EFFECT OF PROBIOTICS ON GASTROINTESTINAL SYMPTOMS AND SMALL INTESTINAL PERMEABILITY IN CHILDREN WITH ATOPIC DERMATITIS

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Objective To determine whether probiotic lactobacilli may alleviate small intestinal inflammation and strengthen the intestinal barrier function in children with atopic dermatitis.

Study design In a double-blinded, placebo-controlled, cross-over study, probiotic lactobacilli (*Lactobacillus rhamnosus* 19070-2 and *L reuteri* DSM 12246) were administered for 6 weeks to 41 children with moderate and severe atopic dermatitis. Gastrointestinal symptoms were registered before and during treatment and small intestinal permeability was measured by the lactulose-mannitol test.

Results During *Lactobacillus* supplementation, there was a significant decrease in the frequency of gastrointestinal symptoms (39% during the placebo period versus 10% during active treatment, $P = .002$). There was a positive association between the lactulose to mannitol ratio and the severity of the eczema ($r = 0.61$, $P = .02$ after placebo and $r = 0.53$, $P = .05$ after active treatment). After probiotic treatment, the lactulose to mannitol ratio was lower (0.073) than after placebo (0.110, $P = .001$).

Conclusions Impairment of the intestinal mucosal barrier appears to be involved in the pathogenesis of atopic dermatitis. The study suggests that probiotic supplementation may stabilize the intestinal barrier function and decrease gastrointestinal symptoms in children with atopic dermatitis. *(J Pediatr 2004;145:612-6)*

Nonpathogenic microorganisms of the indigenous intestinal microflora contribute to the mucosal barrier function and stabilize intestinal permeability.[1] Probiotics, defined as microorganisms, which, when ingested, may have a positive effect in the treatment or prevention of specific diseases,[2] have been shown to modulate the mucosal immune response and reduce gastrointestinal inflammation in infants with food allergy.[3] Suggested probiotic mechanisms include stimulation of the epithelial mucin production,[4] enhanced production of secretory IgA,[5,6] and alleviation of intestinal inflammation by stimulation of anti-inflammatory cytokines.[7,8]

Patients with atopic dermatitis (AD) appear to have an increased intestinal permeability,[9-11] but the pathogenetic role of this finding is unclear. Disruption of the intestinal barrier function may represent a primary abnormality of the gut but may also reflect mucosal damage caused by local inflammatory reactions.

In a randomized trial, we found that probiotic supplementation to children with moderate and severe AD reduced the extent of eczema.[12] In this study, we demonstrated that administration of probiotics also reduced small intestinal permeability and the frequency of gastrointestinal symptoms.

METHODS

We enrolled 41 children with a median age of 4.0 years (1 to 13) with moderate and severe AD. The patients took part in a study investigating the clinical and

AD	Atopic dermatitis	s-IgE	Immunoglobulin E in serum
GI	Gastrointestinal	SCORAD	SCORing Atopic Dermatitis
LM ratio	Ratio of lactulose to mannitol		

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Supported by a grant from FØTEK (The Danish Research and Development Programme for Food Technology), The Danish Dairy Board, and The Dagmar Marshall Foundation. The test preparations were produced by Chr. Hansen, A/S Hørsholm.

None of the authors shared financial or personal relationships with the funding sources.

Submitted for publication Dec 14, 2003; last revision received Jun 2, 2004; accepted Jun 28, 2004.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2004.06.068

Table. Prevalence of gastrointestinal symptoms during placebo and *Lactobacillus* treatment

n = 41	Placebo n (%)	Active n (%)	*P* value
Vomiting only	1	0	
Diarrhea only	6	1	
Abdominal pain only	6	2	
Diarrhea and abdominal pain	3	1	
Any GI symptom	6 (39)	4 (9)	.002

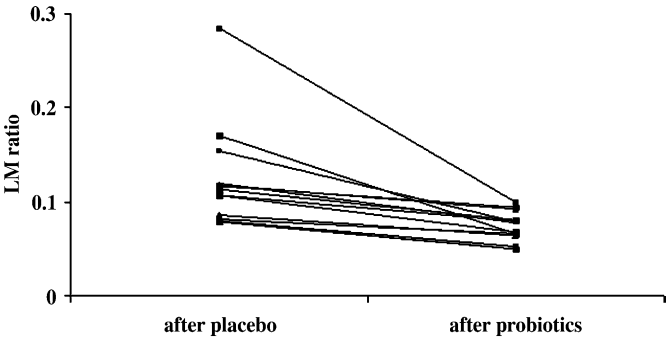

Fig 1. Lactulose:mannitol ratio measured after placebo and after 6 weeks of probiotic supplementation.

anti-inflammatory effects of *Lactobacillus* supplementation.[12] The study protocol included written consent by the parents or guardians of the participating patients and was approved by the Ethics Committee of the Municipality of Copenhagen and Frederiksberg and the Ethics Committee of Copenhagen County (Journal nr. 02-056/99).

