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Clinical Effects of Probiotic *Bifidobacterium breve* Supplementation in Adult Patients with Atopic Dermatitis

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Probiotics (live microbial food supplements) have been shown to be effective in allergic diseases, but the potential of probiotics for treatment of adult atopic dermatitis is not clear. The effect of probiotic *Bifidobacterium breve* (*B. breve*) for atopic dermatitis was assessed in this study. A total of 24 patients were given either *B. breve* strain YY or a placebo for 8 weeks. Clinical severity was evaluated by using the severity scoring of atopic dermatitis and quality of life was assessed by Skindex-29-J. In addition, blood and fecal samples were taken before and after the study. The objective severity scoring for atopic dermatitis significantly improved in the probiotic group compared with the placebo group. In the quality of life assessment, the total score showed significant improvement in the probiotic group. The proportion of *B. breve* in intestinal microflora was increased in the probiotic group. However, no significant change in thymus and activation-regulated chemokine (TARC/CCL17), immunoglobulin E or the number of eosinophils was found. Our results suggest that *B. breve* may be beneficial for the treatment of atopic dermatitis. However, since the severity of disease symptoms in the placebo group at the beginning of the study was milder than those in the probiotic group because the number of patients in the present pilot study was relatively small, further study is needed to determine the clinical value of *B. breve* in adult patients with atopic dermatitis.

Key words: allergy; *Bifidobacterium*; intestinal; probiotics; quality of life

Atopic dermatitis (AD) is characterized by an itchy rash that often appears on the face and neck, in the cubital fossa, and in the popliteal fossa, tending to flare up and spread throughout the body. Patients with AD are also predisposed to other allergic diseases, including bronchial asthma and allergic rhinitis. The basic treatment regimen consists of anti-inflammatory therapy focusing mainly on the use of topical steroids appropriate to disease severity and prevention of relapse or recrudescence after remission by placing an emphasis on skin care and self-control against causal and aggravating factors (Furue et al., 2009). In 80% of patients, symptoms appear by the age of 5 and involve a genetic factor known as "atopic disposition." Recently, filaggrin deficiency is thought to be the major risk factor for AD sufferers (O'Regan et al., 2009). In addition to diminished barrier function, environmental factors such as mites and house dust, perspiration, bacteria or fungi, contact antigens, stress and food have been indicated as causal and aggravating factors. Physicians provide guidance regarding schemes

Abbreviations: AD, atopic dermatitis; IgE, immunoglobulin E; LAB, lactic acid bacteria; QOL, quality of life; SCORAD, severity scoring of atopic dermatitis; TARC, thymus and activation-regulated chemokine

and efforts to alleviate those factors in daily life. Treatment does not aim at providing a complete cure but rather at controlling the disease on a level that causes less of a hindrance to the patient in daily life.

From an epidemiological survey conducted in 1989, Strachan proposed the so-called "hygiene hypothesis" according to which "exposure to infections or unhygienic environments up to infancy reduces the subsequent onset of allergic diseases" (Strachan, 1989). In fact, AD has been increasing in developed countries. If, during infancy, someone is too clean and has too few opportunities to have contact with bacteria and viruses, helper T cells (Th cells) are not stimulated and, consequently, allergies increase. That theory is easy to understand and has been relatively accepted (Flöistrup et al., 2006).

Probiotics are defined as "living microorganisms and products containing microbial metabolites which, in vivo, affect the normal intestinal bacterial flora by improving its balance, and which are consequently biologically beneficial" (Fuller, 1989; Salminen et al., 1998). A large number of lactic acid-producing bacteria (such as *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Bifidobacterium*) are used as probiotics. Owing to their bactericidal and antiproliferative effects against pathogens, they primarily help regulate intestinal functions. Secondly, they also have a stimulating effect on immunity.

