Administration of *Bifidobacterium* to Infants with Atopic Dermatitis: Changes in Fecal Microflora and Clinical Symptoms

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KEY WORDS: atopic dermatitis, probiotics, *Bifidobacterium*

ABSTRACT

Background: We previously reported that the fecal microflora of infants with cow's milk hypersensitivity who were fed casein-hydrolyzed formula supplemented with raffinose (New-MA-1) was normalized. The purpose of this study was to elucidate whether oral administration of bifidobacteria influenced the intestinal microflora of infants with cow's milk hypersensitivity with atopic dermatitis who were fed New-MA-1 and whether their allergic symptoms were thereby improved.

Methods: Seventeen infants with cow milk hypersensitivity with atopic dermatitis who had less than 30% *Bifidobacterium* in their intestinal microflora were selected for this study, and were randomly divided into two groups. Ten subjects were orally given lyophilized bifidobacteria (*Bifidobacterium breve* M-16V strain), and the remaining 7 infants were not.

Results: The changes in fecal microflora and clinical symptoms were compared between the 2 groups. In the bifidobacteria-administered group, the proportion of *Bifidobacterium* in the fecal microflora increased while the proportion of aerobic bacteria decreased after 3 months of the administration, and was associated with a significant improvement of allergic symptoms compared to the beginning of the study. In the control group, there were no significant changes to the overall fecal microflora and total allergic score during the entire study period.

Conclusion: Administration of bifidobacteria to infants with cow's milk hypersensitivity with atopic dermatitis significantly increased the proportion of bifidobacteria in the fecal microflora and also might improve their allergic symptoms.

Table 1. Characteristics of Subjects*

Case	Sex	Age (M)	Clinical symptoms	Total IgE (IU/mL)	Milk
Bifido-group (N = 10)					
1	F	7.1	Erythema	137	1.81
2	M	5.0	Erythema	370	1.3
3	M	18.5	Erythema	453	19.73
4	M	5.0	Erythema	120	0.44
5	F	6.1	Erythema	498	7.92
6	M	15.3	Erythema	161	3.35
7	M	8.3	Erythema, wheezing	126	2.23
8	M	11.3	Erythema	533	3.2
9	M	13.3	Erythema, diarrhea	898	87
10	M	7.1	Erythema, diarrhea	33	0.12
Control group (N = 7)					
11	F	14.7	Erythema	1471	> 100
12	M	5.0	Erythema	553	20
13	M	8.6	Erythema	56	0.44
14	M	11.9	Erythema, wheezing, diarrhea	20	10.09
15	M	13.9	Erythema	884	30.62
16	M	6.6	Erythema	< 2	0.19
17	M	3.1	Erythema	< 2	< .05

*HD indicates house dust; and n.d., not done.

INTRODUCTION

Intestinal microflora is strongly influenced by the dietary habits and physical condition of the host. Several reports have described disturbed fecal microflora in children with allergic diseases,[1] antibiotic use during early infancy as a risk factor for allergic diseases in later life,[2] and the effectiveness of probiotics for the prevention and treatment of allergic diseases[3]. All of these reports indicate disturbances of the intestinal microflora in the development or aggravation of allergic diseases and suggest the possibility that normalization of the intestinal microflora may favorably alter the clinical course of these diseases. However, there have so far been no studies that simultaneously examined the changes in intestinal microflora and alteration of allergic symptoms. Therefore, a direct relationship between these two parameters remains unproven.

