

# Probiotics and prevention of allergic disease

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## Purpose of review

Over the past few decades, public reports have shown increasing enthusiasm for the potential health effects of probiotics. Therefore, the purpose of this review is to focus on studies, which have addressed the use of probiotics for primary prevention of atopic diseases.

## Recent findings

The Finnish study of Kalliomaki was the first report to describe that the frequency of atopic dermatitis in neonates treated with *Lactobacillus rhamnosus* GG (LGG) was half that of the placebo. Recently, these results have been questioned by two trials, which reported no difference in the development of atopic dermatitis in neonates supplemented with LGG and *L. acidophilus*, respectively. In contrast, an unexpected increase in respiratory side-effects was observed in some studies in children who received the probiotics. Up to now no data have been released which report a positive effect of probiotics for the prevention of allergic rhinitis or asthma. Moreover, an allergen-preventive effect of probiotics for the development of atopic dermatitis could not be consistently established.

## Summary

In conclusion, probiotics cannot be generally recommended for primary prevention of atopic disease. Further studies should clarify if any susceptible subgroups exist and how these subgroups benefit from supplementation with certain probiotic strains. Moreover, the selection of the most beneficial probiotic strain or the composition of different probiotics and/or prebiotics, the dose and the timing of supplementation still need to be determined.

## Keywords

allergy, atopic dermatitis, neonates, prevention, probiotics

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## Introduction

Probiotics are defined as products or preparations containing viable microorganisms in numbers thought to alter the host's microflora, thereby bringing about beneficial health effects [1]. In analogy to antibiotics it is important to point out that 'probiotic' is an umbrella term for different bacteriological strains and species with a broad range of various immunological and clinical abilities. Therefore, it is of particular importance to come to a species specific weighting instead of generalized statements. Data from animal models and in-vitro findings can be used as screening instruments to choose new probiotic strains for preventive or therapeutic use. However, the gold standard for evaluating the efficacy of probiotics for primary prevention are randomized, double-blind, placebo-controlled clinical trials. Unfortunately, clinical studies vary considerably in design and the applied probiotics, thereby limiting comparability of the results. The value of probiotics for primary prevention of allergic disorders is controversial. This review

summarizes the clinical effects of probiotics as well as the potential side-effects, which were reported in recently published, clinical prevention trials. Finally, new findings from animal and in-vitro studies and their relevance for further research will be discussed.

## Clinical effects of probiotics

The Finnish study of Kalliomaki was the first report to describe that the frequency of atopic dermatitis in the group supplemented with *L. rhamnosus* GG was half that of the placebo [2]. This finding was confirmed at the ages of 4 and 7 years, where significantly more children in the placebo groups showed symptoms of atopic dermatitis [3,4\*]. The cumulative risk of developing eczema during the first 7 years of life was 42.6% in the LGG group, compared with 66.1% in the placebo group (RR 0.64; 95% CI 0.45–0.92) [4\*]. No differences were found with respect to both, total or specific IgE concentration and skin-prick-test reactivity. There are some issues, which need further comments: first, the number of affected

children was remarkably high: nearly every second child in the placebo group developed an atopic dermatitis. This raises reasonable doubt that any form of bias might have influenced these results. Second, the intake of probiotics after birth is not clearly defined: either the lactating mother or the child could take the probiotics. Third, 17, 25 and 27% of participants were lost to follow-up by age 2, 4 and 7 years, respectively. Again, selection bias cannot be excluded.

Clinical trials vary considerably in terms of study design, the applied probiotics, the dose and the method of administration and the primary endpoint (Fig. 1) [2,3,4\*,5\*\*,6\*\*,9\*\*,10\*,11\*], thereby hampering direct comparison of the results. So far, only one clinical trial from our group has adopted a nearly identical study protocol [5\*\*] to that of the Kalliomaki study. To achieve a more consistent administration of the probiotics, we instructed the families to continue the supplementation of LGG for 3 months after birth to the breast-feeding mothers and to feed the neonates with the probiotics during the following 3 months. We observed no preven-

tive effect of LGG on the development of atopic dermatitis in our population, nor any trend in this direction (Fig. 2). There are some differences in confounding variables, which probably contributed to the discrepancy in these clinical findings. Firstly, the number of atopic family members per individual in our population (LGG: 1.64; placebo: 1.75) was higher than in the Finnish population (LGG: 1.19; placebo 1.11). This may lead to our population being at higher risk compared with the Finnish population, which might account for the differing results. Secondly, we recruited more infants with older siblings (LGG: 50%; placebo: 68.2%) than the Finnish study (LGG: 37%; placebo: 34%), which might be a further potential confounder. Finally, the Finnish and the German population are of different genetic backgrounds.