In 2001, in a double-blind study, Kalliomäki et al. administered the lactic acid bacterium (LAB) *Lactobacillus rhamnosus* (*L. rhamnosus*) to 159 pregnant women with an atopic disposition and their infants aged up to 6 months (Kalliomäki et al., 2001). At the age of 2 years, the incidence of AD was significantly lower in the *Lactobacillus*-treated group than in the placebo-treated group. In a follow-up study, the frequency of AD was also low at 4 years of age in the *Lactobacillus*-treated group (Kalliomäki et al., 2003). In a double-blind study, Kukkonen et al. administered 4 types of encapsulated probiotics (*L. rhamnosus* GG and LC705, *Bifidobacterium breve* (*B. breve*) and *Propionibacterium freudenreichii* ssp. *Shermanii*) to 1,223 pregnant women with an atopic disposition. To the newborns of those women, 0.8 g of galactooligosaccharide (a saccharide that helps the intestinal growth of LAB) or a placebo was added as a prebiotic and administered in addition to *Lactobacillus* for 6 months after birth (Kukkonen et al., 2008). At 2 years of age, the incidence of AD was significantly lower in the probiotics-treated group than in the placebo-treated group. Hattori et al. reported an elevation of the percentage of *Bifidobacterium* of the intestines and an improvement of cutaneous symptoms after administration of *B. breve* to AD infants with low amounts of intestinal *Bifidobacterium* (Hattori et al., 2003). Intestinal flora have a great influence on whether Th cells become Th1 or Th2 type cells. In hygienic environments, administration of probiotics to pregnant women and newborns promotes a healthy differentiation of naive T cells into Th1 cells during infancy and is considered useful in preventing and treating AD (He et al., 2001).

Meanwhile, in adults who have passed beyond the period of body growth and who no longer show any major changes in the balance between Th1 and Th2 cells, improvement of AD symptoms by probiotics has been hitherto considered difficult (Lee et al., 2008). However, it has been reported that *Bifidobacterium* is present in a significantly low number in the intestinal flora of AD patients after infancy (Suzuki et al., 2008). Among probiotics, *Lactobacillus* is often used as fermented milk for its excellent flavor. In Japan, a fermented milk food has been developed based on live *B. longum*, which has a high fixation ratio in the intestines of adults and is excellent for regulation of the intestinal functions; its antiallergic effect is being studied (Xiao et al., 2007; Iwabuchi et al., 2009).

In the present study, *B. breve* was selected on the basis of a report that this organism develops dominantly in the intestines of healthy breast-fed infants but is present in low numbers in the intestines of infants with AD, and because in addition to its regulatory effect on intestinal function, its immunoregulatory effect is potentially useful in

adult patients with AD. Viable *B. breve* or placebo capsules were administered to 24 adult patients with AD in a double-blind manner. Disease severity was assessed using the severity scoring of AD (SCORAD) (Kunz et al., 1997); intestinal flora were examined through feces sampling, serum markers such as serum immunoglobulin E (IgE) and plasma thymus and activation-regulated chemokine (TARC) levels were measured (Kakinuma et al., 2001), and skin-disease-specific quality of life (QOL) was evaluated exhaustively.

Subjects and Methods

The present study has been reviewed by the Ethical Review Board of Tottori University Hospital, and was conducted from January 2008 through May 2009 after being approved on April 11, 2007 (Approval Number: 841). Written voluntary agreement was obtained after a full explanation was provided to the subjects regarding research purposes and methods, prospective benefits and risks, and they understood that a lack of participation would not result in any disadvantage, that consent could be withdrawn at any time, and that their personal information would be protected.

Twenty-four adult patients (8 men, 16 women; mean age: 30.2 ± 9.6 years), diagnosed according to the Guideline for Management of AD by the Japanese Dermatological Association (Furue et al., 2009), were selected as test subjects. During the study, as a general rule, we decided to continue each participant's pre-study treatment regimen.

Test meals for 24 subjects were randomly allocated by the controller as follows: 16 subjects were given test sample A containing *B. breve* (*B. breve*-administered group), and 8 subjects were given test sample B containing a placebo (control group). After sample allocation, the key code was kept by the controller. The test was terminated once the prescribed number of participants was reached. (Test sample A: capsules filled with a lyophilized powder of live *B. breve* strain YY. Test sample B: capsules in which the Test sample A constituent was replaced with a placebo powder. Test samples were provided by Tokiwa Pharmaceutical, Kobe, Japan).

The administered dose of *B. breve* was more than 20 billion per day (2.0×10^{10} colony forming unit). Test sample A was formulated to contain more than 10 billion *B. breve* (1.0×10^{10} colony forming unit) per capsule. The test samples were ingested as one capsule after breakfast and one after dinner. The study period was 8 weeks. Dermatologists rapidly examined subjects' symptoms before study initiation and 8 weeks after its completion. Severity was assessed on the basis of SCORAD.