In a previous study, we reported that the fecal microflora of infants with cow's milk hypersensitivity was normalized by feeding a raffinose-supplemented casein hydrolyzed formula (New-MA-1). However, some infants failed to achieve predominance of bifidobacteria in their fecal flora, and showed persistent clinical symptoms.[4]

The purpose of this study was to investigate whether oral administration of bifidobacteria influences the intestinal microflora in infants with cow's milk hypersensitivity with atopic dermatitis who are deficient in *Bifidobacterium* in

Specific IgE (UA/mL)					Positive skin test
Egg white	Soy bean	Rice	Wheat	HD	
16.99	0.11	0.11	0.09	n.d.	n.d.
49	< 0.05	0.06	4.5	0.24	Milk, egg white
52.33	n.d.	n.d.	8.2	0.93	n.d.
5.9	< 0.05	0.53	< 0.05	4.6	Milk, egg white
39.61	9.52	2.16	44	1.43	n.d.
14.44	11.03	1.4	21.32	0.39	Milk, egg white, soy bean, wheat
12.37	3.2	0.11	8.25	n.d.	n.d.
14.95	9.28	0.66	1.06	n.d.	n.d.
64	3.2	2.2	1.1	21	Milk, egg white, soy bean, HD
5	< 0.05	< .05	< 0.05	0.13	n.d.
> 100	39.4	3.84	5.9	0.7	n.d.
23	11	0.22	33	22	Milk, egg white
7.68	0.71	0.05	0.17	n.d.	n.d.
9.76	< 0.05	< 0.05	0.29	n.d.	n.d.
87.29	13.81	6.47	42.28	n.d.	n.d.
0.18	0.18	0.17	0.18	0.17	Milk, egg white
0.14	< 0.05	< .05	< 0.05	< 0.05	Milk

their microflora despite feeding of New-MA-1, and if it thereby improves their allergic symptoms.

SUBJECTS AND METHODS

Subjects

From a group of 59 infants with cow's milk hypersensitivity and atopic dermatitis who were fed the casein-hydrolyzed formula New-MA-1 for at least 2 weeks, 17 infants who had fecal microflora containing less than 30% *Bifidobacterium* were selected. Characteristics of the 17 infants are listed in Table 1. Cow's milk hypersensitivity was defined as follows: (1) positive RAST score or positive skin prick test and (2) positive allergic symptoms when fed cow's milk or improvement of allergic symptoms upon the elimination of cow's milk from the diet.

Diagnosis of atopic dermatitis was based on the criteria of Hanifin and Rajka[5]. Informed parental consent was obtained from the infants' parents, and the study was approved by the ethics committee of Kansai Medical University.

The 17 infants were divided into 2 groups at random. Ten subjects were given lyophilized bifidobacteria orally, and the remaining 7 infants were not given this preparation.

There was no significant difference of the age, total allergic score, cutaneous symptom score, total IgE, Milk RAST IgE or Eggs RAST IgE between the 2 groups. Lyophilized live bifidobacteria

Table 2. Total Allergic Score

	Score		0	1	2	3
Cutaneous symptoms	Erythema	face	none	mild	severe	
		trunk	none	mild	severe	
		arms	none	mild	severe	
		legs	none	mild	severe	
	Lichenification	face	none	mild	severe	
		trunk	none	mild	severe	
		arms	none	mild	severe	
		legs	none	mild	severe	
	Cracking	face	none	mild	severe	
		trunk	none	mild	severe	
		arms	none	mild	severe	
		legs	none	mild	severe	
	Itching		none	mild	intermediate	severe
	Sleepless		none	mild	intermediate	severe
Gastrointestinal symptoms	Frequency of diarrhea		none	1-3/day	4-5/day	>5/day
Respiratory symptoms			none	cough, mild wheezing	wheezing	severe wheezing
Utilization of ointments			none	non-corticosteroid	corticosteroid	regular use of corticosteroid

(*Bifidobacterium breve* M-16V strain) was added to the casein-hydrolyzed milk formula (New-MA-1), and fed to the infants. Bifidobacteria, 5 x 10^9cfu or 15 x 10^9 cfu per day, were given orally for 3 months.