In a second preventive study in a Finnish population ( $n=925$ ), a mixture of prebiotics and four different probiotic strains failed to reduce the incidence of allergic disease by the age of 2 years, but reduced the manifestation of atopic dermatitis from 32 to 26% [6\*\*]. Even if

**Figure 1 Overview of the different study designs used in clinical trials to elucidate the preventive effect of different strains of probiotics**

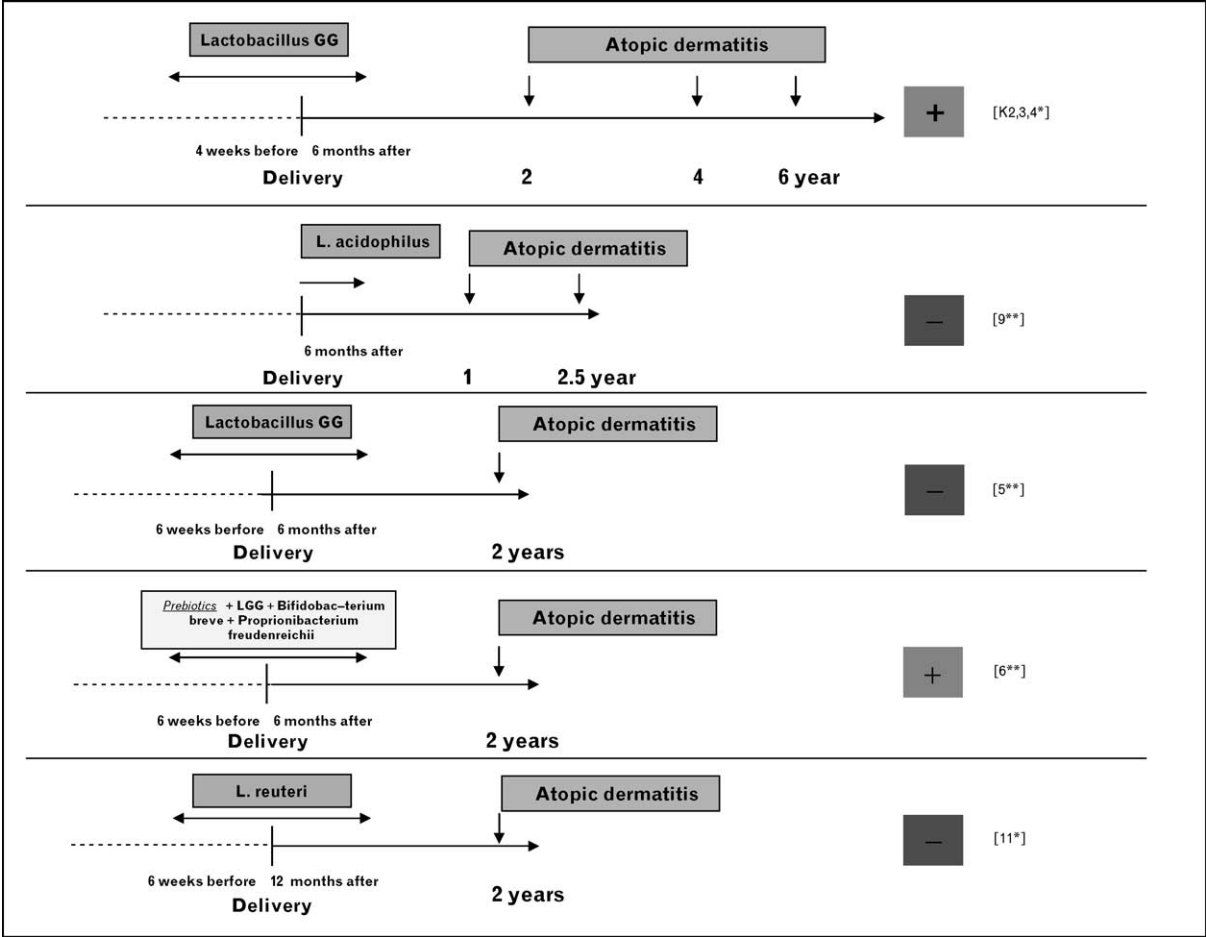
