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Probiotic Supplement Reduces Atopic Dermatitis in Preschool Children

A Randomized, Double-Blind, Placebo-Controlled, Clinical Trial

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Abstract

Background: The role of probiotics in the treatment of atopic dermatitis (AD) remains controversial. A recent systematic review of the available evidence called for further clinical trials with new probiotic formulations.
Objective: To assess the clinical efficacy and impact of *Lactobacillus acidophilus* DDS-1, *Bifidobacterium lactis* UABLA-12 with fructo-oligosaccharide on peripheral blood lymphocyte subsets in preschool children with moderate-to-severe AD.
Method: Randomized, double-blind, placebo-controlled, prospective trial of 90 children aged 1–3 years with moderate-to-severe AD who were treated with a mixture of *L. acidophilus* DDS-1, *B. lactis* UABLA-12 with fructo-oligosaccharide at a dosage of 5 billion colony-forming units twice daily for 8 weeks versus placebo. The primary outcome measure was the percentage change in Scoring of Atopic Dermatitis (SCORAD) value. Other outcome measures were changes in Infant Dermatitis Quality Of Life (IDQOL) and Dermatitis Family Impact (DFI) scores, frequency and amount of topical corticosteroid used, and lymphocyte subsets in peripheral blood measured by laser flow cytometry.
Results: At the final visit, the percentage decrease in SCORAD was 33.7% in the probiotic group compared with 19.4% in the placebo group ($p = 0.001$). Children receiving probiotic showed a greater decrease in the mean [SD] SCORAD score than did children from the placebo group at week 8 (−14.2 [9.9] vs −7.8 [7.7], respectively; $p = 0.001$). IDQOL and DFI scores decreased significantly from baseline by 33.0% and 35.2% in the probiotic group and by 19.0% and 23.8% in the placebo group, respectively ($p = 0.013$, $p = 0.010$). Use of topical corticosteroids during the 8-week trial period averaged 7.7 g less in probiotic patients ($p = 0.006$). CD3, CD16, and CD22 lymphocyte subsets remained unchanged, whereas the percentage of CD4, and the percentage and absolute count of CD25 decreased, and the percentage and absolute count of CD8 increased in the probiotic group at week 8 ($p < 0.007$ vs placebo). There was a significant correlation between CD4 percentage, CD25 percentage, CD25 absolute count, and SCORAD values ($r = 0.642$, $r = 0.746$, $r = 0.733$, respectively; $p < 0.05$) in the probiotic group at week 8.
Conclusion: The administration of a probiotic mixture containing *L. acidophilus* DDS-1, *B. lactis* UABLA-12, and fructo-oligosaccharide was associated with significant clinical improvement in children with AD, with corresponding lymphocyte subset changes in peripheral blood. The efficacy of probiotic therapy in adults with AD requires further investigation.

Background

Atopic dermatitis (AD) is a common inflammatory skin disorder with a prevalence ranging from 15.6% in Europe[1] to 17.2% in the US,[2] and a steady trend towards increase.[3] The disease often presents in early childhood and persists into adult life in 60% of patients.[4] The social influence of AD on children and families is considerable.[5] Current treatment of AD includes skin hydration, emollients, avoidance of allergens and irritants, and use of antihistamine drugs or corticosteroids during exacerbations.[6] These treatments may alleviate the symptoms of AD, but are often not sufficiently effective,

indicating a need for further research on alternative treatments, especially in moderate or severe disease.

The therapeutic potential of probiotics attracted considerable attention after postulation of the hygiene hypothesis,[7] reinforced with suggestions in the literature that bacterial antigens may shift immature immune responses towards an anti-allergic phenotype,[8-10] and trials showing differences in gut microflora between atopic and nonatopic children.[11,12] Several clinical studies[13-16] have demonstrated mild to complete resolution of AD following treatment with probiotics while other studies have suggested that the effect is limited to selected children with atopy[17,18] or is undetectable.[19-21] Recent evidence-based systematic reviews generally concluded that the effect of probiotics was weak to absent,[22,23] and called for investigations of new probiotic preparations given their strain-specific effects.[24]

Published reports indicate that probiotics may have an effect on immune function in AD,[8-10] and alter lymphocyte subsets in experimental animals[25] and clinical settings.[26,27] Patients with active AD had a reduced percentage of CD3 and CD8 peripheral blood lymphocytes,[28,29] and increased CD4 and CD25 counts, without changes in CD16 and CD22 determinants.[26] Hypothetically, the recovery from AD due to the use of probiotics may be accompanied by normalization of CD3, CD4, CD8, and CD25 numbers. However, the impact of probiotics on peripheral lymphocyte subsets in preschool children with AD has not been studied.

The main objective of the present study was to assess the clinical efficacy of a new probiotic preparation and to determine its impact on peripheral lymphocyte subsets in a group of preschool children with moderate-to-severe AD.