The diagnosis of AD was made according to standardized criteria.[13] The clinical severity of the eczema was evaluated by SCORAD (SCORing Atopic Dermatitis).[14] The range of SCORAD is 0 to 80. The eczema was judged as mild (SCORAD, 0 to 15), moderate (SCORAD, 16 to 40), or severe (SCORAD, >40). At inclusion the median (range) SCORAD score was 37 (15 to 66). Before intervention, only 2 patients had mild eczema (SCORAD, <15), 26 had moderate eczema (SCORAD, 16 to 40), and 13 had severe eczema (SCORAD, >40).

Before intervention, a blood sample for analysis of immunoglobulin E in serum (s-IgE) was drawn. The patients were randomly assigned in a double-blinded design to receive either placebo followed by active treatment (group A) or active treatment followed by placebo (group B). Each intervention period was separated by a 6-week washout period.

The bacterial preparation was lyophilized *Lactobacillus rhamnosus* 19070-2 and *L reuteri* DSM 12246. The safety and viability of these strains and their ability to adhere to the intestinal mucosa had been evaluated in vitro and in healthy volunteers.[15,16] *L rhamnosus* 19070-2 and *L reuteri* DSM 12246, given in combination, were shown to be beneficial and in children with acute infectious gastroenteritis.[17,18] A dose of 10^{10} colony-forming units of each strain or the identical-looking placebo preparation was given twice daily for 6 weeks. The placebo preparation consisted of skimmed milk powder (0.28 g; bovine protein, 37 %) and dextrose anhydrate (0.72 g). A study nurse who was not involved in the clinical evaluation or data analyses distributed the test preparations. When administered to the patient, the study preparation (weight, 1.0 g) was dissolved in 2.5 to 5 mL of water or any liquid preferred by the patient. During the 20-week study period, the patients were asked to abstain from any fermented milk products. For assessment of compliance, issues regarding taste and the child's preferred way to ingest the powder were discussed at each scheduled visit.

Gastrointestinal (GI) symptoms before intervention and during the last 14 days of each intervention period were registered through questionnaires. These were filled in before the scheduled visit and delivered to the study nurse. The parents were asked if their child had diarrhea (3 or more loose stools/24 hours that were not accompanied by fever), vomiting without fever, or abdominal pain (children >3 years old, n = 25). The parents/children were asked to indicate whether, before intervention, these symptoms occurred "often" (equivalent to "several times" or "every day" during the last 2 weeks of each treatment period), "once in a while" (equivalent to "a couple of times" during the last 2 weeks of intervention), or "seldom"/never" (equivalent to "did not occur" during the intervention periods). Furthermore, the parents were asked about the possible association of any of these symptoms to specific foods.

Small Intestinal Permeability (Lactulose-Mannitol Test)

Patients from the age of 4 years and above were selected for the permeability test. However, during the study period, there was an unexpected delay in the delivery of sufficient amounts of lactulose and mannitol, causing occasional dropouts with respect to the lactulose-mannitol (LM) test. Therefore, small intestinal permeability was assessed in only 18 patients, 4 to 13 years of age. These patients had moderate and severe eczema [median SCORAD (range) score at inclusion, 50 (18 to 66)]. The IgE levels in patients completing the LM test and in patients without this test were comparable (mean, 625 vs 511 IU/L, $P = .70$). Two patients were noncompliant with respect to urine collection.

Fourteen of the patients had the test performed twice (after placebo and after active treatment). Data from these 14 patients were used to estimate the effect of probiotics on small intestinal permeability.

A test solution consisting of 2 g mannitol and 10 g lactulose in 100 mL of water was prepared. After an overnight fast and after having passed the first morning urine, the patients received 2 mL solution/kg body weight (maximum, 100 mL). Urine was collected over the following 5 hours. An enzymatic method to determine the concentration of lactulose and mannitol was applied.[19] The interassay variation for lactulose was 2.0% and for mannitol was 2.4%.