Fecal sampling was performed and intraintestinal bacterial flora was searched before study initiation and 8 weeks after its completion. Naturally excreted feces collected by the subjects were immediately chilled under anaerobic conditions and bacterial flora was searched for within 24 h (Calpis, Tokyo, Japan).

Blood collection was performed before study initiation and 8 weeks after its completion; blood markers including serum IgE, eosinophils and plasma TARC were examined (Mitsubishi Chemical Medience, Tokyo).

Skindex-29 is a measure using 29 items that can help determine health-related QOL associated with skin disease (Chren et al., 1997). Before study initiation and after its completion, the skin disease-specific QOL questionnaire Skindex-29-J (Japanese version) was completed (Fukuhara, 2004).

Statistical analyses were conducted using SPSS software (SPSS 12.0J for Windows, SPSS, Chicago, IL). For an intergroup comparison between the *B. breve*-administered group and the control group, Student's *t*-test was performed for age, the chi-square test was performed for gender, Mann-Whitney's *U* test was performed for the SCORAD, and the chi-square test was performed among disease-severity-based subgroups. The correlation between the objective SCORAD and each end-point (QOL according to the Skindex-29-J, *Bifidobacterium* in the intestinal flora and blood markers) was examined using Spearman's rank

Table 1. Correlation coefficient between evaluation items at baseline

	Objective SCORAD	
	rs	*P* value
Skindex-29-J	0.547	0.006**
Bifidobacterium (log number)	–0.066	0.758
Bifidobacterium (%)	–0.249	0.241
Eosinophil	0.465	0.022*
IgE (RIST)	0.585	0.003**
TARC	0.569	0.004**

IgE, immunoglobulin E; TARC, thymus and activation-regulated chemokine; RIST, radio-immunosorbent test; *rs*, Spearman rank correlation coefficient; SCORAD, severity scoring of atopic dermatitis.
* $P < 0.05$, ** $P < 0.01$.

correlation coefficient. For comparison within each group before initiation and after completion, the Wilcoxon ranked sum test was used for SCORAD, Skindex-29-J and enterobacteria search. Student's *t*-test was performed for blood tests.

Results

Regarding treatment circumstances, 18 patients used topical preparations (17 used steroids, 7 concomitantly received internal tacrolimus and 1 used Vaseline only) and 15 used orally administered medicines (anti-histamines). During the study period, as a general rule, no pre-study treatment methods were changed.

We examined the relationships between the objective SCORAD of all patients at study initiation and at each end-point (Table 1). Skindex-29-J, number of eosinophils, total IgE levels and plasma TARC levels showed significantly positive correlations to the objective SCORAD. In contrast, *Bifidobacterium* in the intestinal flora showed no correlation in terms of logarithmic number or rate of colonization.

We compared the demographic attributes of the placebo and *B. breve*-administered group subjects (Table 2). There were no differences for gender or age between the 8 subjects in the placebo group and the 16 subjects in the *B. breve*-administered group. The average age of the test subjects was 30.2 years (range, 20–65 years). However, the average placebo group SCORAD score was 25.7 and the average *B. breve*-administered group SCORAD score was 41.0. Patients with high disease severity were present in significantly large numbers in the *B. breve*-administered group. This is attributable to the fact that the study was not randomized according to disease severity. All SCORAD values in the *B. breve*-administered group decreased after the study, and a statistically significant difference was found in the objective SCORAD and in B value, which is indicative of the extent of the rash (Table 3). Meanwhile, the placebo group showed no change (Table 4).

