The effects of a synbiotic in infants with atopic dermatitis
van der Aa, L.B.

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INTRODUCTION

Atopic dermatitis (AD) is a highly prevalent, chronic, itching skin disease that often presents in infancy and greatly affects the quality of life of children and their families (1). Moreover, AD can be the starting point of the so-called allergic march, with subsequent development of other allergic diseases; it has been shown that children with AD have a 40% chance to develop asthma (2). Currently, topical corticosteroids are the mainstay treatment of AD. However, relapses are common and parents often fear possible side effects, leading to non-compliance (3). Therefore, innovative treatment strategies are now the foci of interest.

A promising option is modulation of the intestinal microbiota. It is hypothesized that the intestinal microbiota plays an important role in the development of allergic diseases such as AD (4-7). Differences in intestinal microbiota composition have been shown between atopic and non-atopic children and these differences precede the onset of atopy or clinical symptoms, suggesting a potential causal relationship (8-10). Moreover, the intestinal microbiota interacts with the gut-associated lymphoid tissue (GALT) and seems to be involved in oral tolerance induction, as germ-free mice do not develop oral tolerance after ingestion of ovalbumin (11).

The intestinal microbiota can be modulated with probiotics, prebiotics or a combination of both, i.e. synbiotics. Probiotics are living micro-organisms with immunomodulatory effects. Prebiotics are nondigestible food ingredients (mostly oligosaccharides) that stimulate the growth of certain probiotic bacteria in the colon. Theoretically, optimal synergistic combinations of pro- and prebiotics, so called synbiotics, are most promising for treating AD. In this thesis we have presented the first double-blind placebo-controlled trial that investigates the therapeutic effect of a synbiotic on infant AD.

Summary

In Chapter 1 the theoretical background of the use of probiotics, prebiotics and synbiotics for prevention and treatment of AD is reviewed. Also, an overview of all randomized controlled trials investigating the effect of pro-, pre- or synbiotics on prevention or treatment of AD in children is presented. A total of 7 prevention studies (5 with probiotics, 1 with prebiotics and 1 with synbiotics) and 11 treatment studies (10 with probiotics and 1 with pre- and synbiotics) are reviewed. The results of these studies are inconsistent: some studies show a positive effect on prevention or treatment of AD, others only show a positive effect on prevention or treatment of IgE-associated AD and others don’t find any effect at all. These contradictory results can possibly be explained by the differences in probiotic strains and dosages that were used in these studies or by differences in study population (e.g. age and eczema severity) and study design.

Theoretically, optimal synbiotic preparations are better candidates for AD prevention or treatment than either pro- or prebiotics alone. A large human prevention trial showed that synbiotics significantly reduce the incidence of eczema. The only therapeutic trial with synbiotics was performed in older children, while manipulating the intestinal microbiota probably has more effect in early infancy, when immune programming is initiated.

The aim of this thesis was to investigate the clinical, microbiological and immunological effects of a specific synbiotic, a combination of the probiotic strain Bifidobacterium breve M-16V and a prebiotic mixture of 90% short chain galactooligosaccharides and 10% long chain fructooligosaccharides (Imunofortis®) in infants with atopic dermatitis.

In Chapter 2 we present the results of a randomized, double-blind, placebo-controlled multicenter trial that we performed to investigate the therapeutic effect of an infant formula with an added synbiotic, consisting of Bifidobacterium breve M-16V and a 90% short chain galactooligosaccharides (scGOS) and 10% long chain fructooligosaccharides (lcFOS) mixture (Imunofortis®) on AD in infants. Ninety infants, younger than 7 months and formula fed at time of inclusion, received an extensively hydrolyzed formula with or without (placebo group) synbiotics during 12 weeks. In this period, infants visited the hospital every 4 weeks for evaluation of eczema severity with the SCORAD index. At baseline and after 12 weeks blood and fecal samples were obtained. We did not find a difference in AD severity between the synbiotic and the placebo group at any time point during the study. Also, there were no differences between the two groups in topical corticosteroid usage, total and specific serum IgE concentrations and eosinophil count. The synbiotic group had significantly higher percentages of bifidobacteria and significantly lower percentages of clostridia and E. rectale, potential pathogenic bacteria, in their feces after 12 weeks of intervention than the placebo group. Also, infants in the synbiotic group had less parent-reported constipation and diaper dermatitis. Subgroup analysis showed a modest, but statistically significant positive effect of synbiotics in infants with IgE-associated AD, which has to be confirmed in a future randomized controlled trial, adequately powered to address this group of infants.

