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Therapeutic Effects of Probiotics in Patients with Atopic Dermatitis

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Abstract Recent studies have suggested that oral bacteriotherapy with probiotics might be useful for preventing and managing childhood atopic dermatitis (AD). The purpose of this investigation was to evaluate the efficacy and safety of oral treatment with probiotics for adolescent and adult AD patients as well as for childhood AD patients. Sixty-four patients with mild to moderate AD were recruited for treatment with a mixture of four probiotic strains (*Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Bifidobacterium lactis*) twice daily for 8 weeks. The degree of pruritus was determined by a 10-point visual analog scale every other week, and the patients' global assessments of their clinical responses (*i.e.*, better, unchanged, or worse) was done at the end of intervention. The clinical severity of the eczema was evaluated by eczema area and severity index (EASI) score every other week. As laboratory markers, total immunoglobulin E (IgE), eosinophil cationic protein (ECP) in the serum, and cytokine production [interleukin-4 (IL-4), interleukin-10 (IL-10), and interferon-γ (IFN-γ)] by the peripheral blood mononuclear cells (PBMCs) were measured at the beginning and at the end of intervention. Of the 64 enrolled AD patients, only 50 patients finally completed the 8-week study. After 8-week treatment with probiotics, the EASI score was significantly improved ($p<0.0001$), 50% of the patients experienced improvement of their eczema, and significant improvement of the pruritus was also observed ($p=0.0002$). The effect was more pronounced for the patients with very high IgE levels (>1,000 ku/l) or for the patients with moderate disease severity. There was no significant difference in the therapeutic effects between the childhood AD and adolescent and adult AD patients. There were no significant changes of cytokines, as well as the total IgE and ECP levels, in the patients' serum. Treatment with the mixture of four probiotic strains was generally well tolerated. Our results suggest that the treatment with the mixture of four probiotic strains is beneficial for the management of the adolescent and adult AD patients, as well as for the childhood AD patients.

Key words: Probiotics, adolescent and adult, atopic dermatitis

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Atopic dermatitis (AD) is a common chronically relapsing inflammatory skin disease that is characterized by severe pruritus, dry skin, excoriation, and lichenification. It runs a course of remission and exacerbation, and significantly affects many aspects of the patient's quality of life in a negative fashion; furthermore, the management of AD is complex and challenging [11]. The conventional treatment regimen for AD has included regular skin care with emollients and the use of topical corticosteroids during disease exacerbations [16]. Administration of topical corticosteroids might control the symptoms, especially for mild to moderate eczema, but relapses are common and extensive. Moreover, the prolonged use of corticosteroids carries a risk of local and systemic side effects [15, 16]. Because of these safety limitations, the new nonsteroidal, topical, inflammatory cytokine inhibitors, pimecrolimus cream [2] and tacrolimus ointment [9], have been considered as a therapeutic option for the long-term management of AD. Oral bacteriotherapy with probiotics has recently been explored as a therapeutic option for AD in Western countries.

Probiotics are defined as those products containing a sufficient number of viable microorganisms to alter the host's microflora for producing beneficial health effects [22]. The clinical studies to date have shown that administration of probiotics for childhood atopic disease translates into fewer atopic symptoms and prevents atopic disease in high-risk children [6–8, 12–14, 18–19]. The exact mechanism of action is unknown, but it was suggested that the increased level of transforming growth factor β2 (TGF-β2) [18], IL-10 [13], or IFN-γ [14] may be an explanation for

function. In most studies, *L. rhamnosus* has been used because it has been shown to be safe for use at an early age, it is effective in promoting local antigen and immune responses to pathogen, and it is able to survive passage through and transiently colonize the gastrointestinal tract [5, 7, 12]. The majority of clinical studies have been done using *L. rhamnosus* alone, but mixtures of many probiotics have also been used and they have been proven to be more effective [3, 19].