At the beginning of the study, no difference was found between instestinal flora in the feces of the *B. breve*-administered group and that of the placebo group. Since fecal sampling could not be performed in one *B. breve*-administered group subject (for personal reasons), the subject was excluded; post-study analysis of the intestinal flora was conducted in only 15 *B. breve*-administered

Table 2. Demographics and clinical characteristics of the subjects at the study initiation

Variable	Placebo [*n* = 8]	*B. breve* [*n* = 16]	*P* value†
Age (year)			
Mean	29.3	30.7	0.738
Range	22 – 65	20 – 46	
Gender			
Number of males (%)	3 (37)	5 (31)	0.759
Number of females (%)	5 (63)	11 (69)	
Race			
Asian (%)	100	100	
SCORAD			
Total score	25.7	41.0	0.027*
Objective SCORAD	21.8	33.7	0.016*
Severity of AD‡			
Mild, < 15.0	1	1	0.133
Moderate, 15.0–40	7	9	
Severe, > 40.0	0	6	

AD, atopic dermatitis; *B. breve, Bifidobacterium breve*; SCORAD, severity scoring of atopic dermatitis.
† Student's *t*-test, chi square or Mann-Whitney's *U* test; *$P < 0.05$.
‡ Severity of AD based on objective SCORAD.

Table 3. Change from baseline in the *Bifidobacterium breve*-administered group [*n* = 16]

Variable		Baseline			Final			*P* value†
		Mean	±	SD	Mean	±	SD	
SCORAD	Total score	41.0	±	18.0	35.3	±	16.9	0.109
	Objective SCORAD	33.7	±	13.6	28.9	±	14.0	0.034*
	A: Extent criteria	28.3	±	20.6	25.5	±	23.4	0.437
	B: Intensity criteria	8.0	±	2.9	6.8	±	3.0	0.018*
	C: Subjective symptoms	7.3	±	5.9	6.3	±	5.0	0.861
Fecal microflora (log number)	Total bacteria	10.36	±	0.40‡	10.43	±	0.45‡	0.362
	Bifidobacterium	8.89	±	2.57‡	9.79	±	0.68‡	0.060
	Clostridium lecithinase (+)	1.90	±	2.29‡	1.49	±	2.05‡	0.208
	Enterobacteriaceae	7.31	±	1.25‡	7.33	±	1.01‡	0.834
Fecal microflora (%)	*Bifidobacterium*	22.11	±	20.2‡	36.39	±	27.28‡	0.031*
	Clostridium lecithinase (+)	0.00	±	0.00‡	0.00	±	0.00‡	0.180
	Enterobacteriaceae	1.38	±	3.13‡	0.46	±	0.66‡	0.400
Diagnostic marker	Plasma TARC (pg/mL)	760.8	±	1356.3	511.7	±	1099.2	0.133
	Serum IgE (RIST) (IU/mL)	5694.6	±	8087.0	5463.2	±	8164.4	0.444
	Eosinophil (/μL)	528.8	±	503.6	475.6	±	426.1	0.418
QOL	Skindex-29-J	40.0	±	21.2	30.4	±	14.1	0.019*
	Emotions	42.7	±	23.9	32.3	±	16.2	0.030*
	Symptoms	57.8	±	25.8	44.6	±	13.5	0.016*
	Functioning	27.5	±	21.1	20.6	±	15.6	0.087

QOL, quality of life; RIST, radio-immunosorbent test; SCORAD, severity scoring of atopic dermatitis; TARC, thymus and activation-regulated chemokine.

† Wilcoxon signed rank test or Student's *t*-test; *$P < 0.05$.

‡ $n = 15$.

Table 4. Change from baseline in the placebo group [*n* = 8]

Variable		Baseline			Final			*P* value†
		Mean	±	SD	Mean	±	SD	
SCORAD	Total score	25.7	±	6.6	26.1	±	12.1	0.866
	Objective SCORAD	21.8	±	6.8	21.1	±	9.3	0.440
	A: Extent criteria	14.9	±	13.6	13.6	±	12.0	0.292
	B: Intensity criteria	5.4	±	1.4	5.3	±	2.1	0.888
	C: Subjective symptoms	3.9	±	2.0	5.0	±	3.5	0.323
Fecal microflora (log number)	Total bacteria	10.53	±	0.33	10.61	±	0.46	0.389
	Bifidobacterium	9.88	±	0.53	9.88	±	0.71	0.866
	Clostridium lecithinase (+)	1.48	±	2.08	2.06	±	2.58	0.500
	Enterobacteriaceae	6.78	±	1.33	7.29	±	0.98	0.834
Fecal microflora (%)	*Bifidobacterium*	29.93	±	17.57	21.83	±	13.43	0.123
	Clostridium lecithinase (+)	0.00	±	0.00	0.00	±	0.00	1.000
	Enterobacteriaceae	0.21	±	0.36	0.61	±	1.36	1.000
Diagnostic marker	Plasma TARC (pg/mL)	179.3	±	281.6	292.3	±	577.4	0.332
	Serum IgE (RIST) (IU/mL)	699.4	±	742.2	629.6	±	605.0	0.250
	Eosinophil (/μL)	332.5	±	364.1	336.3	±	245.0	0.973
QOL	Skindex-29-J	27.8	±	14.8	24.9	±	17.1	0.401
	Emotions	32.5	±	21.3	30.9	±	19.2	0.574
	Symptoms	39.7	±	11.5	34.2	±	19.1	0.527
	Functioning	16.8	±	16.0	14.6	±	17.7	0.600