Material and Methods

Study Population

The study population was selected from the patients attending the ambulatory consultation clinic at Lviv City Children Hospital from June 2007 to June 2008. We recruited both boys and girls aged 12–36 months with AD established using Hanifin and Rajka criteria.[30] Other inclusion criteria were presence of moderate or severe disease; ability of the parent or legal guardian to comprehend the study requirements and to provide informed consent; and direct telephone access.

Exclusion criteria were mild AD; clinically evident bacterial skin lesions; use of systemic corticosteroids; food allergy other than to egg or cow's milk; chronic concomitant disease that would likely require the use of immunosuppressants or antihistamine drugs during the research period; presence of severe systemic disease or cancer at any site and stage; and suspected or established primary or secondary immune deficiency.

Assignments

Patients were randomized using computer-generated random codes to receive either probiotic or placebo treatment. The probiotic formulation was a mixture of *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium lactis* UABLA-12 with fructo-oligosaccharide in a rice maltodextrin powder (DDS® Junior; UAS Laboratories, Eden Prairie, MN, USA). A single dose of the preparation, measured as one-quarter of a teaspoon or approximately 1 g, contains 5 billion colony-forming units (CFU) of *L. acidophilus* DDS-1 and *B. lactis* UABLA-12 and 50 mg of fructo-oligosaccharide. The mixture was reconstituted in tepid water, juice, or baby food and immediately fed twice a day (10 billion CFU daily) for an 8-week trial period. Parents or guardians were provided with 140 doses of probiotic, and instructed to administer 112 doses over 8 weeks.

The placebo constituted a pure powder of rice maltodextrin. Probiotic and placebo were identical in appearance, taste, smell, packing, and manner of administration. All formulations were dispensed by a technician, with the investigator and patient blinded regarding the identity of the treatment. Compliance was assessed from the parental report and the weight of remaining powder.

Treatment of AD was maintained with skin hydration, emollients, and avoidance of allergens and irritants according to PRACTALL (Practical Allergology) recommendations.[6] Hydrocortisone 1% or mometasone 0.1% ointments were allowed as rescue medications when indicated in both groups.

Food Allergy and Elimination Diet

As food allergy is the major confounder in an AD clinical trial, we carefully selected patients with no food allergy or with a well documented allergy only to egg or cow's milk. Allergy was diagnosed on the basis of a thoroughly collected food history followed by a double-blind, placebo-controlled, food challenge[31] in suspected cases. The food challenge was performed 2–3 months before the enrollment during visits for the parental education program. Briefly, egg or cow's milk and systemic antihistamine drugs were eliminated for at least 5 days before the challenge, and topical corticosteroids were reduced to a minimum. Then, depending on age, one-fourth to one-half of a whole fresh egg or 25–50 mL of milk was mixed with potato mash in a proportion of 1 : 2 by volume. The mixture was given in a fasting state and the observation period was 48 hours for

immediate and late-phase allergic reactions. Once a clear skin reaction was documented, the patient was placed on an egg- or milk-free diet. The diet was maintained for at least 2 months and during the whole trial period. Compliance to the diet was documented in the food diary. Patients with other food allergies were not included in the study.

Clinical Examination

The status of AD was assessed at weeks 0, 2, 4, and 8 with the following validated instruments: severity of cutaneous involvement using the Scoring of Atopic Dermatitis (SCORAD) questionnaire,[32] quality of life using the Infant Dermatitis Quality of Life (IDQOL) questionnaire,[33] and impact of AD on the family using the Dermatitis Family Impact (DFI)[34] questionnaire.

The SCORAD index included an assessment of the extent and intensity of the rash, pruritus, and sleep disturbance. The extent was calculated using the 'rule of nine' and expressed the skin area involved. The intensity of rash was graded from 0 to 3 as compared with the standard pictures. Pruritus and sleep disturbance were plotted on a 10-unit analogue scale. The final score was calculated using the following formula: $A/5 + 7B/2 + C$, where A = extent, B = intensity, and C = subjective symptoms. AD was considered moderate or severe if the SCORAD score was within the range of 25–50 units or >50 units, respectively.[35]

The IDQOL and DFI questionnaires each included ten questions specific to the infant or family activity as assessed over the last week. Each answer to the question was graded from 0 to 3, giving 30 units as the highest score reflecting the worst quality of life or the greatest family impact.

Frequency and cumulative use of topical corticosteroids were assessed by parental report and by weighing the medications remaining in application tubes.

Laboratory Tests

Baseline total IgE levels were determined using the chemiluminescent immunoassay (DPC Immulite® 1000; Diagnostic Products Corporation, Los Angeles, CA, USA) and eosinophil count by conventional automatic cell counter (QBC II Plus 4452; Becton Dickinson, Franklin Lakes, NJ, USA). Major lymphocyte subsets were counted in heparinized blood samples exposed to fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, CD16, CD22, and CD25 (Becton Dickinson, USA) at weeks 0 and 8. Samples were analyzed on a flow cytometer FACScan using the blue-green excitation light (488 nm argon-ion laser) with SimulSET software (Becton Dickinson, USA).