In animal models it has been shown that B. breve M-16V suppresses Th2 immune responses (12). In Chapter 3 we investigated if the synbiotic mixture had such a suppressive immune effect in the infants with AD participating in our randomized trial. Blood samples were obtained at baseline and at week 12, the end of intervention, for determination of plasma IL-5, IgG1 and IgG4 concentrations, chemokine (CTACK and TARC) concentrations, ex vivo PBMC cytokine responses (IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL17, TGF-β and IFN-γ) and regulatory T cell percentages. In line with our clinical observations, we did not find any differences in plasma IL-5, IgG1, IgG4, CTACK, and TARC concentrations, ex vivo cytokine production (except for decreased IL-12 response in the synbiotic group after stimulation with egg and peanut allergen, but not with antiCD3/antiCD28 and cow’s milk allergen), and regulatory T cell percentage between the synbiotic and the placebo group. Also, in the subgroup with IgE-associated AD there were no immunological differences between the two groups. Possibly, synbiotics only modulate the local, intestinal immunological milieu. It could also be that systemic immunological effects are transient, affect other immunomarkers than the ones we investigated, or that these effects are only evident after a “hit” with an allergen. In conclusion, we could not demonstrate any beneficial systemic immunological effect of this specific synbiotic.
In Chapter 4 we studied the immunological differences between infants with IgE-associated AD and infants with non-IgE-associated AD. The infants that participated in our synbiotic trial were divided into an IgE-associated (defined as an elevated total or specific IgE at baseline) group and a non-IgE-associated group. Baseline plasma eosinophilic granulocyte count, IL-5, IgG1, IgG4, CTACK and TARC concentrations, ex vivo PBMC cytokine responses, and regulatory T cell percentages were compared between these two groups. We demonstrated that infants with IgE-associated AD display a different, much more Th2-dominated immunological profile, with high IL-4, IL-5 and IL-13 responses and high concentrations of eosinophils, IgG1 and CTACK, than infants with non-IgE-associated AD, who show a profile with high IL-12 responses. Since infants with IgE-associated AD demonstrate a more pronounced Th2-profile, it seems conceivable that these children have more severe and persistent eczema and a higher risk for developing other allergic (Th2-related) diseases than infants with non-IgE-associated AD, as was suggested by previous studies (14-16). If this is indeed true, it would be sensible to test for allergic sensitization in all infants with AD to identify infants with IgE-associated AD, in order to provide better, tailor-made advice regarding treatment and prognosis.

Infants with AD have an increased risk of 40% to develop asthma (2). Early intervention with synbiotics might prevent subsequent development of asthma in these children. In Chapter 5 we explored if the prevalence of asthma-like symptoms and asthma medication use at one-year follow-up differed between those infants with AD that had received synbiotics and those that had received placebo. Information on airway symptoms and medication use was obtained with a questionnaire. In the 75 children that returned for follow-up, we found that the prevalence of frequent wheezing (≥3 episodes after the intervention period) and wheezing and/or noisy breathing apart from colds was significantly lower in the synbiotic than in the placebo group (13.9% vs. 34.2% and 2.8% vs. 30.8% respectively). Also, the percentage of children that had started to use asthma medication after the intervention period was significantly lower in the syndbiotic than in the placebo group (5.6% vs. 25.6%). These results suggest that this synbiotic can prevent asthma-like symptoms, and possibly also the subsequent development of asthma, in infants with AD. The children will be followed up to age 5, when they are old enough to perform lung function tests and bronchial hyperresponsiveness measurements, in order to confirm or reject the diagnosis of asthma.