QOL, quality of life; RIST, radio-immunosorbent test; SCORAD, severity scoring of atopic dermatitis; TARC, thymus and activation-regulated chemokine.

† Wilcoxon signed rank test or Student's *t*-test.

group subjects (Table 3). The logarithmic number of *Bifidobacterium* increased, and a remarkable and statistically significant increase in colonization rate was seen (Table 3). Meanwhile, no change was observed in intestinal flora of the control group (Table 4). On the other hand, no significant difference was found in blood variables after the study in either group.

At study initiation, the Skindex-29-J score was 40.0 in the *B. breve*-administered group and 27.8 in the placebo group; likewise, there was also a difference in the Skindex-29-J score (Tables 3 and 4). Overall Skindex-29-J values decreased significantly as a result of *Bifidobacterium* administration (Table 3). Among the measured Skindex-29-J items (emotions, symptoms and functions), emotions and symptoms also significantly decreased. Meanwhile, the Skindex-29-J of the placebo group showed no change (Table 4).

Discussion

In 1899, *Bifidobacterium* was isolated from the feces of breast-fed children and was used as a probiotic for the first time (Tissier, 1899). While in the mother's womb, human beings live in an aseptic environment, but by the 6th or 7th day after birth, *Bifidobacterium* accounts for as much as 95% of the enteric bacteria in infants' intestines. From then on, under the influence of various factors such as age, eating habits and stress, *Bifidobacterium* decreases to 15% in adults and < 1% in the elderly. Generally, LAB produce lactic acid by degrading sugar, but *Bifidobacterium* has strong bactericidal activity in addition to producing acetic acid. *Bifidobacterium* regulates intestinal function but, unlike LAB, it cannot grow in the presence of oxygen; for that reason, it must be carefully processed.

The predominant types of *Bifidobacterium* present in human intestines vary from *B. breve* in infants, *B. longum* in adults and *B. adlescentis* in the elderly (Mitsuoka, 2005). Previous reports have also mentioned administration of *B. breve* via bottle-feeding to ensure the formation of normal intestinal bacterial flora in low-weight children (Akiyama et al., 1994).

Although prevention of AD by administration of probiotics to mothers and infants has already been reported (Kalliomäki et al., 2003), most reports on the treatment of patients with AD by probiotics were based on studies in infants. A randomized double-blind crossover study of a combination of *L. rhamnosus* and *L. reuteri* in patients with childhood AD (average 5.2 years of age) was also reported (Rosenfeldt et al., 2003). Cutaneous eruption size according to the SCORAD (A values) decreased significantly in probiotic-administered groups. Meanwhile, only a few studies have confirmed the efficacy of probiotic administration in adult AD patients. Roessler et al. recently reported that SCORAD scores tended to improve after administration of *L. paracasei* and *B. lactis* to adult AD patients (Roessler et al., 2008). In addition, an epidemiologic study reported that the number of *Bifidobacterium* was significantly lower in AD patients than in healthy subjects (Watanabe et al., 2003).

Therefore, in this study, we examined the efficacy of administration of live encapsulated *B. breve* to adult AD patients. Throughout the study period, we found no exacerbation of allergic symptoms or adverse reactions (digestive symptoms) caused by the administration of test meals, suggesting that live *B. breve* is safe for adult AD patients. In addition to a significant decrease in objective SCORAD after administration of live *B. breve*, a significant decrease of the extent of cutaneous eruption (B value) was found as well as elevated *Bifidobacterium* colonization rate in feces. This also indicates that *B. breve* administration improves skin disease-specific QOL of adult patients with AD. However, blood tests showed no significant difference in TARC level, an AD blood variable. Overall, the present study suggests that administration of live *B. breve* is a safe and useful adjuvant therapy for adult AD patients.