Outcome Measures

The primary outcome measure was the percentage change in the SCORAD index at week 8. Secondary outcome measures were changes in IDQOL and DFI scores at weeks 2, 4, and 8, frequency and amount of topical corticosteroid use, and absolute number and percentage of peripheral blood lymphocyte subsets at week 8.

Statistical Analysis

Sample size was calculated assuming a SCORAD index change of 34% in the probiotic group and 17% in the placebo group at week 8.[16] To reject the null hypothesis with statistical significance and an α type I error of 0.05 and a β type II error of 0.5, approximately 48 patients were required in each group. To account for possible withdrawals and losses during follow-up, it was necessary to recruit approximately 120 patients.

The incidence of factors affecting the course of AD in the two groups was assessed by the chi-squared test. The two-sided, unpaired t-test for independent variables was used to compare numeric variables with normal distribution. SCORAD, IDQOL, and DFI scores were assessed with the Mann-Whitney U test. Friedman ANOVA was applied to analyze change in the listed scores over the time. A time series analysis employing an exponential smoothing model was used to determine the time-point at which the SCORAD score reached a particular value. Analysis of covariance was used to control for the confounding factors. Statistical analyses were performed using Statistica v.5.0 (StatSoft, Tulsa, OK, USA) software. A value of 0.05 or less was considered statistically significant.

Ethics

The study received institutional review board approval at the Lviv National Medical University (approval #1 of 22 January 2007). Parents signed the informed consent form before children entered the trial.

Results

As shown in figure 1, 123 children were initially screened; 27 were excluded as either not meeting inclusion criteria, refusal to participate, or suspected food allergy.

The remaining 96 patients were randomized and evenly distributed in probiotic and placebo groups. A small number of subjects were lost to the study due to intercurrent respiratory tract infections requiring antibacterial therapy, refusal to

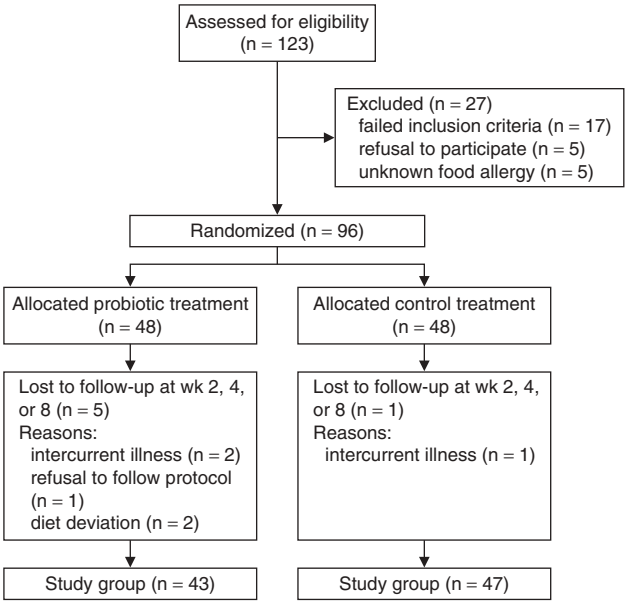

Fig. 1. Selection and distribution of study subjects.

participate, or deviation from the diet with resultant exacerbation. The final trial consisted of 43 and 47 children in the probiotic and placebo groups, respectively.

Baseline major demographic and clinical parameters pertinent to the study outcomes are shown in table I.

According to parental report and powder remaining after treatment, the median number of doses administered was 107 (5–95th percentile range 98–111) in the probiotic group and 104 (5–95th percentile range 96–109) in the placebo group ($p = 0.505$). Forty-one children (95%) in the probiotic group and 43 children (92%) in the placebo group were compliant and received more than 90% of the allocated doses ($p = 0.723$).

Clinical Outcomes

At follow-up visits, patients displayed a progressive decline in SCORAD indexes, reaching a significant difference compared with baseline at week 4 in both the probiotic and placebo groups (figure 2).

However, children receiving probiotics experienced a more rapid decline in mean (SD) SCORAD scores, with values of –4.7 (8.1), –8.7 (9.0), and –14.2 (9.9) versus –2.5 (7.3), –5.1 (8.2), and –7.8 (7.7) in the placebo group at weeks 2, 4, and 8, respectively. At the final visit, the primary outcome measure, the percentage change in SCORAD, approached 33.7% in the probiotic group and 19.4% in the placebo group ($p = 0.001$). The effect was still significant after controlling for the effect of the baseline SCORAD score $F(1.87) = 13.9$ ($p < 0.05$), level of total IgE $F(1.87) = 11.7$ ($p < 0.05$), or both confounders $F(1.87) = 12.7$ ($p < 0.05$). Further analysis indicated that in the probiotic group a 19% decrease in the SCORAD score occurred at week 3.9, while in the placebo group this only occurred at week 7.9, i.e. this reduction occurred twice as rapidly in children receiving probiotics. The percentage of patients that achieved a >25% improvement of AD was greater in the probiotic group than in the placebo group: at week 2, 23.3% versus 10.6% ($p = 0.110$); at week 8, 67.4% versus 36.2% ($p = 0.004$).

