	4.0 ± 3.0	0.853
SCORAD*	30.6 ± 7.7	30.3 ± 6.8	0.844
Itching*	6.5 ± 2.2	6.3 ± 1.8	0.655
Insomnia*	6.1 ± 2.6	6.2 ± 2.3	0.803
Eosinophil count (/mm ³)*	545.1 ± 386.4	542.9 ± 355.7	0.977
Log (total IgE)*	5.2 ± 1.7	5.2 ± 1.6	0.944
Sensitization, No. (%)			
<i>Dermatophagoides farinae</i>	25/44 (56.8)	28/39 (71.8)	0.156
<i>Dermatophagoides pteronyssinus</i>	21/44 (47.7)	26/39 (66.7)	0.082
Egg white	16/44 (36.4)	16/39 (41.0)	0.663
Cow's milk	10/44 (22.7)	15/39 (38.5)	0.119
Peanut	9/44 (20.5)	10/39 (25.6)	0.575
Soy	8/44 (18.2)	8/39 (20.5)	0.788
Wheat	7/44 (15.9)	11/39 (28.2)	0.175
Any of above	29/44 (65.9)	31/39 (79.5)	0.168

*Mean $\pm$ standard deviation.

AD, atopic dermatitis; SCORAD, SCORing of Atopic Dermatitis.

ments ($p = 0.023$; Fig. 3). There were no differences in the eosinophil counts between pre- and post-treatment in the placebo group ($p = 0.059$). No relevant changes in logarithmic total IgE levels were found at week 14 compared to week 2 in the probiotic and placebo groups ($p = 0.054$ and $p = 0.800$). Logarithmic IFN- γ and IL-4 were significantly lower at week 14 compared to baseline measurements in the probiotic group ($p < 0.001$ and 0.049 ; Fig. 3). However, there were no differences in logarithmic IFN- γ , TNF- α , and IL-4 between pre- and post-treatment in the placebo group ($p = 0.386$, $p = 0.081$, and $p = 0.209$).