Although the previous clinical studies showed promising results for treating childhood AD, it is not known what effect probiotic supplementation has on the adolescent and adult AD patients. To address this issue in this study, we evaluated the clinical effects of a mixture of four probiotic strains, *L. rhamnosus*, *L. plantarum*, *L. casei*, and *Bifidobacterium bifidum*, for treating adolescent and adult AD patients, as well as for treating childhood AD patients, and we also measured the levels of serum eosinophil cationic protein (sECP), total serum total IgE (sIgE), and cytokine production from the PBMCs to elucidate the possible therapeutic mechanisms.

MATERIALS AND METHODS

Patients and Study Design

The study was performed from October 2004 to August 2005. A total of 64 patients were recruited to the Department of Dermatology, Kangnam St. Mary's Hospital, College of Medicine, the Catholic University of Korea. AD was diagnosed by the criteria of Hanifin and Rajka [4]. Patients of either sex, aged more than 2 years with mild to moderate AD, according to the severity criteria developed by Rajka and Langeland [17], were recruited. The exclusion criteria applied at recruitment were as follows; any other serious skin disorder, clinically infected AD, any systemic disease, pregnancy or lactation, any chronic condition that was not well controlled, any allergy to probiotics, or if the patients were taking any fermented agents. No systemic immunosuppressive therapy, phototherapy, antibiotics, and systemic corticosteroid therapy were allowed from 4 weeks prior to start of the study.

The patients received one sachet of probiotics twice daily for 8 weeks. A sachet of probiotics contained 1×10^9 colony forming units (CFU); a mixture of 4 bacterial species: *L. rhamnosus* 2×10^8 CFU, *L. plantarum* 2×10^8 CFU, *L. casei* 4×10^8 CFU, and *B. lactis* 3×10^8 CFU. During the 8-week study period, the patients were asked to abstain from any fermented food products containing live microorganisms, any systemic or topical corticosteroids, or any immunosuppressive drugs. Patients were permitted to take oral antihistamines and to use emollients that had been used previous to the study. Otherwise, none of the patients changed their diet during the study period. The study protocol included the consent form written by the patients or by the parents of the participating patients who were aged less than 18 years. This study was approved by Kangnam St. Mary's Hospital's institutional review board (IRB).

Bacterial Strains

The majority of previous clinical studies have been done using *L. rhamnosus* because of its safety, immunologic effect, and stability [5, 7, 13, 14]. We used a mixture of four strains of probiotic bacteria including *L. rhamnosus* because it is well known that a mixture of many probiotics might reinforce any beneficial effect of *L. rhamnosus* [3, 19]. The other three strains of lactic acid bacteria (LAB) were selected according to their function in the human digestive tract. *L. plantarum* was chosen because it is a representative LAB strain that inhabits the small intestine. *B. lactis* was selected as a main occupant in the large intestine and *L. casei* was chosen because of its high protease activity. It was expected that the proteases would operate to cut off the antigenic proteins and so relieve the symptoms of atopy.

The obtained nucleotide sequence of the LAB strains were accessed from the National Center for Biotechnology Information (NCBI) GenBank as follows; *L. rhamnosus* LR(3) (AY675253), *L. plantarum* LP(1) (AY735405), *L. casei* LC(2) (AY699577), and *B. lactis* BL(2) (AY700230). The strains were cultured separately in liquid medium containing enzymatically hydrolyzed casein (3.0%), glucose (2.0%), and lactose (4.0%) at 37°C under anaerobic conditions. The cultured cells were harvested and coated with protein and polysaccharides and freeze-dried to improve their stability and shelf life by the method of the dual-protein coating process (DUOLAC method, Korean Patent No. 10-0429495). The prepared dual-protein coated mixed LAB products that were specifically designed for AD obtained approval (No. 2004-0039-120) from the Korea Food and Drug Administration (KFDA) as health promoting food products and they were used in the clinical tests. The probiotic products were stored for the clinical test at 4°C until use.

Clinical Evaluation

The patients were evaluated at baseline and at weeks 2, 4, 6, and 8. The clinical evaluation parameters included the EASI score and the patients' subjective assessments of pruritus and the clinical responses. The EASI score combined the clinical evaluation of the intensity and extent of the eczema. The range of the EASI score was from 0 to 72. The patients were asked to assess their pruritus using a visual analog scale, with 0 indicating no itching sense and 10 indicating the worst itch imaginable, and they provided a global assessment of the clinical response as better, unchanged, or worse.