There are 2 types of Th cells: those that inhibit allergy (Th1 cells) and those that promote allergy (Th2 cells). Allergies do not occur when

the balance between Th1 and Th2 is preserved, but when Th2 dominates, allergies occur easily. Neonatal T cells are naive but during an individual's period of growth, they gradually differentiate into Th1 and Th2 cells. Production of Th1 cells is facilitated by gram-positive bacteria, whereas production of Th2 cells is facilitated by gram-negative bacteria (Takeuchi et al., 1999). In the differentiation process, dendritic cells are stimulated through Toll-like receptors (TLR, membrane protein receptors that activate intracellular transcription factors such as NF-κB) when molecules derived from viruses or bacteria are present. The probiotic LAB is a gram-positive bacterium that stimulates TLR2, which recognizes peptidoglycans in gram-positive bacteria and causes the differentiation of naive T cells into Th1 cells. In sterile environments where TLR are not stimulated, naive T cells differentiate into Th2 cells. In a clean environment, *B. breve* is thought to promote differentiation of naive T cells to Th1 during infancy. In addition, stimulation with *B. breve* causes maturation of dendritic cells and an increase of immunosuppressive IL-10 through TLR2 (Hoarau et al., 2006). It is from *B. breve* (the major portion of intestinal flora) that healthy infants receive the stimulation required for immune cell maturation during early childhood. In contrast, it can be hypothesized that *B. breve* is absent in children with AD, resulting in insufficient stimulation.

In a double-blind, placebo-controlled trial using yogurt containing *B. longum* on adult patients with pollen allergy, the *B. longum*-administered group showed significant improvement of symptoms and significant inhibition of increased TARC levels (Xiao et al., 2007; Iwabuchi et al., 2009). Even in adults, *Bifidobacterium* is suggested to improve the balance between Th1/Th2 when dominated by the Th1 side. In experiments conducted on mice orally administered *B. breve*, allergy-induced increases in IgE levels were inhibited, IL-4 was significantly inhibited, and, consequently, Th2 cell proliferation was suppressed, suggesting that the balance between Th1/Th2 might have shifted to the Th1 side (Inoue et al., 2009). Recently, a comparative study of 6 different types of *Lactobacillus* with potential antiallergic properties was conducted on oval albumin-sensitized BALB/c mice. As a result, it was concluded that *B. breve* was the most promising probiotic (Hougee et al., 2010).

In adult AD patients, whose Th cells are already mature, the improving effect of *B. breve* might be difficult to explain in terms of Th1/Th2 balance alone. Recently, other T-helper cell subsets that differentiate from naive T cells have attracted attention as clues to immunostimulatory activity phenomena that cannot be explained by Th1/Th2 balance. Typical examples include regulatory T cells, which release the immunodepressive cytokine IL-10 (Sakaguchi, 2000), and Th17 cells, which release the inflammatory cytokine IL-17 (Weaver et al., 2006). Recent probiotic research has also reported that the LAB *L. casei* increases IL-10 secretion from regulatory T cells (Hacini-Rachinel et al., 2009) and that *B. infantis* has an inhibitory effect on Th17 (Tanabe et al., 2008). It might have been through this kind of immunoregulatory effect that *B. breve* improved adult AD symptoms.

In conclusion, we conducted a study of *B. breve* administration on adult AD patients. Through exhaustive evaluation of cutaneous symptom severity, intestinal flora detected in the feces, blood allergy indicators, and patients' psychological status (QOL), we found that cutaneous symptoms improved, the share of *Bifidobacteria* in the feces increased significantly, and skin disease-specific patient QOL improved significantly. The above results suggest that administration of *B. breve* might be a useful adjuvant therapy for adult AD. However, the severity of disease symptom in the placebo group was milder than that in the probiotic group because the number of patients in the present pilot study was relatively small. We plan to perform further large study in order to determine the definite clinical value of *B. breve* in adult patients with AD.

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