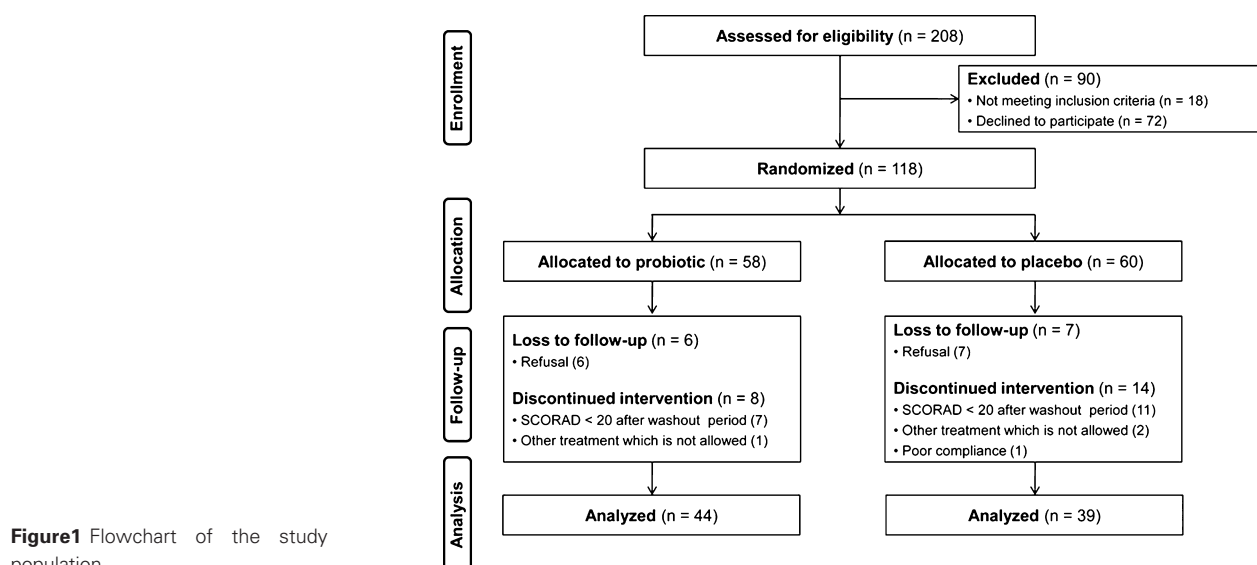


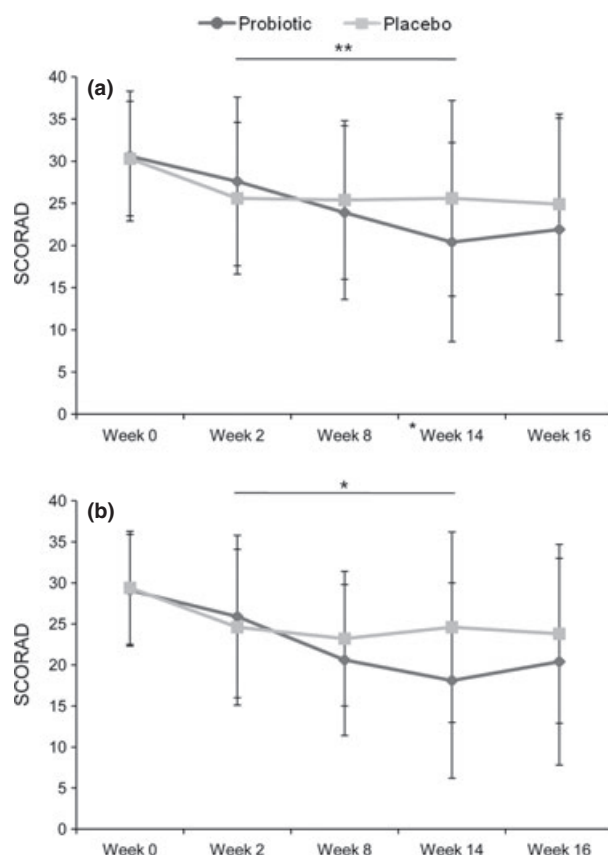
Figure 1 Flowchart of the study population.

Table 2 Intention-to-treat analysis of SCORAD scores after treatment with probiotics or placebo in total patients

	Probiotic (n = 58) (95% CI)*	Placebo (n = 60) (95% CI)*	p Value
Week 2			
SCORAD	27.6 ± 10.0 (25.0 to 30.2)	25.6 ± 9.0 (23.2 to 27.9)	0.252
Week 8			
SCORAD	23.9 ± 10.3 (20.9 to 26.9)	25.4 ± 9.4 (22.6 to 28.2)	0.471
Change (weeks 2–8)	6.0 ± 9.2 (3.3 to 8.7)	1.8 ± 8.9 (–0.8 to 4.4)	0.033
Week 14			
SCORAD	20.4 ± 11.8 (16.9 to 23.9)	25.6 ± 11.6 (22.0 to 29.2)	0.044
Change (weeks 2–14)	9.1 ± 10.6 (6.0 to 12.3)	1.8 ± 12.1 (–1.9 to 5.6)	0.004
Week 16			
SCORAD	21.9 ± 13.2 (18.0 to 25.8)	24.9 ± 10.7 (21.6 to 28.2)	0.261
Change (weeks 2–16)	7.6 ± 12.0 (4.1 to 11.2)	2.6 ± 10.1 (–0.5 to 5.7)	0.041

*Mean ± standard deviation.

SCORAD, SCORing of Atopic Dermatitis; Week 0–2, wash-out period; week 14–16, follow-up period.

**Figure 2** SCORing of Atopic Dermatitis (SCORAD) scores from weeks 0 to 16 in total patients (a) and in patients who did not use topical corticosteroids (b). * $p < 0.05$, ** $p < 0.01$.

Fifty-one fecal samples were collected during study period. Fecal samples from the probiotic group consisted of 8, 10, 11, and 12 samples collected at weeks 2, 8, 14, and 16, respectively. From the placebo group, 10 fecal samples were obtained at week 2. *L. plantarum* was not detected in fecal DNA samples at week 2 in both groups. However, the median percentage of *L. plantarum* DNA among total bacteria significantly increased

up to 0.480% at week 8 and 1.296% at week 14 compared to week 2 ($p = 0.043$ and $p = 0.007$; Fig. S1 in the Supporting information). The percentage of *L. plantarum* significantly decreased to 0.001% at week 16 ($p = 0.037$).