IgE and ECP in Serum

At the beginning and end of the intervention, a blood sample for the analysis of the total sIgE and sECP levels was drawn. Three ml of blood was collected from each patient. The serum was separated in a refrigerated centrifuge at 1,100 ×*g* at 4°C for 10 min. All the samples were stored at −20°C until use. The total sIgE and sECP levels were measured from the serum samples using the UniCAP Total IgE Fluoroimmunoassay kit and UniCAP ECP Fluoroimmunoassay kit (PharmaciaDiagnostics, Uppsala, Sweden), respectively, on a UniCAP 100 automatic analyzer (PharmaciaDiagnostics) according to the manufacturer's instructions. The ranges of measurement of the total sIgE and the sECP levels were from 2 to 5,000 ku/l and from 2 to 200 μg/l, respectively. The normal reference range for the total sIgE and the sECP levels were from 0 to 200 ku/l and from 0 to 15 μg/l, respectively.

Production of Cytokines from PBMCs

Isolation and Culture of PBMCs. Analysis of the production of cytokines from the PBMCs was performed at the beginning and the end of the intervention. PBMCs were isolated by centrifugation over Ficoll-Hypaque density gradients, according to the manufacturer's instruction (Sigma-Aldrich, St. Louis, MO, U.S.A.). The cells were washed with phosphate-buffered saline (PBS), and then cell viability was checked by the trypan blue exclusion method. The cells (2×10^6/well) were cultured in 6-well flat-bottom plates that contained RPMI-1640 supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, U.S.A.), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Five ng/ml of phorbol myristate acetate (PMA) and 5 μg/ml of phytohemagglutinin (PHA) were added into each well and the cells were incubated at 37°C in 5% CO_2 for 2 days.

Cytokine mRNA and Protein Detection. The mRNA expression for cytokines (IL-4, IL-10, and IFN-γ) was investigated by performing the semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The total RNA was extracted from the cultured PBMCs using commercial Tri reagent (Sigma-Aldrich, St. Louis, MO, U.S.A.). Reverse transcription into cDNA was performed with the cDNA synthesis kit (Promega, Madison, WI, U.S.A.) in a 20-μl reaction volume, according to the manufacturer's instruction. PCR was carried out using gene-specific primer sets in 20-μl reaction volumes [1, 21] (Table 1). RT-PCR amplifications were performed under the following conditions; 94°C for 30 sec, 60°C (for glyceraldehyde-3-phosphate dehydrogenase and GAPDH), 56°C (for IL-10), 52°C (for IL-4), and 50°C (for IFN-γ) for 30 sec, and then 72°C for 45 sec. The PCR products were analyzed by electrophoresis in a 2% agarose gel in 0.5× Tris-Boric-EDTA (TBE) buffer, and the products were visualized in a UV transilluminator by staining with 0.5 μg/ml of ethidium bromide. To quantify the intensity of the ethidium bromide signals, the gels were photographed and the images were analyzed by densitometry using ImageMaster software.

Furthermore, the quantities of IL-4 and IL-10 in the culture supernatants were measured with ELISA (BD Biosciences, San Jose, CA, U.S.A.). For all the assays, the background absorbance was subtracted from all the data points and the amount of cytokine in each sample was determined by extrapolating the OD values to the cytokine concentrations using the linear range of the standard curve. All the cytokine levels were determined by a commercial method according to the manufacturer's instruction.

Table 1. The primers used in the PCR for cytokines.

Cytokines	Primers
IFN-γ	5'-TCTGCATCGTTTTGGGTTCT-3', 5'-CAGCTTTTCGAAGTCATCTC-3'
IL-4	5'-ACTGCTTCCCCCTCTGTTCTTCC-3', 5'-GAGGTTCCTGTCGAGCCGTTTCA-3'
IL-10	5'-TGCTCTGTTGCCTGGTCCTCCTGA-3', 5'-GCTCCACGGCCTTGCTCTTGTTTT-3'
GAPDH	5'-ACCACAGTCCATGCCATCAC-3', 5'-TCCACCACCCTGTTGCTGTA-3'

Statistics

The data were presented as means with 95% CI or medians with an interquartile range (IQR). The Wilcoxon signed rank test was used for comparison between baseline and at 8 weeks of treatment for the EASI score, the visual analog scale for pruritus, the sIgE, the sECP, and the cytokines. Repeated measures of ANOVA were used to test interactions between the EASI score and different baseline characteristics. *p*-Values less than .05 were considered significant.