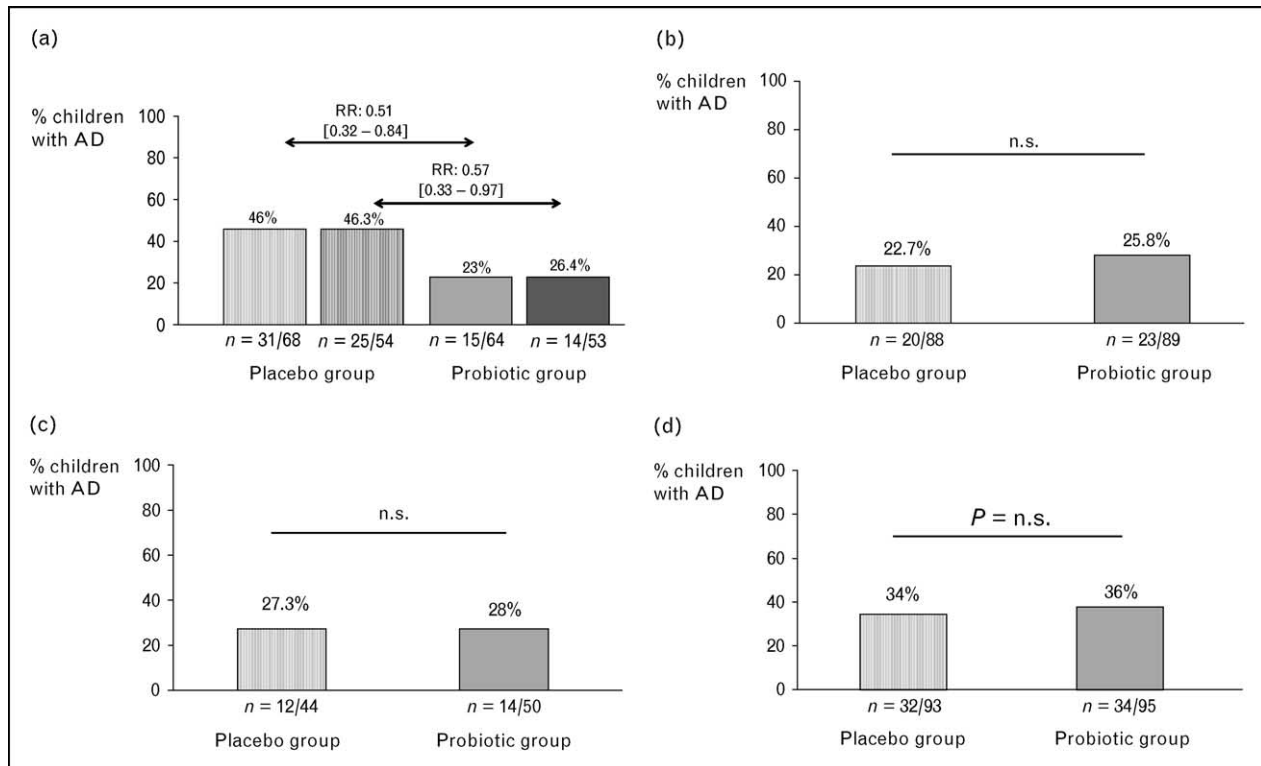


**Figure 2 Overview of the effect of probiotics on the development of atopic dermatitis**

Only trials which have investigated the effect of probiotics exclusively have been considered. AD, Atopic dermatitis; B, bifidobacterium; L, lactobaccillus. Reproduced with permission (a) [2,3]; (b) [9\*\*]; (c) [5\*\*]; (d) [11\*].