In Chapter 6 we respond to the paper ‘The impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization’ of Huurre and colleagues, published in Clinical & Experimental Allergy (17). The authors reported that long-term breastfeeding (> 6 months) by allergic mothers increased the risk of infant sensitization. However, in our opinion this finding could be influenced by reverse causation: the presence of allergic symptoms in their infants could cause mothers to continue breastfeeding for a longer period of time, which would also explain the observed relation between breastfeeding and increased sensitization. Also, the authors gave considerable attention to their finding that TGF-β2 concentration was somewhat higher in breast milk of the probiotic group than that of the placebo group. This was only true for the first breast milk sample and not for the second sample that was obtained after one month. Moreover, the difference in breast milk TGF-β2 concentration between the probiotic and the placebo group was not statistically significant. Therefore, we disagree with the authors that maternal probiotic supplementation results in an increased TGF-β2 concentration in breast milk.

In Chapter 7 an overview is presented of the current European feeding guidelines for prevention of allergic disease. This overview is part of the EuroPrevall study, a multi centre research project that focuses on the prevalence and causes of food allergy across different countries in Europe. The department of Pediatric Respiratory Medicine and Allergy of the Emma Children's Hospital AMC participates in this project. In this chapter, the guidelines concerning breastfeeding, cow’s milk formula, introduction of complimentary foods and use of infant formulas with added probiotics or prebiotics are described. Most of these guidelines were found to differ between countries and to lack support by definitive scientific evidence.

General discussion

In this thesis, we have investigated for the first time the clinical, microbiological and immunological effects of a specific synbiotic, consisting of Bifidobacterium breve M-16V and a specific prebiotic scGOS/lcFOS mixture (Immunofortis®), in infants with atopic dermatitis (AD). Up to now, most studies in children with AD focussed on probiotics, with conflicting results. In most of these trials lactobacilli were used, while it can be argued that bifidobacteria are better candidates for AD treatment, since low bifidobacteria levels appear to be associated with AD (7). Moreover, it has been shown that Bifidobacterium breve M-16V reduces allergic symptoms in mice more effectively than lactobacilli (18). Since this specific strain has also been shown to reduce AD severity in children in a small study (19), we chose to use it for our randomized trial. We hypothesized that adding a prebiotic oligosaccharide mixture would increase survival of B. breve M-16V in the gut and would also stimulate other probiotic bacteria already present in the infant gut, which could generate extra immunomodulatory effects. An animal study showed that this synbiotic combination reduces the clinical allergic response in a cow’s milk allergy model more effectively than either the pre- or the probiotic compound alone (20). However, in our clinical trial we found no effect of this synbiotic on the severity of AD in infants. In general, there are several reasons for this negative result/outcome of our clinical trial. First, intake or dosage of the study product could have been inadequate because of non-compliance. In our trial, synbiotics were already added to the powder milk, which was supplied to the parents free of charge and could be prepared as a usual infant formula. This indicates that the chance of non-compliance was small. In addition, we found significant differences in microbiota composition between the synbiotic and the placebo group, which also indicates adequate synbiotic intake. The fecal detection rate of the specific probiotic strain that was part of the synbiotic mixture (B. breve M-16V) was relatively low. This could have been caused by the detection method, it is also possible that the M-16V strain did not adequately survive passage of the upper gastrointestinal tract in all...
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infants. Possibly, a higher dosage would increase the number of surviving bacteria and the clinical effect. Although a treatment period of 12 weeks seems adequate and even relatively long compared to other AD treatment studies with probiotics, one could argue that this period might have been too short to elicit a clinical effect.

Second, a positive effect could have been obscured by the simultaneous improvement of the placebo group. In most cases infant AD gradually improves during the first year, which can cause a significant improvement of the placebo group, as was seen in our and many other trials (21-24). Even if the natural course of the disease indeed masked a synbiotic effect, this would not change the conclusion that synbiotics are of no additional value in the treatment of AD. Besides the natural disease course, the use of topical corticosteroids and of an extensively hydrolyzed formula could also have concealed a beneficial synbiotic effect. However, corticosteroid use was similar in the synbiotic and the placebo group and the extensive hydrolysate would only have a beneficial effect in those infants in whom AD was caused by cow’s milk allergy. The number of participants with cow’s milk allergy is likely to have been small (infants did not have other symptoms besides eczema and most infants did not have elevated specific IgE against cow’s milk) and equally divided over both groups due to randomization. Therefore, these factors probably had no substantial influence on our results.