RESULTS AND DISCUSSION

Study Population

Initially, 64 patients were included in the study, but 14 patients (21.9%) did not complete the study. Discontinuation of the study was mostly secondary to follow-up loss (11 patients), whereas 2 patients discontinued because of adverse effects, and 1 patient discontinued because of exacerbation of the eczema. Thus, 50 patients (58% male patients; median age, 19.5 years; age range, 5–42 years; severity, 72% moderate) finally completed the study and the demographic and clinical characteristics of these patients are presented in Table 2.

Clinical Effects

A statistically significant clinical improvement was observed over the treatment period. At inclusion, the median EASI

Table 2. Demographic data of 50 patients.

		Patients
Age (y)		19.5 (5–42)
	2–12y	16 (32%)
	>12y	34 (68%)
Gender	male	29 (58%)
	female	21 (42%)
Severity	mild	14 (28%)
	moderate	36 (72%)
Food allergy		15 (30%)
Skin prick test or RAST*		
	Pollen	4
	House dust mite	16
	Molds	6
	Pets with fur	13
	Cow's milk	1
	Egg	2
	Soy	0
	Wheat	0
EASI score		10.5 (0.7–33.8)
VAS for pruritus		3.25 (0–10)
sIgE (ku/l)†		485.5 (7.37–15060)
	very high (>1,000 ku/l)	16 (33.3%)
	high (200–1,000 ku/l)	16 (33.3%)
	normal (<200 ku/l)	16 (33.3%)
sECP (μg/l)†		18.1 (2–200)

Values are presented as median (range).
EASI, eczema area and severity index; VAS, visual analog scale.
*Data available: 27 patients.
†Data available: 48 patients.

score was 10.5 (range, 0.7–33.8). As the study progressed, the median EASI score decreased to 8.04 (range, 0–28.3) at week 2, 6.64 (range, 0–27) at week 4, and 6.28 (range, 0.2–22.9) at week 6, and at the end of the study the median EASI score was reduced to 5.65 (range, 0–22.8) (Fig. 1, Table 3). Patients treated with probiotics showed a significant improvement in their median EASI score after 8 weeks of treatment ($p<.0001$). There was a significant improvement in the mean EASI score 2 weeks after starting intervention, which was sustained throughout the study period (Fig. 1).

The patients' assessments for pruritus using the visual analog scale showed a decrease in the score from 3.25 (range, 0–10.00) at baseline to 3.00 (range, 0–8.50) at the end of the study ($p=.0002$) (Table 3). The patients' self-assessments of the clinical response also showed mild to moderate improvement throughout the study (better, 50%; unchanged, 40%; worse, 10%) (Table 3).

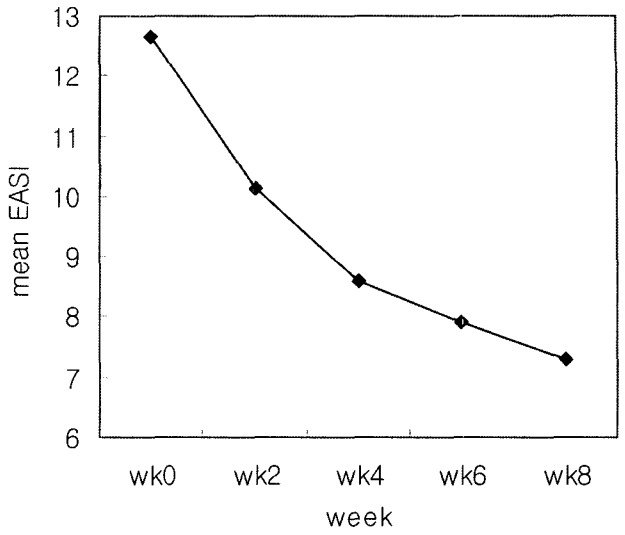

Fig. 1. Significant decrease in the mean EASI scores: changes from baseline until end of treatment ($p<.0001$).