Analysis of components of the SCORAD index also showed a steady trend towards improvement with both forms of treatment (table II). The area of skin involvement became significantly smaller compared with baseline by week 4 in the probiotic group and by week 8 in the placebo group. This difference between the two groups was detectable beginning at week 4. Significant decrease in the intensity of skin lesions was observed by week 4 in the probiotic group, and by week 8 in the placebo group. Itching and sleep disturbance scores changed from baseline at weeks 4 and 8, and differed significantly between the two groups at the final visit.

Evaluation of the courses of AD in individual patients revealed a consistent pattern of general decrease in SCORAD scores, with mean values significantly lower in patients receiving probiotic treatment (figure 3).

During the trial period of 8 weeks, the manifestations of AD assumed a milder form in 19 patients (44.2%) in the probiotic group and in 10 patients (21.3%) in the placebo group ($p = 0.019$). A shift from severe to moderate AD was observed in 12 (27.9%) and 8 (17.0%) patients in the probiotic and placebo groups, respectively ($p = 0.199$). The disease progressed in five children (11.6%) in the probiotic group and in seven children (14.9%) in the placebo group, a difference that was not statistically significant ($p = 0.550$).

Stratification of patients by severity of AD, IgE level, and eosinophil count indicated differences in the extent of SCORAD changes (table III). Children with severe disease manifested greater changes in SCORAD indexes in both probiotic and placebo groups. Generally, the changes were more pronounced in the probiotic group regardless of stratification domain.

Quality-of-Life Measures

In both groups, IDQOL changed significantly at week 4 compared with baseline values (table IV). Compared with baseline values, DFI decreased significantly at week 2 in the probiotic group and week 4 in the placebo group. By week 8, IDQOL and DFI changed from baseline by 33.0% and 35.2% in the probiotic group, and by 19.0% and 23.8% in the placebo group ($p < 0.05$).

Use of Topical Corticosteroids

As reported by parents, use of topical corticosteroids decreased in frequency from 1.9 (SD 1.5, median 2.0, range 0.0–4.9) at baseline to 0.8 (SD 0.9, median 0.0, range 0.0–2.0) [$p < 0.001$] per week at the final visit in the probiotic group; and from 1.7 (SD 1.5, median 2.0, range 0.0–4.0) to 1.2 (SD 1.4, median 1.0, range 0.0–3.0) [$p < 0.089$] in the placebo group, with no significant difference between the groups at week 8 ($p = 0.130$). Weighing the total amount of corticosteroids used during the entire trial period indicated a statistically significant difference between the groups. At week 8, 25.6 g (SD 14.5, median 25.0 g,

Table I. Baseline demographic and clinical characteristics of probiotic and placebo groups

Characteristic	Probiotic (n = 43)	Placebo (n = 47)	p-Value (probiotic vs placebo)
Age, mo, mean (SD)	25.6 (7.7)	24.1 (6.3)	0.345
Sex, n (%)			
male	28 (65.1)	28 (59.6)	0.626
female	15 (34.9)	19 (40.4)	0.626
SCORAD index			
mean (SD)	42.1 (12.6)	40.2 (10.5)	0.437
median (5–95th percentile)	38.3 (26.2–63.4)	37.6 (27.4–61.4)	0.750
moderate AD, n (%)	31 (72.1)	38 (80.9)	0.316
severe AD, n (%)	12 (27.9)	9 (19.1)	0.316
IDQOL score			
mean (SD)	11.2 (4.4)	12.1 (3.6)	0.305
median (5–95th percentile)	12.0 (5.1–17.9)	12.0 (7.0–17.0)	0.873
DFI score			
mean (SD)	12.2 (4.0)	12.6 (4.7)	0.679
median (5–95th percentile)	13.0 (5.2–18.9)	11.0 (6.0–20.7)	0.430
Birth weight, g, mean (SD)	3384 (487)	3294 (357)	0.317
Breast fed, mo, mean (SD)	9.0 (4.4)	8.5 (3.7)	0.533
Solid food introduced, mo, mean (SD)	6.0 (2.1)	5.7 (1.7)	0.453
Food allergy[a], n (%)	9 (20.9)	8 (17.0)	0.676
Parental allergy[b], n (%)	22 (51.2)	18 (38.3)	0.221
Parental AD, n (%)	13 (30.2)	10 (21.3)	0.336
Topical corticosteroids, n (%)	32 (74.4)	32 (68.1)	0.512
Topical corticosteroids, d/wk[c], mean (SD)	1.9 (1.5)	1.7 (1.5)	0.474
Oral antihistamines[d], n (%)	14 (32.6)	11 (23.4)	0.333
Total IgE, IU/mL			
mean (SD)	53.8 (25.9)	59.1 (23.7)	0.318
median (5–95th percentile)	59.0 (16.3–92.8)	61.0 (15.9–90.1)	0.225
>50 IU/mL, n (%)	25 (58.1)	28 (59.6)	0.892
Eosinophil count, cells/mm^3			
mean (SD)	247 (75)	263 (74)	0.292
>250 cells/mm^3, n (%)	19 (44.2)	26 (55.3)	0.296

a Allergy to egg and milk according to double-blind, placebo-controlled, food challenge.

b Presence of asthma, hay fever, urticaria, or AD.

c Use in the last wk.

d Use in the last 2 wk.