New MA-1

New MA-R is an extensively hydrolyzed and ultrafiltrated bovine casein formula. Although lactose is completely eliminated, New MA-R contains 0.13 g of raffinose per 100 mL as a bifidobacterial growth factor.[4]

***Bifidobacterium breve* M-16V**

Bifidobacterium breve M-16V is a strain originally isolated from a healthy infant and identified as *Bifidobacterium breve* based on its sugar fermentation spectrum.

The *B. breve* M-16V preparation does not contain any milk protein, whereas the other 3 probiotics tested contained a small amount of milk protein when examined by using a Sandwich ELISA with rabbit-anti-lactoglobulin.

Fecal Microflora Analysis

Fecal microflora analysis was performed before and at 1, 2, and 3 months after the initiation of the study using Mitsuoka's method.[6] Fecal samples were collected at home and immediately cooled under anaerobic conditions, and analyzed for microflora within 24 hours. The proportion of *Bifidobacterium* is expressed as the percent bifidobacterial count relative to the total bacterial count.

Determination of Allergic Symptom Scores

Allergic symptom scores were assessed by questioning the parents and examining the parents' diaries during the study period. The total allergic score was obtained from the cutaneous symptom score and scores for gastrointestinal symptoms, respiratory symptoms and utilization of ointments (Table 2). The latter 3 categories were scored on a scale (0-3) in ascending order of severity.

Atopic dermatitis was assessed by a scoring system modified from Kimata's

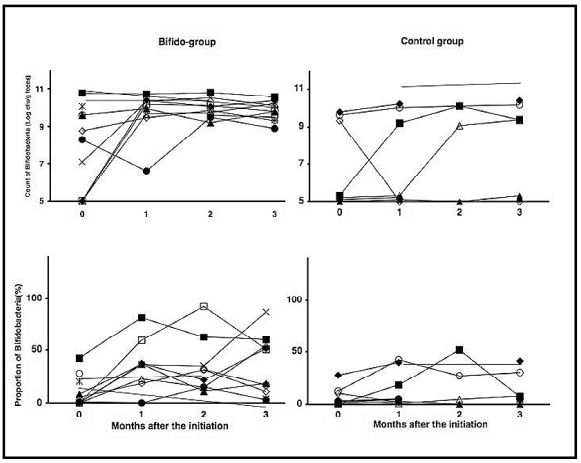

Figure 1. Changes of fecal microflora in bifidobacteria- administered group and control group. Data are presented as the log 10 number of *Bifidobacterium* per gram feces (upper) and the proportion of *Bifidobacterium* in total fecal microbial counts (lower). The *P* values were calculated by the Wilcoxon matched-pair sign-rank test.

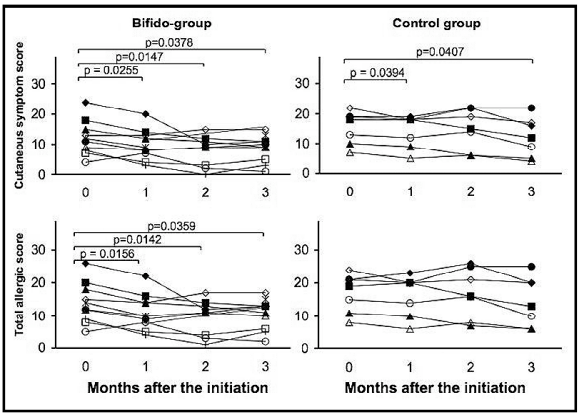

Figure 2. Changes in severity of cutaneous symptom score and total allergic score in the bifidobacteria-administered group and control group. The P values were calculated by the Wilcoxon matched-pair sign-rank test.

score.[7] Erythema, lichenification, and cracking were scored on a scale (0-2) in ascending order of severity. These symptoms were assessed on four regions of the body, the face, trunk, arms, and legs. Subjective symptoms of itching and sleep disturbance were graded from zero to three (0-3).

Statistical Analysis

Statistical analysis was performed with the non-parametric Mann-Whitney U-test, Wilcoxon test and Spearman test. A P value < 0.05 was considered to be significant.