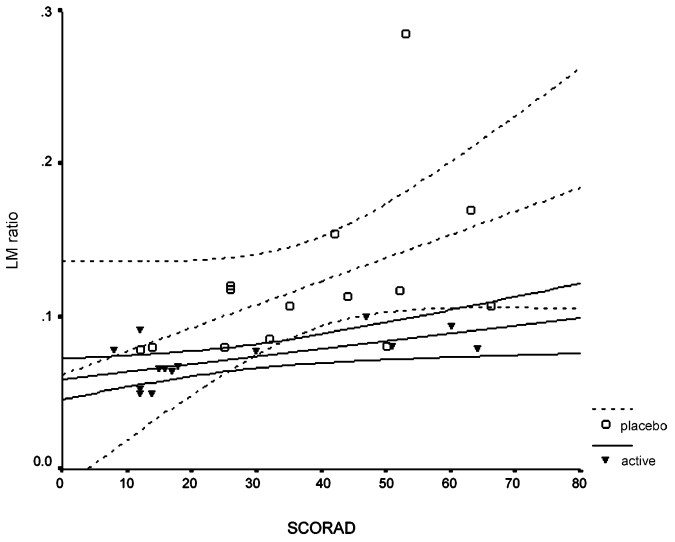

Fig 2. Association between clinical severity of atopic dermatitis and LM ratio in all patients with at least one LM test. Values are median and range of LM ratio in mild (SCORAD, 0-15), moderate (SCORAD, 16-40), and severe eczema (SCORAD,>40). Four patients completed only one test and were not included in the analysis of probiotic effects.

The urinary recovery of lactulose and mannitol was calculated as percentage of the dose administered to the child and expressed as the ratio of recovered lactulose to mannitol (LM ratio).

Statistics

The basic data are presented as medians and ranges. The Wilcoxon signed rank test, the Mann-Whitney *U* test, and χ^2 test were used when appropriate. Correlations were expressed by the Spearman correlation coefficient. Values of $P < .05$ were considered significant.

RESULTS

Gastrointestinal Symptoms

Before intervention, 12 of 41 patients (27%) reported diarrhea, vomiting, and/or abdominal pain to occur "once in a while," whereas 6 of 41 patients (14%) stated that these symptoms occurred "often." No association between the clinical severity of eczema and the prevalence of GI symptoms was demonstrated (Spearman correlation between SCORAD at inclusion and presence of GI symptoms, −0.079, $P = .63$). However, there was a positive association between the baseline SCORAD scores and the s-IgE levels (Spearman correlation coefficient, 0.586, $P = .035$). Two patients with a positive skin prick test to cow's milk reported diarrhea when drinking milk. Before and during the study period, they abstained from milk products. Otherwise, no relations between specific foods and GI symptoms were reported. The median (range) s-IgE was higher in the group reporting GI symptoms before intervention compared with patients with no GI symptoms [1225 (55 to 2035) kIU/L vs 183 (14 to 1008) kIU/L, $P = .01$].

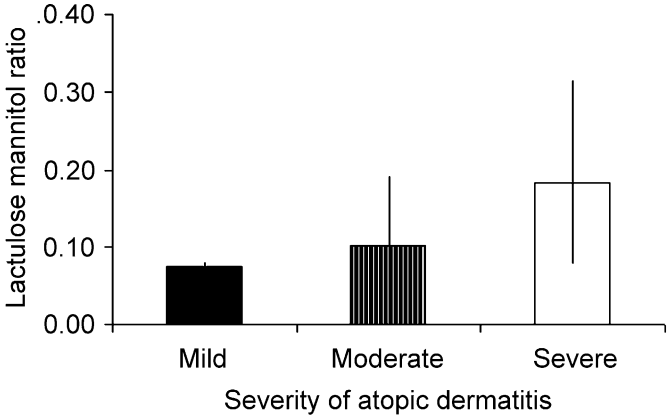

Fig 3. Association between clinical severity of atopic dermatitis and LM ratio after placebo *(open circles)* and after *Lactobacillus* treatment *(dark squares),* with 95% CI for both parameters.

A significant difference in the frequency of "any GI symptom" during placebo versus active treatment was noted. During the last 14 days of the placebo period, 16 of 41 patients (39%) reported diarrhea, vomiting, and/or abdominal pain occurring at least "a couple of times" or "several times a week." In contrast, during the last 14 days of the active treatment, only 4 of 41 patients (10%) reported GI symptoms ($P = .002$) (Table). Five patients with no GI symptoms before intervention complained of such symptoms during placebo. Among patients who, during placebo, stated they did not have GI complaints, none complained of such symptoms during active treatment. During interventions, no episodes of acute gastroenteritis were reported. One patient received a 10-day course of dicloxacillin for treatment of impetigo. Otherwise, no antibiotics were prescribed during the study period.

Lactulose:Mannitol Ratio

After placebo and probiotic treatment, the median (range) LM ratios were 0.110 (0.079-0.285) and 0.073 (0.050-0.100) respectively ($P = .001$). Figure 1 shows the LM ratio for each child after placebo and after active treatment.

There was no carryover effect because the difference between the placebo and the active period was not different between group A (starting with placebo) and group B (starting with active) (0.0047 [0.016-0.007] and 0.057 [−0.094-0.123]), respectively ($P = .41$). Furthermore, there was no bias in random assignment because the average of the LM test for the active and the placebo group for each child was not different in group A and B (0.089 [0.079-0.107] and 0.105 [0.064-0.152]), respectively ($P = .66$).