AD = atopic dermatitis; **DFI** = Dermatitis Family Impact; **IDQOL** = Infant Dermatitis Quality of Life; **SCORAD** = Scoring of Atopic Dermatitis.

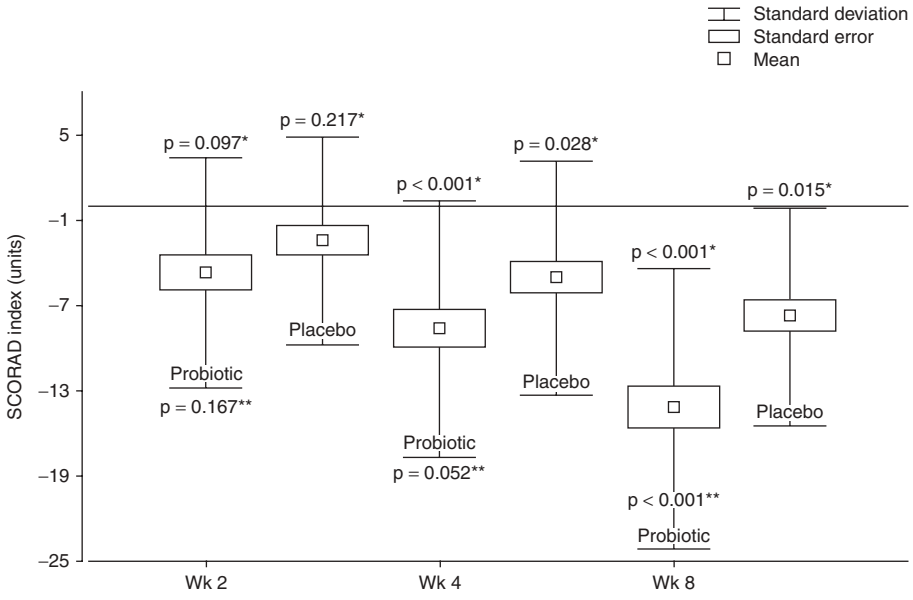

Fig. 2. Change in the Scoring of Atopic Dermatitis (SCORAD) index from baseline in probiotic (n = 43) and placebo (n = 47) groups over time. * Upper probabilities vs baseline values, ** probiotic vs placebo.

range 0.0–45.0 g) was used in probiotic patients versus 33.3 g (SD 11.4, median 35.0 g, range 15.0–50.0 g) in placebo patients (p = 0.006). Thus, the probiotic group used 7.7 g less topical corticosteroids during the 8 weeks of study (p = 0.006).

Lymphocytes Subsets in Venous Blood

Absolute and relative counts in the major lymphocyte subsets were comparable in both study groups at week 0 (table V).

At week 8, CD3, CD16, and CD22 subsets remained unchanged, whereas the percentage and/or absolute count of CD4 and CD25 decreased, and the percentage of CD8 increased in the probiotic group. The correlation matrix of clinical and immune parameters showed a significant association only between CD4 percentage, CD25 percentage, and CD25 absolute number and SCORAD values (r = 0.642, r = 0.746, r = 0.733, respectively; p < 0.05) at week 8 in the probiotic group.

Table II. Changes from baseline in Scoring of Atopic Dermatitis (SCORAD) index components (A = extent, B = intensity, C = scratching, insomnia) in probiotic and placebo groups over the 8-week study period

SCORAD variables	Week	Probiotic (n = 43)		Placebo (n = 47)		p-Value (probiotic vs placebo)
		units[a]	p-value vs wk 0	units[a]	p-value vs wk 0	
A	0	38.2 (13.7)		40.2 (10.5)		0.945
	2	−6.6 (9.3)	0.053	−2.5 (7.3)	0.131	0.164
	4	−9.0 (11.0)	0.002	−5.1 (8.2)	0.121	0.029
	8	−12.2 (0.3)	0.001	−7.8 (7.7)	0.016	0.018
B	0	7.3 (2.4)		6.6 (1.9)		0.178
	2	−0.7 (1.9)	0.180	−0.3 (1.6)	0.422	0.340
	4	−1.3 (2.2)	0.005	−1.0 (1.8)	0.020	0.483
	8	−2.4 (2.3)	0.001	−1.4 (2.0)	0.001	0.020
C	0	8.7 (2.9)		8.0 (2.4)		0.453
	2	−0.7 (2.0)	0.237	−0.5 (1.6)	0.370	0.696
	4	−1.7 (2.4)	0.001	−1.1 (1.8)	0.038	0.188
	8	−3.0 (2.7)	0.001	−1.4 (2.0)	0.006	0.004

a Mean (SD).