the effect in the intervention group was much smaller and the supplementation strategy was different from the one in the study of Kalliomaki, these data support the hypothesis that susceptibility to probiotics might differ between individuals due to different genetic backgrounds. On the basis of the fact that a combined supplementation of different probiotics and prebiotics was given, it is difficult to distinguish the effectiveness of the particular components. A possible preventive effect of prebiotics was also affirmed by Moro [7], who enrolled 259 infants in a double-blind, placebo controlled trial to study the effect of galacto and long-chain fructooligosaccharides on the incidence of atopic dermatitis during the first 6 months. They showed a beneficial effect of prebiotics on the development of atopic dermatitis: only 10 infants (9.8%) in the intervention group compared with 24 infants (23.1%) in the control group developed atopic dermatitis. However, at the age of 6 months the reported prevalence of atopic dermatitis in the control group of infants with a family history receiving a protein hydrolysate is unusually high (23.1%) as compared with only 14.8% at 12 months in infants with a similar familial risk receiving a regular cows' milk protein formula in the GINI study [8]. Moreover, the severity of atopic dermatitis was rather low as indicated by reported SCORAD scores. Therefore, the results of this single study do not

allow conclusions concerning the mechanism of the effect of prebiotics. However, the data do support a potential role for prebiotics as dietary manipulation for primary allergy prevention during infancy.

In support of our findings, a double-blind, placebo-controlled trial in a large Australian population ( $n = 178$ ) failed to demonstrate a preventive effect of *L. acidophilus* on atopic disease [9\*\*]. Taylor *et al.* [9\*\*] recruited only families in which the mother had an allergic disease; the supplementation of probiotics was started directly after birth. Similar to our results, not even a trend of different rates of atopic dermatitis was observed between the groups. After 12 months, 34 of 88 (43.2%) in the probiotic group and 34 of 87 (39.1%) in the placebo group developed an atopic dermatitis. Consistent with these results, there was no significant reduction in atopic dermatitis (42%) compared with the placebo group (34%) at the follow-up examination with 2.5 years [10\*]. There was no difference in the rate of allergic sensitization or in any other allergic disease.

In a recently published study [11\*], Abrahamsson could not confirm a preventive effect of *L. reuteri* on infant eczema. However, he observed that the treated infants had less IgE-associated eczema at 2 years. Moreover,

skin-prick test reactivity was also less common in the treated than in the placebo group, but this difference was only of significance for infants with allergic mothers. Again, there are differences in the administered probiotics and the supplementation period (1 year), which might explain the discrepancy compared with the Taylor study [9<sup>••</sup>] and to ours [5<sup>••</sup>] (Fig. 1).

Very recently, Lee *et al.* [12<sup>•</sup>] published a meta-analysis. They included the data of six prevention studies [2,3,6<sup>••</sup>,9<sup>••</sup>,11<sup>•</sup>,13]. However, data from three out of six studies derive from the same group and from one study population [2,3,13]: The trial, published by Rautava *et al.* [13] was assigned as 'part of a trial evaluating the preventive potential of probiotics in allergy, as described in detail elsewhere'. Again, the clinical data published by Kalliomaki 2001 and 2003 [2,3] comprised the same population, which was examined at 2 and 4 years of age. Altogether, a cumulative weight of 33.19% is based on data from one single population. Moreover, the only trial, which showed an effectiveness of the preventive strategies, except the data of the Isolauri group [16], studied the combined effect of probiotics and prebiotics [6<sup>••</sup>]. This trial was included in the meta-analysis, even though a preventive effect of 'probiotics' cannot be deduced based solely on the study design. Basically, a reliable analysis of the preventive effects of probiotics should only include the data of Kalliomaki, Taylor and Abrahamsson. Therefore, the authors' statement that they have calculated a summary effect size of a reduced relative risk ratio of 0.69 (0.57–0.86) in the probiotic group is based on an invalid data set. Consequently, the conclusion that the data supports a preventive potential of probiotics on pediatric atopic dermatitis should be considered with caution.