It appears that the treatment response was more pronounced in the group with moderate disease, as compared with the group with mild disease ($p=.0003$), or it was more pronounced in the group with a very high IgE level as compared with the group with a high or normal IgE level ($p<.0001$) (Table 4). In addition, a significant relation between the EASI score and the disease severity or the sIgE level was observed ($p=.0158$ and $p=.0006$, respectively). However, it is likely that the treatment response showed no significant difference between children and adults ($p>.6$), nor between patients with food allergy and those without food allergy ($p>.2$) (Table 4).

Serum IgE, ECP, and Cytokines

After intervention, the sIgE levels decreased from 485.5 (range, 737–15,060) ku/l to 462.5 (range, 7.21–16,180) ku/l, but no significant changes were found ($p=.1994$) (Table 5). After probiotics treatment, the sECP levels decreased from

Table 3. Comparison of clinical scores between baseline and week 8.

	Baseline	Week 8	*p*-Value*
EASI score	10.50 (0.70–33.80)	5.65 (0–22.80)	<.0001
VAS for pruritus	3.25 (0–10.00)	3.00 (0–8.50)	.0002
Patient's global assessment (Patient No., %)		better (25, 50%) unchanged (20, 40%) worse (5, 10%)	

Values are presented as median (range).
*Wilcoxon signed rank test.

Table 4. Comparison of EASI score among the different subgroups.

Group		EASI		*p*-Value*
		Baseline	Week 8	
Age	>12y	13.976 (±9.417)	8.388 (±6.625)	.6014
	2–12y	9.856 (±9.566)	5.019 (±5.546)	
Severity	Mild	5.300 (±5.575)	2.471 (±2.642)	.0158†
	Moderate	15.519 (±9.300)	9.192 (±6.519)	
sIgE	Very high	21.363 (±9.154)	12.375 (±7.277)	.0006†
	High	10.106 (±6.662)	6.481 (±4.856)	
	Normal	7.594 (±6.730)	3.675 (±3.698)	
Food allergy	Yes	14.640 (±9.371)	7.960 (±6.595)	.2492
	No	12.282 (±9.740)	7.306 (±6.545)	

Values are presented as mean (±SD).
*Repeated measures of ANOVA.
†*p*-Value indicates the significance of the relation between the EASI score and subgroups.

18.1 (range, 2–200) μg/l to 14.3 (range, 2–172) μg/l, but no significant changes were found (p=.1359) (Table 5). No significant changes were found in the mRNA expression levels of IL-4, IL-10, and IFN-γ, and similar results were observed in the analysis of the production of cytokines (IL-4 and IL-10) by ELISA.

Safety

Adverse events were monitored by the investigators at each visit. In most patients, the probiotics were well tolerated and only one patient complained of constipation, but that patient completed the study.

In this study, we evaluated the therapeutic effects of probiotics for the patients with mild to moderate AD by administering a mixture of four probiotic strains. This probiotic treatment was effective for treating adolescent and adult AD, as well as childhood AD, and there was no significant difference in the therapeutic response between the two age groups. However, according to the clinical and laboratory parameters, the therapeutic efficacy was more pronounced for the patients with very high sIgE levels or for the patients with moderate disease severity. In addition, there were no significant changes of the cytokines (IL-4, IL-10, and IFN-γ), sIgE, and sECP at the beginning and at the end of the probiotic treatment.