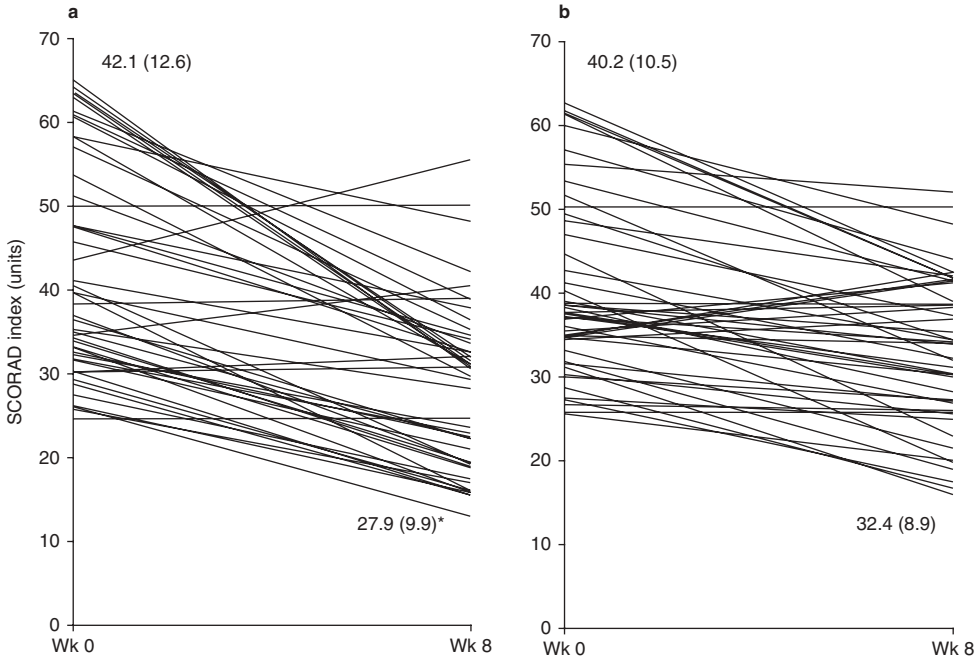

Fig. 3. Change in the Scoring of Atopic Dermatitis (SCORAD) index from baseline to week 8 in individual patients in the probiotic (n = 43) [**a**] and placebo (n = 47) [**b**] groups. Figures are mean (SD) SCORAD values at weeks 0 and 8. * $p < 0.05$ vs placebo.

Adverse Events

Probiotic and placebo groups were comparable in the frequency of adverse events (60.5%, n = 26 vs 51.1%, n = 24, respectively; p = 0.372). Upper respiratory tract infections were documented in 11 (25.6%) versus 10 (21.3%) [p = 0.631], lower respiratory tract infections occurred in 4 (9.3%) versus 5 (10.6%) [p = 0.838], herpetic stomatitis in 7 (16.3%) versus 5 (10.6%) [p = 0.429], diarrhea in 3 (7.0%) versus 2 (4.3%) [p = 0.579], constipation in 6 (14.0%) versus 6 (12.8%) [p = 0.868], and abdominal colic in 5 (11.6%) versus 4 patients (8.5%) [p = 0.625], respectively. Two children (4.7%) in the probiotic group (burn, croup) and three (6.4%) in the placebo group (head injury, food poisoning in two children) experienced severe adverse events. None was related to the medications under investigation.

Discussion

Our study provides evidence that certain strains of probiotic bacteria may have a beneficial effect on the course of AD in preschool children. The probiotic effect was not attributable to age, sex, dose, or duration of probiotic supplementation, as these parameters were similar in children in whom probiotic formulations succeeded or failed to demonstrate clinical benefit.

Table III. Baseline values and changes in the Scoring of Atopic Dermatitis (SCORAD) index in subgroups of patients with atopic dermatitis (AD) at week 8 of probiotic or placebo treatment

Subgroup	Probiotic			Placebo			p-Value (probiotic vs placebo)
	n	baseline[a]	change[a]	n	baseline[a]	change[a]	
Moderate AD	31	35.2 (6.5)	−10.2 (7.6)	38	31.9 (5.9)	−5.9 (6.7)	0.014
Severe AD	12	59.7 (4.2)	−24.7 (7.2)*	9	53.9 (3.9)	−15.8 (6.2)*	0.008
IgE ≤50 IU/mL	18	42.7 (12.7)	−14.3 (10.7)	19	32.3 (10.5)	−6.6 (9.5)	0.068
IgE >50 IU/mL	25	41.7 (12.3)	−15.2 (9.4)	28	39.2 (10.6)	−8.3 (7.3)	0.006
Eosinophil count ≤250 cells/mm^3	24	40.7 (11.9)	−13.1 (10.2)	21	43.9 (11.4)	−7.7 (8.4)	0.071
Eosinophil count >250 cells/mm^3	19	43.9 (13.2)	−15.7 (9.6)	26	40.3 (10.0)	−7.8 (7.3)	0.003

a Mean (SD).

* $p < 0.05$ vs moderate AD.