RESULTS

Fecal Microflora

The results of fecal microflora analyses are shown in Tables 3 and 4. In case 6 (Table 1), *Bifidobaterium* was not detected in the preliminary microflora analysis for the purpose of selection, but the proportion of *Bifidobacterium* increased to 40% before the initiation of the study. Even including this case, no difference in fecal microflora before initiation of the study was observed between the 2 groups with and without bifidobacteria administration. The mean proportion of *Bifidobacterium* was 7% to 10% in both groups, which was markedly decreased compared to the *Bifidobacterium*-predominant fecal microflora of healthy infants.[8]

In the bifidobacteria-administered group (bifido-group), the proportion of *Bifidobacterium* increased compared to the pre-administration level (10.62 ± 14.8) to 36.7 ± 25 (P = 0.0173), 35.19 ± 26.5 (P = 0.0077) and 35.54 ± 28.6, at 1, 2, and 3 months of administration, respectively (Figure 1, Table 3). This was accompanied by a significant increase in the proportion of total anaerobic bacteria and a significant decrease in the proportion of total aerobic bacteria. There were no remarkable dose-dependent differences (at the doses of 5 x 10^9 cfu/day and 1.5 x 10^{10} cfu/day). In the control group, there was no increase in the proportion of *Bifidobacterium* during the study period (Table 4). There were no changes of the overall fecal

Table 3. Fecal Microflora of the 10 Infants in the Bifido Group

Months after the initiation	0			1	
Bacterial group	Frequency of positive cultures	Counts*	Proportion† (%)	Frequency of positive culture	Counts*
Total bacteria	10/10	10.36 ± 0.5	100.00	8/8	10.48 ± .3
Total aerobic bacteria	10/10	9.57 ± 0.6	31.58 ± 28.1	8/8	9.52 ± 0.3
Enterobacteriaceae	10/10	9.09 ± 0.4	13.42 ± 18.7	8/8	9.03 ± 0.4
Streptococcus/ Enterococcus	9/10	9.14 ± 0.9	17.33 ± 22.6	8/8	9.16 ± 0.5
Staphylococcus	10/10	5.26 ± 1.8	0.84 ± 2.6	8/8	5.17 ± 1.3
Yeasts	3/10	3.50 ± 0.7	0.00 ± 0.0	1/8	3.78
Candida	6/10	3.32 ± 0.9	0.00 ± 0.0	4/8	2.95 ± 0.6
Corynebacterium	1/10	6.30	0.00 ± 0.0	0/8	
Total anaerobic bacteria	10/10	10.16 ± 0.6	68.42 ± 28.1	8/8	10.40 ± 0.3
Lactobacillus	2/10	2.45 ± 0.2	0.00 ± 0.0	0/8	
Bifidobacterium	7/10	9.16 ± 1.2	10.62 ± 14.9	8/8	9.64 ± 1.3
Eubacterium	4/10	8.73 ± 0.8	2.85 ± 5.9	5/8	9.03 ± 0.3
Bacteroidaceae	8/10	9.90 ± 0.5	25.95 ± 18.0	7/8	9.76 ± 0.4
Peptococcaceae	7/10	9.37 ± 0.8	10.78 ± 15.1	5/8	9.44 ± 0.4
Clostridium-others	9/10	9.48 ± 0.7	17.24 ± 14.8	8/8	9.05 ± 1.1
Clost.perfringens	4/10	5.43 ± 2.2	0.02 ± 0.1	3/8	5.93 ± 2.1
Veillonella	8/10	7.19 ± 2.2	0.96 ± 1.5	6/8	8.72 ± 0.6
Megasphaera	0/10			0/8	