The change in LM ratio after active treatment was due to decreased excretion of lactulose. After active treatment, the median excretion of lactulose decreased by 50%, from 0.30% to 0.15% ($P = .02$). The median excretion of mannitol remained unchanged (12.9% after placebo vs 10.2 % after active treatment).

There was a positive correlation between the LM ratio and the clinical severity of eczema (Spearman correlation

coefficient between SCORAD after placebo and LM ratio after placebo: 0.609, $P = .021$, and between SCORAD and LM ratio after active treatment: 0.528, $P = .052$). Figure 2 illustrates the association between the clinical severity of the eczema and small intestinal permeability after placebo and after active treatment.

Figure 3 shows pooled data of all available LM ratios and the corresponding SCORAD scores.

DISCUSSION

We have shown previously that administration of probiotic *Lactobacilli* reduces the clinical severity of AD.[12] When evaluating the clinical effects of *L rhamnosus* 19070-2 and *L reuteri* DSM 12246,[12] we were able to demonstrate a reduction in the extent of the eczema, and we found a more pronounced effect in patients with elevated IgE. We now demonstrate that probiotic supplementation is also associated with a reduction in the frequency of GI complaints and a decrease in small intestinal permeability.

In an animal model, *L rhamnosus* GG (ATCC 53103) (*L* GG) was shown to reverse increased intestinal permeability in sucking rats exposed to cow's milk.[20] More recently, another probiotic *Lactobacillus* strain, *L plantarum* 299v, was shown to abolish increased intestinal permeability in rats exposed to *Escherichia coli*.[21] Few clinical trials examining the relation between probiotic microorganisms and intestinal permeability in human beings have been published. Administration of *L* GG to 4 children and adolescents with Crohn disease decreased the small intestinal permeability in parallel with a reduction in the clinical severity scores.[22]

The urinary excretion of a large molecule (usually lactulose), compared with that of a smaller molecule (mannitol or rhamnose), provides a noninvasive estimate of intestinal permeability. In the current study, GI symptoms and small intestinal permeability improved after probiotic supplementation. Mannitol is absorbed through small pores in the enterocyte membrane (transcellular pathway). Decreased excretion of mannitol would indicate that the mucosal surface area is reduced. In contrast, lactulose is unable to pass through the healthy mucosa but may, under certain conditions, be absorbed through the paracellular pathway.[23] Accordingly, the demonstration of increased excretion of lactulose in AD would indicate a "leaky" mucosa.

In children with AD, GI symptoms are common.[24] In the current study, GI complaints were more common in children with high s-IgE levels. Increased counts of immunoreactive IgE cells have been demonstrated in duodenal biopsy specimens from adult patients with AD.[25] Many patients with AD have elevated s-IgE levels, but the pathogenetic role of this finding is unsettled. In young children particularly, food allergens may exacerbate skin eruptions. Still, clearance of the eczema very rarely occurs with allergen avoidance. Likewise, in older children, reduced exposure to airborne or food-borne allergens seldom improves the eczema.

Our findings suggest that in AD, the integrity of the intestinal mucosal barrier is disturbed. This allows for an increased antigen transfer, which may stimulate specific IgE production and promote local inflammatory reactions. Thus, a therapy directed toward reversing the increased intestinal permeability is logical. In the current study, we were able to demonstrate a direct relation between decreasing intestinal permeability and improvement in the SCORAD score. We also showed that LM ratio was significantly higher in children with severe eczema compared with children with mild and moderate eczema. This finding supports the theory of an association between AD and the disruption of the intestinal mucosal barrier.

For oral bacteriotherapy to be most effective, the question of timing may be important. In epidemiologic studies, age-dependent variations in small intestinal permeability have been found.[26] Small intestinal permeability is increased in infants compared with older children. Moreover, the composition of the intestinal flora in infancy is supposed to influence maturation of the intestinal immune system. Early intestinal microflora might influence the development of allergic sensitization.[27] In a study examining intestinal flora in 1-year old infants, a reduced ratio of bifidobacteria and lactobacilli to clostridia and *Staphylococcus aureus* was found to precede the development of atopic disease.[28] Thus, exogenous administration of beneficial bacteria might be useful in the prevention of AD. A beneficial effect of *L* GG as prophylaxis against atopic eczema has recently been suggested.[29]

Our results indicate that disruption of the intestinal barrier function may contribute to the pathogenesis of AD. Future studies should address particularly the optimal timing of probiotic supplementation and should prospectively evaluate the effect of long-term supplementation and the potentials of probiotic microorganisms to prevent atopic diseases.

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