Table IV. Changes from baseline in the Infant Dermatitis Quality of Life (IDQOL) and Dermatitis Family Impact (DFI) scores in probiotic and placebo groups over the 8-week trial period

Questionnaire	Wk	Probiotic (n = 43)		Placebo (n = 47)		p-Value (probiotic vs placebo)
		units[a]	p-value vs wk 0	units[a]	p-value vs wk 0	
IDQOL	0	11.2 (4.4)		12.1 (3.6)		
	2	−1.0 (2.3)	0.234	−1.0 (1.7)	0.129	0.963
	4	−2.4 (3.0)	0.002	−1.6 (1.0)	0.038	0.095
	8	−3.7 (3.3)	<0.001	−2.3 (1.6)	0.001	0.013
DFI	0	12.2 (4.0)		12.6 (4.7)		
	2	−1.7 (1.5)	0.041	−1.1 (3.4)	0.228	0.280
	4	−2.5 (1.5)	0.002	−1.7 (3.6)	0.049	0.153
	8	−4.3 (1.7)	<0.001	−3.0 (2.7)	0.001	0.010

a Mean (SD).

However, subgroup analysis revealed that children with increased IgE levels and eosinophil counts were more likely to benefit from probiotics. Such patients constituted more than half of the study population, and contributed markedly to the overall therapeutic success rate. This finding is in agreement with reports suggesting that children with atopy are particularly sensitive to probiotic supplementation,[15,17] perhaps reflecting the shift in T helper-1/T helper-2 (T_h1/T_h2) balance observed in allergic patients treated with probiotics.[36,37] Changes in immune function mediated by probiotics may also play a role, as they develop gradually, by week 4 according to our data. Pessi et al.,[38] in a study of children with AD treated with *Lactobacillus rhamnosus* GG, observed that an increase in the level of interleukin-10 occurred not earlier than 4 weeks.

The reported discrepancies in clinical effectiveness of probiotic treatments may be linked to processing during the manufacture of probiotic preparations. Formulations based on *Lactobacillus* species may have been cultivated on media containing milk. After freeze drying, such preparations may retain a trace amount of milk allergens, which may have potentially reduced or obscured the effectiveness of probiotics in patients with milk allergy in several therapeutic trials.[13,17,21] The fact that the probiotic formulation employed in the present trial was free of dairy products and egg allergens assumes particular importance as approximately 20% of the studied children proved to be allergic to cow's milk or egg.

In view of the disparate therapeutic results obtained with probiotics in pediatric AD, it may be pertinent to discuss possible differences among specific bacteria and/or their combinations.[24] *Lactobacillus* GG was reported to be clinically effective in a mixture with *B. lactis*,[14] while *Lactobacillus* GG alone was comparable to placebo in more recent studies.[17,19]

Table V. Lymphocyte subsets in peripheral blood in children with atopic dermatitis at baseline and after 8 weeks of probiotic or placebo treatment

Subset	Probiotic (n = 16)		Placebo (n = 16)		p-Value (probiotic vs placebo)	
	wk 0	wk 8	wk 0	wk 8	wk 0	wk 8
CD3, mean % (SD)	69.7 (7.5)	64.3 (7.2)	70.7 (5.1)	62.0 (9.7)	0.662	0.461
CD3, mean absolute count (SD)	3294 (554)	2716 (579)	3322 (707)	2631 (627)	0.905	0.692
CD4, mean % (SD)	39.8 (3.0)	36.1 (1.7)	39.9 (5.9)	39.3 (2.2)	0.970	<0.001
CD4, mean absolute count (SD)	1901 (391)	1530 (288)	1855 (402)	1667 (298)	0.746	0.196
CD8, mean % (SD)	22.1 (3.1)	27.8 (3.9)	23.9 (3.5)	22.8 (3.8)	0.131	0.001
CD8, mean absolute count (SD)	1052 (232)	1165 (233)	1121 (262)	956 (175)	0.438	0.007
CD16, mean % (SD)	16.9 (4.5)	16.7 (4.3)	15.0 (3.1)	14.8 (4.1)	0.168	0.215
CD16, mean absolute count (SD)	814 (289)	708 (213)	698 (171)	639 (244)	0.176	0.403
CD22, mean % (SD)	28.2 (3.8)	27.3 (4.3)	27.3 (3.2)	24.3 (5.2)	0.456	0.085
CD22, mean absolute count (SD)	1343 (291)	1168 (357)	1290 (339)	1023 (260)	0.637	0.200
CD25, mean % (SD)	16.7 (3.8)	13.0 (1.7)	17.8 (4.2)	17.5 (2.6)	0.433	<0.001
CD25, mean absolute count (SD)	804 (259)	548 (117)	825 (209)	750 (187)	0.799	0.001

We tested the DDS-1 strain of *L. acidophilus* in combination with *B. lactis* UABLA-12, in contrast with previously studied bacteria that included various strains of *Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus fermentum* alone or in combination with *Bifidobacterium breve,* and *B. lactis*.[22] In addition, our formulation included fructo-oligosaccharide, a carbohydrate that may protect against infections during the course of the disease.[39] A preparation containing neutral short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides was shown to reduce the incidence of AD during the first 6 months of life,[39] and the severity of the disorder in preschool children.[40] As the tested formulation contained a mixture of three theoretically active ingredients, *L. acidophilus* DDS-1, *B. lactis* UABLA-12, and fructo-oligosaccharide, this makes it difficult to ascribe the clinical effect to one or all of its particular components, and to compare the clinical effects with those found in other studies. The changes in the SCORAD index observed in the present trial were comparable to those documented in a study in which *L. rhamnosus* Lcr35 was supplemented with a prebiotic.[40]