Discussion

Immunomodulatory effects of gut flora on the development of AD have been reported (1, 2). Adequate microbial stimuli are necessary for the development of the immune systems such as in the maturation of Th1/Th2 cells in early childhood (19). Special microbial composition and microbial diversity prevent Th2-shifted immunity and the development of allergic diseases in later life (6, 20, 21). In our randomized, double-blind, placebo-controlled study, we found that *L. plantarum* CJLP133 was beneficial in reducing the clinical severity of pediatric AD. This clinical improvement is correlated with changes in eosinophil counts of the peripheral blood and mRNA expression of IFN- γ and IL-4, suggesting that immunologic alteration is involved in the therapeutic effect of the probiotics. This result is consistent with our animal experiment in which *L. plantarum* CJLP133 suppressed house dust mite-induced dermatitis (14).

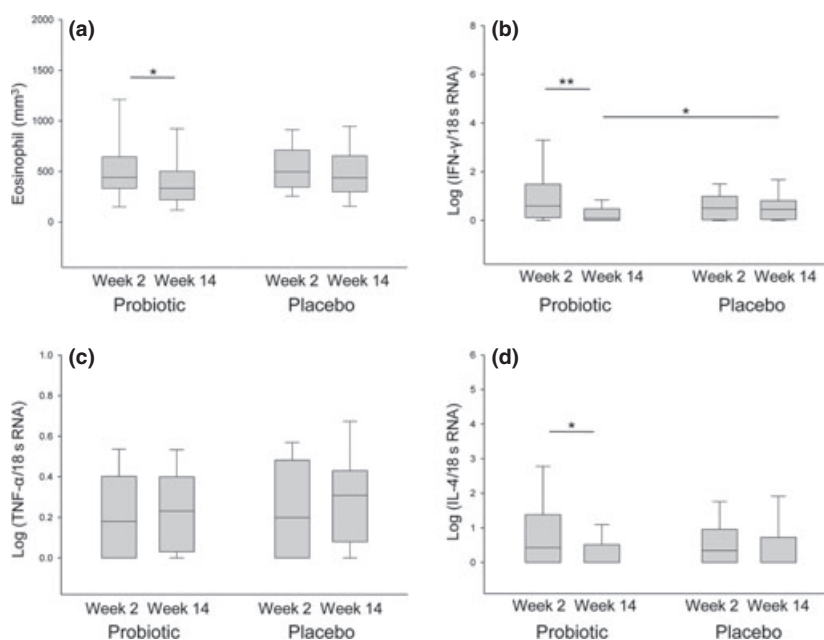
In the present study, reductions in eosinophils, IFN- γ , and IL-4 were found in the probiotic group during *L. plantarum* CJLP133 administration. A reduction in eosinophil counts represents decreased disease activity, as there is a positive correlation between clinical severity and total eosinophil counts in acute exacerbation phase of AD (22). The type 1 cytokine IFN- γ is related to the pathogenesis of eczematous dermatitis through the induction of Fas-induced keratinocyte apoptosis (23, 24), and the increment of IFN- γ is involved in the chronic lesions of AD (25), while Th2 cytokines including IL-4 predominate in the acute phase of the lesions (26). Most patients in our study have chronic eczematous lesions, with a mean period of more than 4 yr elapsing after the onset of AD. Therefore, reduction in IFN- γ may be related to the improvement of chronic AD lesions. The exact mechanism remains unclear, but it is plausible that increasing the production of regulatory cytokines such as IL-10 or TGF- β might reduce

Table 3 Intention-to-treat analysis of SCORAD scores after treatment with probiotics or placebo in patients who did not use topical corticosteroids