### Possible side-effects of probiotics

Overall, prenatal and postnatal administration of probiotics appears to be a well tolerated intervention without severe systemic side-effects. However, in recently conducted clinical trials, some conspicuously unexpected findings turned up which need to be considered. In the Kalliomaki study [3], the authors reported that allergic rhinitis and asthma tended to be more common in the probiotic group. At the age of four, 10 of 53 (18.8%) and five of 54 (9.2%) developed allergic rhinitis, and three of 53 (5.6%) and one of 54 (1.8%) developed asthma in the LGG-group and placebo-group, respectively. Allergic rhinitis and asthma also tended to be more common in the probiotic group at the age of 7 years [4<sup>•</sup>]. Similarly, significantly more children in the probiotic group developed wheezing during the first 6 months of life in the Taylor study [9<sup>••</sup>]. This finding also corresponds with data of our population, where we observed a significantly higher proportion of children with recurrent ( $\geq 5$  epi-

sodes) wheezing bronchitis during the first 2 years in the LGG group (26%), compared with placebo (9%;  $P=0.03$ ) [7].

In addition more respiratory symptoms in neonates supplemented with probiotics, unexpectedly higher numbers of children with allergic sensitization and spitting-ups were reported. In the Taylor study [9<sup>••</sup>], the rate of sensitization to common allergens was significantly higher in the probiotic group (skin prick test positive children: 39.8 versus 24% placebo;  $P=0.03$ ). Moreover, the proportion of children with atopic dermatitis and allergic sensitization was significantly higher in the probiotic group (26%) compared with placebo (14%;  $P=0.045$ ) [5<sup>••</sup>]. In the Abrahamson study [11<sup>•</sup>], more infants in the *L. reuteri* group were reported having spitting-ups at 1 and 2 months than in the placebo group (1 month: 26 vs. 14%,  $P=0.04$ ; 2 months: 33 vs. 19%,  $P=0.04$ ). At the age of 12 months, however, no difference was found in the cumulative incidence of mild adverse events such as spitting-up, colic, and constipation [9<sup>••</sup>]. One must be careful when concluding that there is a causal relationship between probiotic supplementation and the reported broad range of possible side effects. Further studies are necessary to investigate the effect of different probiotic strains, especially on the reported airway symptoms.

### Data from animal models and in-vitro studies

The local effects of probiotics directly in the gut include the reduction of local inflammation [14] and of systemic antigen load by maintaining the integrity of the intestinal barrier [15]. In sucking rats exposed to cows' milk, *L. rhamnosus* GG was shown to reverse increased intestinal permeability [16]. Similarly, *L. plantarum* was shown to eradicate increased permeability in rats exposed to *Escherichia coli* [17]. Recent studies of murine models of mucosal inflammation suggest that probiotics are able to modulate the TH1/TH2 immune response. In the context of an experiment-induced Th1 T cell colitis in mice, a daily administration of probiotics resulted in a milder form of recurrent colitis [18]. In addition, probiotic administration was associated with an early increase in the production of IL-10 and an increased number of regulatory CD4<sup>+</sup> T cells bearing surface TGF- $\beta$  [18].

In line with these findings, using an animal model with Balb-c-mice [19], probiotic treatment induced the expression of transforming growth factor beta (TGF- $\beta$ ) and IL-10 in the colon. The possible beneficial effects of lactobacillus is further supported by results from in-vitro studies, which show specific species of lactobacilli prime dendritic cells driving the development of regulatory T cells in order to produce increased levels of IL-10 [20]. Moreover, these regulatory T cells were able to inhibit the proliferation of bystander T cells in an

IL-10-dependent fashion. Incidentally, there are substantial biological differences between probiotic strains [20]. In addition, further studies in humans also showed a *Bifidobacterium*-induced increase in secretion of the anti-inflammatory IL-10 by mononuclear cells [21].

In an experimental model using OVA-sensitized female C3H/HeJ mice, Kim assessed the in-vivo response of *L. acidophilus* AD031 and *Bifidobacterium lactis* AD011 [22]. These two probiotic strains were selected because they were able to induce IFN- $\gamma$  and IL-10 in an in-vitro model. The OVA-sensitized mice, which had been treated with probiotics, had significantly decreased serum levels of OVA-specific IgE, IgA, and IgG1. The IL-4 level was significantly lower and IFN- $\gamma$  and IL-10 was higher compared with placebo-treated mice in the spleen cells. However, no synergistic effect of both *L. acidophilus* and *Bifidobacterium lactis* was observed under experimental conditions [22].