***Log $_{10}$ number of bacteria/g feces.**
***†Data are presented as mean ± SD.**

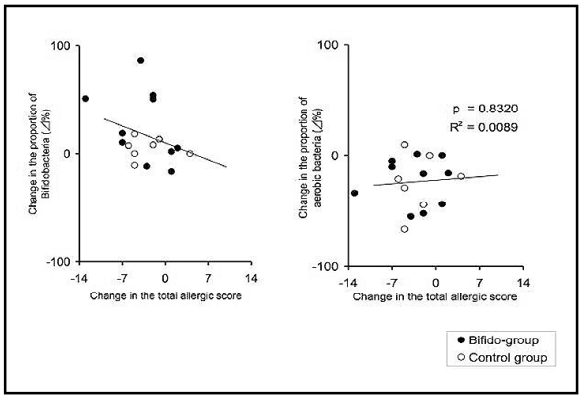

Figure 3. Correlation of changes in the proportion of bifidobacteria (left) and aerobic bacteria (right), and changes in severity of allergic symptoms. Changes in severity were evaluated by the difference in total allergic score during the 3 months of the study period. Open circles represent subjects in the bifidobacteria-administered group. Closed circles indicated subjects in the control group. The P values were calculated by Spearman's rank correlation test.

microflora in the control group. Three months after the start of the study period, *Bifidobacterium* remained undetectable in 3 of the 7 control infants. The proportion of aerobic bacteria significantly decreased gradually during 3 months in the bifido-group (Table 3), while a significant decrease was observed in the control group only at 3 months (Table 4). *Lactobacillus* was detected in a few subjects at a very low count.

Allergic Symptoms

Figure 2 shows the changes of cutaneous symptom scores and the total allergic scores in the 2 groups. There were no significant differences in cutaneous symptom scores and total allergic scores

	2			3		
Proportion† (%)	Frequency of positive culture	Counts*	Proportion† (%)	Frequency of positive culture	Counts*	Proportion† (%)
100.00	9/9	10.46 ± 0.3	100.00	10/10	10.43 ± 0.3	100.00
14.33 ± 11.2	9/9	9.52 ± 0.5	17.10 ± 17.0	10/10	9.14 ± 0.5	8.84 ± 10.0
6.26 ± 7.1	9/9	8.87 ± 0.5	4.56 ± 4.4	10/10	8.56 ± 0.7	2.61 ± 2.9
8.03 ± 8.1	8/9	9.36 ± 0.6	12.16 ± 15.5	10/10	8.70 ± 0.8	6.23 ± 9.1
0.05 ± 0.1	9/9	4.88 ± 2.0	0.36 ± 1.1	9/10	4.47 ± 1.1	0.00 ± 0.0
0.00 ± 0.00	1/9	2.60	0.00 ± 0.0	1/10	3.30	0.00 ± 0.0
0.00 ± 0.00	6/9	3.18 ± 0.9	0.00 ± 0.0	4/10	3.30 ± 0.2	0.00 ± 0.0
	1/9	8.30	0.02 ± 0.1	0/10		
85.67 ± 11.2	9/9	10.37 ± 0.4	82.90 ± 17.0	10/10	10.39 ± 0.3	91.16 ± 10.0
	2/9	2.78 ± 0.7	0.00 ± 0.0	1/10	3.65 ± 0.9	0.00 ± 0.0
36.68 ± 25.0	9/9	9.91 ± 0.5	35.19 ± 26.5	10/10	9.77 ± 0.5	35.54 ± 29.6
2.86 ± 4.8	2/9	9.75 ± 0.2	4.66 ± 12.4	4/10	9.20 ± 0.5	3.93 ± 7.0
23.96 ± 22.9	7/9	9.81 ± 0.5	19.73 ± 17.5	9/10	9.60 ± 1.1	34.33 ± 31.0
7.85 ± 8.0	5/9	9.57 ± 0.3	9.86 ± 11.0	9/10	8.91 ± 1.0	9.78 ± 13.0
12.11 ± 14.4	8/9	9.29 ± 0.5	9.53 ± 14.0	10/10	9.09 ± 0.4	6.12 ± 4.0
0.04 ± 0.1	1/9	8.60	0.13 ± 0.4	2/10	5.30 ± 1.4	0.00 ± 0.0
2.17 ± 3.4	8/9	8.72 ± 0.8	3.81 ± 4.8	8/10	7.74 ± 1.6	1.43 ± 2.5
	0/9			0/10		

between the 2 groups before the study. In the bifido-group, the cutaneous symptom scores at 1, 2, and 3 months were significantly decreased compared to the pre-administration score. In the control group, the cutaneous symptom scores were significantly decreased only at 1 and 3 months.