A growing body of evidence has recently demonstrated that the administration of probiotics is effective for improving the atopic symptoms [6, 12–14, 18] and for preventing atopic disease in the high-risk group [7, 8]. These previous studies were performed in selected atopic patients (age <2 years, with or without food allergy, and mild to moderate disease) who may have been responsive to dietary intervention with probiotics [6–8, 12–14, 18]. They received *L. rhamnosus* GG (LGG) or *B. lactis* Bb-12 and they showed a diminished SCORAD score. In a study by Rosenfeldt *et al.* [19], the administration of probiotic *Lactobacillus* strains (a mixture of *L. rhamnosus* 19070-2 and *L. reuteri* DSM 12246) to an unselected group of children (age range, 1–13 years; mean age, 5.2 years) with moderate to severe eczema improved the clinical severity of the eczema and the level of sECP, and the effect was more pronounced for the allergic patients. However, compared with the previous studies, the intervention of that study showed only modest improvement of atopic eczema; this may be attributable to the older age of the subjects and the severity of the eczema, which may be less responsive to dietary intervention.

According to these previous studies, the possible mechanisms of probiotics in children suffering with AD suggested that probiotics can reverse any increased intestinal permeability, enhance the gut-specific IgA responses,

Table 5. Changes of sIgE, sECP, and cytokines at baseline and at week 8.

	Baseline	Week 8	*p*-Value*
sIgE (ku/l)	485.50 (7.37–15,060)	462.50 (7.21–16,180)	.1994
sECP (μg/l)	18.10 (2–200)	14.30 (2–172)	.1359
Cytokines			
IFN-γ	1.00 (0.02–111.48)	1.00 (0.02–35.26)	.5709
IL-4	1.39 (0.02–83.82)	1.00 (0.03–45.63)	.7478†
IL-10	1.045 (0.02–12.33)	1.00 (0.02–22.53)	.8246†

Values are presented as median (range).
*Wilcoxon signed rank test.
†Similar results were observed with ELISA.

help to promote the gut barrier function and restore the normal gut microecology, and probiotics can enhance the production of TGF-β2, IL-10, and IFN-γ [5, 6, 13, 14, 18, 20]. Contrary to the children with AD, the probable mechanisms for the effects of the probiotic preparation on adults with AD have not been elucidated. In this study, we measured the levels of cytokines to examine the possible mechanisms, but there was no significant correlation between the clinical effects and the levels of cytokines in the adolescent and adult AD patients as well as in the childhood AD patients. Therefore, further studies are needed to elucidate the mechanisms of probiotics in adolescent and adult AD patients.

In this study, most of the patients who completed the study were in the chronic stable stage of eczema and showed clinical improvements, but one patient who had active eczema at the beginning of the study had to stop the intervention because of an exacerbation of disease and this patient was then treated with systemic immunosuppressive therapy. From this clinical experience, we may suggest that the probiotics have a convincing effect as a maintenance therapy for chronic stable eczema and for prevention of acute flare-up, but the probiotics failed to show any benefit for the treatment of acute exacerbated AD. This preventive effect for pruritus was also applied to the inflammatory bowel conditions. According to the study by Kuisma *et al.* [10], the administration of LGG was inefficient for the clinical improvement of pouch inflammation as the primary therapy, because the fast transit time, the high stool frequency, watery stool, and the change in mucosal morphology might have disturbed the adherence and colonization of probiotic bacteria. Likewise, we think the probiotics may also be inefficient for treating acute exacerbated AD. The more disturbed gut microecology and barrier function or using a lower dose of probiotics that is less than what is needed for their intestinal survival in active AD patients may prevent the effect of probiotics on the gut.

Unlike the previous probiotic studies, we could not compare the effect of each of the four probiotic strains with placebo or with the other strains. For this purpose, more patients and a longer study time are needed. This may prevent us from fully appreciating the effects of the probiotic preparations in this trial. It could also be argued that the treatment or follow-up period was a bit too short.

Although this trial has several drawbacks, our results constitute the first clinically documented demonstration of a possible role for the mixture of four probiotic strains in controlling adolescent and adult AD, as well as childhood AD. In the future, further studies are needed to explore the possible strain-specific effects, the effects of long-term probiotic supplementation, and the mechanisms of probiotic bacteria on adolescent and adult AD.

In conclusion, the mixture of four probiotic strains was effective and safe for helping manage adolescent and adult AD, as well as childhood AD. Our results suggest that the probiotic approach may offer beneficial effects as an adjuvant therapy for the management of patients suffering with chronic stable AD.

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