Using a similar approach, Nonaka *et al.* [23<sup>•</sup>] selected the strongest IL-12-inducing strain from *L. pentosus* strain S-PT84 from 16 lactic-acid bacteria of plant origins. They were able to show that heat-killed *L. pentosus* preparations induced production of IL-12 and IL-10 *in vitro*, activated natural killer cells and modulated the T helper 1/T helper 2 balance toward a Th1-dominated state. In the OVA-induced allergy model, *L. pentosus* lowered IgE levels and splenic IL-4 production. Similar effects were observed in recently published in-vitro studies. Recent studies have confirmed the immunological effects of *Lactobacillus* GG. We investigated immunological changes in LGG supplemented mothers at birth and in neonates, and compared cytokine release and proliferative capacity of mononuclear cells in response to various stimuli with the control group [24<sup>••</sup>]. Here we were able to demonstrate that in comparison with unstimulated controls, in-vitro stimulation of mononuclear cells with LGG leads to a significant increase of IL-10 and IFN- $\gamma$  release but not IL-13 in cell supernatants. However, this phenomenon was observed in maternal as well as cord blood mononuclear cells, independently of the supplementation with LGG or placebo. This indicates that the increase of TH1 cytokines is an in-vitro effect, which is not influenced by prior LGG ingestion. Moreover, in addition to our described experiments, we stimulated mononuclear cells with other microbial products such as LPS and peptidoglycan. Here we observed a similar pattern with enhanced IL-10 and IFN- $\gamma$ , but not IL-13 release in response to these stimuli. This indicates that the observed in-vitro effects might reflect a general response towards a microbial stimulus rather than a specific response to LGG. Parallel to the clinical trial, Taylor *et al.* [25<sup>••</sup>] examined regulatory T-cell function in relation to probiotic supplementation and the subsequent development of an early allergic phenotype [25<sup>••</sup>]. They

observed that the administration of *L. reuteri* was not associated with any significant differences in the proportion of circulating CD4+CD25+CTLA4+ regulatory T-cells or in the resting expression of the forkhead transcription factor, FOXP3, which plays a critical role in the development and function of regulatory T cells. In both, the probiotic and the placebo group, children with atopic dermatitis at the age of 6 months had significantly higher allergen-induced FOXP3 expression. These results were interpreted as an activation of a regulatory pathway, which was obviously not influenced by probiotic supplementation and gut colonization.

## Conclusion

In conclusion, it has been concordantly shown that supplementation with probiotics during pregnancy and early infancy did not reduce the incidence of allergic rhinitis or asthma during childhood. Moreover, an allergen-preventive effect of various probiotics for the prevention of atopic dermatitis could not be consistently established. Therefore, probiotics cannot be generally recommended for primary prevention. Further studies should clarify if any susceptible subgroups exist and how these subgroups benefit from a specific supplementation with probiotics. All together, the concept of using probiotics for primary prevention of allergy still seems to be plausible. However, the selection of the most beneficial probiotic strain or the composition of different probiotics and/or prebiotics, the timing of supplementation, the optimal dose and method of administration still needs to be determined.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Havenaar R, Huis in 't Veld JHJ. Probiotics: a general view. In: Wood BJ, editor. Lactic acid bacteria in health and disease. Vol 1 of the series, The lactic acid bacteria. Amsterdam: Elsevier Applied Science Publishers; 1992.
- 2 Kalliomaki M, Salminen S, Arvilommi H, *et al.* Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076–1079.
- 3 Kalliomaki M, Salminen S, Poussa T, *et al.* Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003; 361:1869–1871.
- 4 Kalliomaki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first
  - 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119:1019–1021.
- 5 Kopp MV, Hennemuth I, Dietschek A, *et al.* A randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical or immunological effects of *Lactobacillus* GG supplementation. *Pediatrics* 2008; 121:e850–e856.

The only trial, which used a nearly identical study protocol to that of the Kalliomaki study. However, the effects were not reproducible. Not even a trend of a protective effect of probiotics on atopic dermatitis could be shown.

- 6 Kukkonen K, Savilahti E, Haahtela T, *et al.* Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119: 192–198.

This study included the largest population including 925 families, who completed the trial. Unfortunately, the combination of four different probiotics and prebiotics limit comparability to other trials.