In the bifido-group, the total allergic scores at 1, 2, and 3 months were significantly decreased compared to the pre-administration score. In the control group, there was no significant change in total allergic scores during the whole study period.

Relationship Between Change in Microflora and Improvement of Allergic Symptoms

To examine the relationship between changes in microflora and improvement of allergic symptoms for each subject, the changes of the proportion of *Bifidobacterium* proportion of aerobes, and allergic scores during the study period for each infant were plotted, including those for the control group (Figure 3). The reduction in symptom score was not intensified with a greater increase in *Bifidobacterium*or a decrease in total aerobes, and no significant correlation was observed between the change in fecal microflora and changes in total allergic score. Also, the same result was obtained when the cutaneous symptoms score was used (data not shown).

DISCUSSION

In the present study, the administration of bifidobacteria to infants with cow's milk hypersensitivity with atopic dermatitis significantly increased the pro-

Table 4. Fecal Microflora of the 7 infants in the Control Group

Months after the initiation	0			1	
	Frequency of Positive culture	Counts*	Proportion† (%)	Frequency of positive culture	Counts*
Total bacteria	7/7	10.44 ± 0.3	100.00	7/7	10.35 ± 0.2
Total aerobic bacteria	7/7	9.85 ± 0.6	39.68 ± 32.7	7/7	9.75 ± 0.4
Enterobacteriaceae	7/7	9.47 ± 0.5	21.29 ± 25.3	7/7	9.18 ± 0.6
Streptococcus/ Enterococcus	7/7	8.94 ± 1.3	16.77 ± 18.1	7/7	9.45 ± 0.6
Staphylococcus	7/7	5.04 ± 1.1	0.00 ± 0.0	7/7	4.72 ± 0.7
Yeasts	2/7	3.78 ± 2.1	0.00 ± 0.0	1/7	2.60
Candida	4/7	4.22 ± 2.1	0.00 ± 0.0	3/7	4.24 ± 1.5
Corynebacterium	1/7	10.00	1.62 ± 4.3	0/7	
Total anaerobic bacteria	7/7	10.05 ± 0.7	60.32 ± 32.7	7/7	10.17 ± 0.3
Lactobacillus	1/7	6.60	0.00 ± 0.0	2/7	2.30 ± 0.00
Bifidobacterium	3/7	9.58 ± 0.2	7.30 ± 10.7	3/7	9.80 ± 0.5
Eubacterium	2/7	3.80 ± 2.1	0.00 ± 0.0	3/7	6.30 ± 3.6
Bacteroidaceae	6/7	9.38 ± 1.6	23.49 ± 22.5	7/7	9.53 ± 0.5
Peptococcaceae	5/7	8.89 ± 1.4	13.00 ± 24.4	6/7	9.56 ± 0.3
Clostridium-others	6/7	9.45 ± 0.6	15.97 ± 15.1	7/7	9.22 ± 0.5
Clost.perfringens	2/7	7.60 ± 1.4	0.24 ± 0.6	3/7	5.59 ± 2.2
Veillonella	7/7	6.48 ± 2.1	0.31 ± 0.7	7/7	7.86 ± 1.1
Megasphaera	0/7			0/7	

*Log_{10} number of bacteria/g feces.
*†Data are presented as mean ± SD.

portion of bifidobacteria in the fecal microflora and also might have improved their allergic symptoms.