	Probiotic (n = 45) (95% CI)*	Placebo (n = 46) (95% CI)*	p Value
Week 2			
SCORAD	25.9 ± 9.9 (22.9 to 28.8)	24.6 ± 9.5 (21.8 to 27.4)	1.000
Week 8			
SCORAD	20.6 ± 9.2 (17.4 to 23.8)	23.2 ± 8.2 (20.3 to 26.2)	0.474
Change (weeks 2–8)	7.8 ± 7.8 (5.1 to 10.5)	3.3 ± 7.2 (0.7 to 5.9)	0.043
Week 14			
SCORAD	18.1 ± 11.9 (13.9 to 22.2)	24.6 ± 11.6 (20.3 to 29.0)	0.076
Change (weeks 2–14)	9.8 ± 10.0 (6.3 to 13.3)	2.0 ± 11.9 (–2.5 to 6.5)	0.017
Week 16			
SCORAD	20.4 ± 12.6 (16.0 to 24.9)	23.8 ± 10.9 (19.7 to 27.9)	0.558
Change (weeks 2–16)	7.5 ± 10.7 (3.7 to 11.2)	2.8 ± 9.9 (–0.9 to 6.5)	0.183

*Mean ± standard deviation.

CI, confidence interval; SCORAD, SCORing of Atopic Dermatitis; Week 0–2, wash-out period; week 14–16, follow-up period.

**Figure 3** Eosinophil counts (a), IFN- γ (b), TNF- α (c), and IL-4 (d) at weeks 2 and 14. * $p < 0.05$, ** $p < 0.01$.

IFN- γ (27–29). It is also possible that the generation of regulatory dendritic cells and CD4⁺ Foxp3⁺ T cells might be involved (30). Unfortunately, the level of IL-10 expression at week 2 and 14 was too low to detect, suggesting that the detection of mRNA expression of regulatory cytokines has limitations in peripheral blood.

In this clinical trial, fecal samples were analyzed to examine the relationship between the distribution of gut flora and changes in clinical severity. It was found that clinical severity and blood eosinophils decreased in the probiotic group, and the microbial composition ratios of *L. plantarum* increased during supplementation of probiotics. The results indicate that the clinical improvement is related to administration of the probiotics, as the clinical severity and fecal prevalence of *L. plantarum* were aligned with each other over the course of

the study. Interestingly, *L. plantarum* rapidly disappeared in the fecal samples after discontinuation, demonstrating that *L. plantarum* does not persist in the gut. Accordingly, the SCORAD score increased after probiotics supplementation discontinued, and the SCORAD scores at week 16 lost statistical significance between the probiotic and the placebo groups. This is possible because *L. plantarum* CJLP133 is not of human origin but originates in the traditional Korean food Kimchi. In contrast, a study by Weston et al. (7) showed effects persisting 2 months after ceasing probiotics supplementation. Further research is needed to investigate the therapeutic effect of *L. plantarum* CJLP133 after long-term administration.

Our results correspond with those of earlier studies in which probiotics were beneficial in the treatment of AD (7–9, 13). When *L. fermentum* was given for 8 wk to children with

moderate to severe AD between the ages of 6–18 months, reduction in the clinical severity was significant in the probiotic group but not the placebo group (7). Administration of *L. rhamnosus* Lcr35 for 3 months in children aged 2–12 yr showed reduction of clinical severity in AD (13). Another study has shown that supplementation of *L. sakei* is associated with a reduction in the SCORAD score and lower pre-treatment-adjusted serum levels of CCL17 and CCL27 (9). However, in a study to investigate the efficacy of *L. rhamnosus* GG in infants under the age of 12 months suffering from AD, treatment with the probiotics did not show any significant changes with respect to eczema severity or immunological effects (10, 11). Disagreement between the studies on the therapeutic effect of probiotics in AD might depend on the type of probiotics or the study population. Subjects in studies for the positive effects of probiotics on AD including ours seem to be older, have higher SCORAD scores, and to be more sensitized (7–9, 13), suggesting that the therapeutic effect of probiotics might be expected in specific subgroup of pediatric AD. Our present study did not show statistical differences in clinical improvement between probiotic and placebo groups in patients who

had used TCS during study period (data not shown). The result might be due to the low number of patients, anti-inflammatory effects of TCS, or lower SCORAD.