- 7 Moro G, Arslanoglu S, Stahl B, *et al.* A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006; 91:814–819.
- 8 von Berg A, Koletzko S, Grühl A, *et al.*, German Infant Nutritional Intervention Study Group. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003; 111:533–540.
- 9 Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007; 119:184–191.

Using a double-blind, placebo-controlled randomized format, the investigators found that supplementation with *L. acidophilus* for the first 6 weeks did not reduce the incidence of atopic dermatitis.

- 10 Prescott SL, Witschut J, Taylor A, *et al.* Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy* 2008; 63:1481–1490.

Follow-up data of reference 2 at the age of 7 years.

- 11 Abrahamsson TR, Jakobsson T, Bottcher MF, *et al.* Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119:1174–1180.

The trial examines the use of *L. reuteri* in the prevention of allergic disease in the first 2 years of life.

- 12 Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. *J Allergy Clin Immunol* 2008; 121:116.e11–121.e11.

The conclusion of the meta-analysis is that the administration of probiotics is an effective option for the prevention of atopic diseases. However, this statement should be considered with reservation, as data of one study population were included three-fold in the analysis.

- 13 Rautava S, Kalliomäki M, Isolauri E. Probiotics during pregnancy and breastfeeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; 109:119–121.
- 14 Kelly D, Campbell JI, King TP, *et al.* Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 2004; 5:104–112.
- 15 Rosenfeldt V, Benfeldt E, Valerius NH, *et al.* Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr* 2004; 145:612–616.

- 16 Isolauri E, Majamaa H, Arvola T, *et al.* Lactobacillus casei strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 1993; 105:1643–1650.

- 17 Mangell P, Nejdfor P, Wang M, *et al.* Lactobacillus plantarum 299v inhibits Escherichia coli-induced intestinal permeability. *Dig Dis Sci* 2002; 47:511–516.

- 18 Di Giacinto C, Marinaro M, Sanchez M, *et al.* Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. *J Immunol* 2005; 174:3237–3246.

- 19 Chen CC, Louie S, Shi HN, Walker WA. Preinoculation with the probiotic Lactobacillus acidophilus early in life effectively inhibits murine Citrobacter rodentium colitis. *Pediatr Res* 2005; 58:1185–1191.

- 20 Smits HH, Engering A, van der Kleij D, *et al.* Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005; 115:1260–1267.

- 21 Lammers KM, Brigidi P, Vitali B, *et al.* Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol* 2003; 38:165–172.

- 22 Kim JY, Choi YO, Ji GE. Effect of oral probiotics (Bifidobacterium lactis AD011 and Lactobacillus acidophilus AD031) administration on ovalbumin-induced food allergy mouse model. *J Microbiol Biotechnol* 2008; 18:1393–1400.

- 23 Nonaka Y, Izumi T, Izumi F, *et al.* Antiallergic effects of Lactobacillus pentosus strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. *Int Arch Allergy Immunol* 2008; 145:249–257.

The animal studies of Kim and Nonaka offer an approach to handpick probiotics with potential health effects for humans for prospectively clinical trials. They elected certain probiotic strains based on their in vitro ability to induce regulatory cytokines (IL-10, IL12) and showed in OVA-sensitized mice decreased specific IgE and increased IgG production.

- 24 Kopp MV, Goldstein M, Dietschek A, *et al.* Lactobacillus GG has in-vitro effects on enhanced IL-10 and IFN-γ release of mononuclear cells but no in-vivo effects in supplemented mothers and their neonates. *Clin Exp Allergy* 2008; 38:602–610.

The charm of this study is that in parallel to the clinical trial, cytokine levels and proliferative response of mononuclear cells isolated from mothers and their neonates were analyzed. This study was able to confirm in-vitro effects of probiotics on enhanced IL-10 and IFN-γ production. However, these results were independently of the antedated supplementation.

- 25 Taylor AL, Hale J, Hales BJ, *et al.* FOXP3 mRNA expression at 6 months of age is higher in infants who develop atopic dermatitis, but is not affected by giving probiotics from birth. *Pediatr Allergy Immunol* 2007; 18:10–19.

Again, the charm of this study is that in parallel to the clinical trial potential immunological effects were analyzed. They showed that *L. reuteri* was not associated with any significant differences neither in the proportion of circulating regulatory T-cells nor in the expression of FOXP3.