Administration of bifidobacteria significantly improved both cutaneous score and total allergic score. In the control group, there was no significant improvement of the total allergic score, but a significant decrease of cutaneous score was found at 1 month and 3 months after the initiation of the study. This cutaneous improvement may be explained by the increased frequency of use of ointment, such as corticosteroid, which would not result in improvement of the total allergic score.

The mechanism of improvement of the allergic symptoms by the administration of *Bifidobacterium* is thought to be as follows: (1) Lipoteichoic acid in the cell wall of gram-positive bacteria such as Bifidobacterium binds to Toll like receptor 2 on the antigen presenting cells, which produce IL-12 to activate the type 1 CD4 + lymphocytes (Th1);[9] (2) *Bifidobacterium* may regulate the Th1/Th2 balance through regulatory T cells. (3) Administration of probiotics is likely to suppress the increase of the gut permeability induced by milk allergy.[10]

Recent studies have indicated that the administration of probiotics, including *Lactobacillus rhamnosus* GG (*L.rhamnosus* GG) or *Bifidobacterium lactis* Bb-12, is effective for the improvement or prevention of atopic dermatitis.[3] However, those studies did not demonstrate the improvement of the fecal microflora. Kirjavaninen et al[11] also reported that supplementation of hydrolyzed whey formula with viable *L. rhamnosus* GG improved the (Scoring Atopic Dermatitis) (SCORAD) cutaneous score for atopic infants, but did not increase the quantity of fecal *Lactobacillus GG* detected using 16s rRNA-specific gene probes. In the present study, by simultaneously examining

	2			3		
Proportion† (%)	Frequency of positive culture	Counts*	Proportion† (%)	Frequency of positive culture	Counts	Proportion† (%)
100.00	4/4	10.20 ± 0.6	100.00	7/7	10.50 ± 0.2	100.00
31.61 ± 18.8	4/4	9.65 ± 0.4	39.36 ± 38.1	7/7	9.44 ± 0.6	15.40 ± 14.8
11.53 ± 10.5	4/4	9.08 ± 0.19	13.82 ± 17.4	7/7	9.05 ± 0.4	4.77 ± 4.0
20.08 ± 15.6	4/4	9.43 ± 0.5	25.60 ± 22.2	7/7	8.79 ± 1.2	10.63 ± 12.7
0.00 ± 0.0	4/4	4.57 ± 1.1	0.00 ± 0.0	7/7	4.51 ± 0.7	0.00 ± 0.0
0.00 ± 0.0	1/4	2.30	0.00 ± 0.0	1/7	2.60	0.00 ± 0.0
0.00 ± 0.0	2/4	2.60 ± 0.4	0.00 ± 0.0	3/7	4.28 ± 1.4	0.00 ± 0.0
	0/4			0/7		0.00 ± 0.0
68.39 ± 18.8	4/4	9.80 ± 0.1	60.64 ± 38.05	7/7	10.42 ± 0.2	84.60 ± 14.8
0.00 ± 0.0	1/4	2.30	0.00 ± 0.0	1/7	2.60	0.00 ± 0.0
14.40 ± 19.5	3/4	9.75 ± 0.6	21.00 ± 23.7	4/7	9.82 ± 0.5	12.48 ± 16.7
1.24 ± 3.2	0/4			1/7	9.30	0.50 ± 1.3
23.97 ± 18.8	3/4	9.93 ± 0.3	25.66 ± 26.9	7/7	9.92 ± 0.3	31.00 ± 18.2
18.37 ± 17.7	3/4	8.80 ± 1.1	4.28 ± 4.2	5/7	9.75 ± 0.4	13.93 ± 12.0
9.49 ± 6.1	3/4	9.10 ± 1.0	8.41 ± 8.0	7/7	9.61 ± 0.5	18.08 ± 14.1
0.01 ± 0.0	0/4		0.00 ± 0.6	0/7	2.90	0.00 ± 0.0
0.90 ± 0.90	3/4	8.60 ± 0.3	1.29 ± 1.5	7/7	8.90 ± 1.0	8.60 ± 8.1
	0/4			0/7		