In conclusion, our results suggest that supplementation with probiotic *L. plantarum* CJLP133 is beneficial in the treatment of AD in children. However, its long-term effect remains unclear, as *L. plantarum* does not persist in the gut. The exact mechanism by which the probiotics modulate the immune system also needs to be studied.

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Conflict of interest

Bongjoon Kim and Sehee Hwang are employed by CJ CheilJedang Corporation. Other authors declare that there are no potential conflicts of interests with respect to the authorship and/or publication of this article.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Methods for cytokine measurements and microbial DNA preparation from fecal samples.

Figure S1. Percentage of *Lactobacillus plantarum* DNA among total bacteria in the probiotics group.

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SUPPORTING INFORMATION

Appendix S1. Methods for cytokine measurements and microbial DNA preparation from fecal samples

Real-time PCR for cytokine measurements

At weeks 2 and 14, patients' blood was drawn to measure cytokine expression. RNeasy Mini Kits (Qiagen, Valencia, CA) were used according to the manufacturer's protocol to isolate RNA from human blood. Primers and probes for human IL-4, TNF- α , IFN- γ , and 18S rRNA were purchased from PerkinElmer/Applied Biosystems (Norwalk, CT). One microgram of RNA was reverse-transcribed in a 20- μ L reaction containing Random Primers (Invitrogen, Carlsbad, CA), and Superscript II enzyme (Invitrogen, Carlsbad, CA). Each PCR system (25 μ L) contained 1 μ L of cDNA, 2 $\times$ TaqMan Master Mix (Applied Biosystems), 900 nM forward primer, 900 nM reverse primer, and 200 nM probe (PerkinElmer/Applied Biosystems). Real-time PCR was performed and analyzed by the dual-labeled fluorogenic probe method using an ABI Prism 7300 sequence detector (PerkinElmer/Applied Biosystems). Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer's technical bulletin. A standard curve was generated using the fluorescent data from the 10-fold serial dilutions of total RNA from the highest expression sample. This was then used to calculate the relative amounts of target mRNA in test samples. Quantities of all targets in test samples were normalized to the corresponding 18S rRNA transcript in the blood samples.

Microbial composition of *L. plantarum* in fecal samples

Bacterial DNA of 250 mg fecal samples was extracted by PowerSoil DNA isolation kit (Cat. #12880, CA, US). For analyzing the total bacterial 16S gene copy number, the 16S universal

primer (BACT1369F, PROK1492R) and probe (5'-FAM-CTTGTACACACCGCCCGTC-BHQ1-3') were used (1). Through a comparative genomics approach with *L. plantarum* CJLP133 and other *Lactobacillus* strains, specific genes of *L. plantarum* and *TagB* (putative glycosyl/glycerophosphate transferase involved in teichoic acid biosynthesis) were detected. Specific primers of *TagB* (TagBF: 5'-ACCTGCTTATGTGGATGC-3', TagBR: 5'-TACCCGAGTGTGTTTCAG-3') and probe (FAM-TCGTCCATCATCGGTTCGCCAC-BHQ1) were used to detect *L. plantarum*. A mixture of real-time PCR contained 10 µL of the 2 × THUNDERBIRD Probe qPCR Mix, 3 µL of DNA template, 1 µL of primer 1 and 2, 1 µL of probe, 0.4 µL of 50 × ROX reference dye and 3.6 µL of distilled water. Reactions took place after 3 min at 95°C, followed by 45 thermocycles: 30 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C (CFX96 REAL TIME SYSTEM, Bio-Rad).

Data analysis was performed by CFX Manager Software (Bio-Rad) Ver 2.1. The percentage of *L. plantarum* among total bacteria is calculated as (starting quantity of *TagB* real time results) × (average of 16S copy number) / (starting quantity of 16S real time results). An average of the 16S copy number was 4 as reported before (2).

REFERENCES

1. Rousselon N, Delgenes JP, Godon JJ. A new real time PCR (TaqMan PCR) system for detection of the 16S rDNA gene associated with fecal bacteria. J Microbiol Methods 2004; 59: 15-22.
2. Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res 2001; 29: 181-4.

Figure legend

Figure S1. Percentage of *L. plantarum* DNA among total bacteria in the probiotics group