The significant decline in the SCORAD index was due to a consistent decrease in all index components. Grüber et al.[20] attributed improvements in the affected area of skin to the natural course of AD, postulating that this SCORAD-linked modality is not sufficiently sensitive to detect differences in outcomes resulting from short-term interventions with a probiotic. The present study demonstrates that the 8-week period of probiotic treatment was sufficient to induce significant changes in the extent of the rash compared with placebo, as other investigators have found for the same or even shorter treatment periods.[15,16] Similarly, Rosenfeldt et al.[15] reported that change in the extent of rash was the most sensitive measure of improvement during probiotic treatment.

To our knowledge, this is the first report of the effects of probiotic treatment on quality of life in patients with AD. Despite its clinical importance, this outcome measure has been generally omitted from previous studies, or reported results were inconsistent with SCORAD changes.[15,17,19,20] As IDQOL shares several components with SCORAD, such as itching and interference with night-time sleep, a correlation between the two indexes appears reasonable. A previous study also reported no significant change in the DFI score in spite of the decrease in SCORAD score, linking the negative findings with heavy demands on family time and effort and the increased financial burden experienced by parents managing their child's skin condition.[41] The decrease in DFI score in children treated with probiotics documented in the present study may reflect improved child health, and a reduction in the use of drugs such as topical corticosteroids.

We are unaware of reports concerning corticosteroid-sparing effects associated with probiotics in the management of AD. In previous trials that failed to demonstrate beneficial outcomes, the frequency of corticosteroid applications per week was utilized as a measure of efficacy.[16,19] In the present study, a decline in the frequency of application of topical corticosteroids was also not statistically significant between probiotic- and placebo-treated groups. Topical corticosteroid usage, estimated by weight, registered a significant decrease by week 8 of probiotic therapy, indicating that SCORAD changes reflected the use of probiotics rather than concurrent medications. All patients in the study received topical corticosteroid applications of the same potency, allowing direct dosage comparisons. However, the absence of a pretrial run-in period during which the number of applications could be followed prospectively, and amounts of a particular topical corticosteroid preparation in use prior to probiotic supplementation determined precisely, is a potential flaw in our study design. A run-in phase would also be useful if the patients took different drugs, probiotic supplements requiring wash-out, used different non-pharmacologic treatments, were not on an elimination diet, or if the patient required clarification of the daily course of AD. In our study, the patients were stable on regular long-term follow-up, received the standard recommendations, and successfully maintained the elimination diet at least 2 months before enrollment. IDQOL, DFI, and the subjective part of SCORAD are designed for retrospective assessment, so the absence of a run-in period may not have significantly affected the results of the study.

Changes in CD4, CD8, and CD25 blood lymphocyte subsets may reflect an immune regulatory role of probiotics in AD. Immune modulation is likely to be mediated by gut-associated lymphoid tissue, which is in close contact with bacteria. Compared with healthy subjects, CD4 and CD25 counts increased and the percentage of CD8-bearing lymphocytes decreased in children with AD.[26] In experimental mice, an increase in the percentage of CD4/CD25-containing cells correlated positively with severity of dermatitis, yet there was no effect of *L. rhamnosus* GG supplementation on this marker.[25] Our findings suggest that probiotics exert their beneficial effects by decreasing the percentage and/or absolute counts of CD4 and CD25 and by increasing CD8 determinants. A previous trial also showed that CD4/CD54 markers decreased significantly in adult patients with AD receiving probiotic preparations containing a combination of *L. acidophilus* 74-2 and *B. lactis*.[27] While we did not study CD54 as a co-marker to CD4, the

decrease in the CD4 counts observed in children treated with probiotics also appeared to be associated with clinical improvement, reflected in significant positive correlations between CD4 determinants and SCORAD indexes in the probiotic group.

Unfortunately, in this study we have not been able to test the T_h1/T_h2 shift hypothesis, using CD determinants as endpoints, as these only reflect gross stages and directions of lymphocyte differentiation, and do not correlate with cytokine T_h1/T_h2 profiles. Actually, T_h1 and T_h2 cells represent polarized forms of the CD4 lymphocytes. However, the decrease of CD4 and CD25 cell counts found in our trial may at least suggest that probiotics decreased maturation of T lymphocytes toward effector/active cells due to as yet unknown mechanisms.

Conclusion

The administration of a probiotic mixture containing *L. acidophilus* DDS-1, *B. lactis* UABLA-12, and fructo-oligosaccharide was associated with significant clinical improvement in children with AD, and with corresponding lymphocyte subset changes in peripheral blood. The efficacy of probiotic therapy in adults with AD requires further investigation.

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