allergic symptoms and fecal microflora, we did not find a clear correlation between the degree of allergic improvement and changes of fecal microflora for each subject. The data of intestinal microflora obtained from analysis of naturally passed feces reflect the microflora in the lower intestinal tract. Considering the fact that Peyer patches, the major tissue responsible for intestinal mucosal immunity, are located mainly in the small intestine, it is reasonable to assume that the lyophilized bifidobacteria taken orally act directly on the small intestine and that the proportion of bifidobacteria in the large intestine does not influence the host immunity.

Hypoallergenic infant formula does not include lactate, and thus is not suitable for fermentation by probiotics such as *Lactobacillus* or *Bifidobacterium*. We have already developed New-MA-1 supplemented with raffinose, which is indigestible in the small intestine and fermented by *Bifidobacterium* in the colon. This formula was previously reported to be effective for improvement of the fecal microflora.[4]

B. breve M-16V is a strain originally isolated from a healthy infant and was identified as *Bifidobacterium breve* based on its sugar fermentation spectrum. We measured the antigeneity of 4 probiotic products including our *B. breve* M-16V preparation, against rabbit anti-bovine β-lactoglobulin antibody using sandwich ELISA. The lyophilized *B. breve* M-16 V preparation did not contain any cow's milk β-lactoglobulin, but the other 3 commercial probiotics contained small amounts of the milk protein. Therefore, our *B. breve* M-16V preparation is completely safe for infants with cow's milk hypersensitivity.

In conclusion, the administration of viable bifidobacteria is effective for normalizing fecal microflora in infants with atopic dermatitis with cow's milk hyper-

sensitivity who are fed lactose-free raffinose-supplemented casein-hydrolyzed formula, and also it may improve their allergic symptoms.

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REFERENCES

1. Björkstén B, Naaber P, Sepp E, et al. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy.* 1999;29:342-346.
2. Wickens K, Pearce N, Crane J, et al. Antibiotic use in early childhood and the development of asthma. *Clin Exp Allergy*. 1999;29:766-771.
3. Kalliomäki M, Salminen S, Arvilommi H, et al. Probiotics in primary prevention of atopic disease : A randomized placebo-controlled trial. *Lancet.* 2001:357;1076-1079.
4. Hattori K, Sasai M, Yamamoto A, et al. Intestinal flora of infants with cow milk hypersensitivity fed on casein-hydrolyzed formula supplemented raffinose [in Japanese]. *Arerugi.* 2000;49:1146-1155.
5. Hanifin JM, Rajaka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*. 1980;92(suppl):44-47.
6. Yaeshima T, Takahashi S, Matsumoto N, et al. Effect of yogurt containing *Bifidobacterium longum* BB536 on the intestinal environment, fecal characteristics and defecation frequency: A comparison with standard yogurt. *Bioscience Microflora.* 1997;16:73-77.
7. Kimata H, Igarashi M. Topical cromolyn (disodium cromoglycate) solution in the treatment of young children with atopic dermatitis. *Clin Exp Allergy.* 1990;20:281-283.
8. Fukushima Y, Li ST, Hara H, et al. Effect of follow-up formula containing bifidobacteria (NAN BF) on fecal flora and fecal metabolites in healthy children. *Bioscience Microflora.* 1997;16:65-72.
9. Cleveland MG, Gorham JD, Murphy TL, et al. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infec Immun.* 1996;64:1906-1912.
10. Isolauri E, Majamaa H, Arvola T, et al. Lactobacillus casein strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology.* 1993;105:1643-1645.
11. Kirjavainen PV, Salminen S, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. *J Pediatr Gastroenterol Nutr.* 2003;36:223-227.