Finally, the most likely cause for the negative result of the trial is that the tested product indeed had no effect on AD severity. Although in vitro and animal studies show that probiotics can stimulate production of immunosuppressive cytokines, particularly IL-10, and induce development of regulatory T cells (25), which seem promising for treatment of AD and other allergic diseases, immunological results of clinical trials in children with AD are inconsistent (26,27). We also could not demonstrate any beneficial systemic immunological effects of the synbiotic mixture that we used. Apparently it is difficult to create a systemic immunotolerant milieu with probiotics in humans, which may explain the lack of clinical effect seen in our and many other trials.

Several studies indicate that probiotic administration has to be accompanied by allergen exposure in order to elicit an allergen-specific immunosuppressive effect, and it has also been suggested that probiotic administration during the first “hit” with an allergen is essential for adequate immune modulation (28-31). One can hypothesize that the effect of pro- or synbiotics on already established allergic disease is limited and that the window of opportunity to manipulate the immune system is before the onset of allergic diseases. This hypothesis is supported by the decrease in AD incidence that is seen in some of the prevention studies with pro- and synbiotics (32-34). Also, it could explain why, while we did not find an effect on AD severity, we did show a very interesting beneficial effect on prevalence of asthma-like symptoms and asthma medication use in the synbiotic group at one-year follow-up. One could speculate that the administration of synbiotics in early infancy concurred with first aeroallergen encounters in our study participants, which prevented them from developing asthma-like symptoms.

Although we did not find an effect on AD severity in our total study population, subgroup analysis showed a significant beneficial effect of synbiotics in IgE-associated AD, this is in agreement with several trials studying the effect of probiotics on AD (22,26,35). Subgroup analyses in randomized clinical trials must be viewed cautiously and should be seen as hypothesis-generating. The new hypothesis would be that pro- and synbiotics are only effective in children with IgE-associated AD and not in children with non-IgE-associated AD. We showed that infants with IgE-associated AD display a significant Th2-dominated immunological profile compared to infants with non-IgE-associated AD. This immunological profile could explain the tendency of infants with IgE-associated AD to exhibit more persistent AD and a higher chance of developing other allergic diseases, which was shown in earlier studies (14-16). Since the underlying mechanism of pro- or synbiotic effects on allergic diseases is considered to be immuno-suppression of Th2-responses, it seems conceivable that pro- or synbiotic effects would be limited to the children that display those responses, i.e. the children with IgE-associated AD. Unfortunately, we did not demonstrate any immunological effects of the synbiotic mixture in the IgE-associated subgroup and therefore cannot confirm this hypothesis.

Conclusions and future perspectives

The synbiotic combination of B. breve M-16V and the prebiotic mixture of 90% short chain galactooligosaccharides and 10% long chain fructooligosaccharides (Immunofortis®) does not reduce eczema severity in infants with AD, although it does successfully modulate the intestinal microbiota composition of these infants. Also, it does not induce systemic immunological changes in cytokine pattern or number of regulatory T cells. However, infants with AD that had received synbiotics showed a lower prevalence of asthma-like symptoms and asthma medication use at one-year follow-up than infants that had received placebo, indicating that this synbiotic mixture might prevent asthma-like symptoms or even asthma. The children will be followed up to age 5, when they are old enough to perform lung function tests and bronchial hyperresponsiveness measurements, in order to confirm or reject the diagnosis of asthma. Also, subgroup analysis suggested that this mixture could have a beneficial effect on AD severity in infants with IgE-associated AD. These results have to be confirmed in future randomized controlled trials with the same synbiotic, adequately powered to address these new hypotheses.

In conclusion, our results do not support the use of an infant formula with this specific synbiotic for AD treatment. Our results on prevention of asthma-like symptoms have to be confirmed before this infant formula can be advised for that purpose in clinical practice. Previous clinical trials on treatment or prevention of allergic diseases with probiotics, prebiotics and synbiotics have shown inconclusive results and systematic reviews conclude that, at this moment, there is not enough evidence to support adding these agents to infant formulas in order to treat or prevent allergic diseases (36-38). More clinical trials are needed in this area. Trials with promising results need to be repeated (same probiotic strain, similar study population) and study populations have to be followed in order to evaluate long term effects. In this way, the efficacy of probiotics, prebiotics and synbiotics in allergy treatment or prevention can be elucidated and their possible place in future feeding guidelines for infants with or at risk for allergic disease can be determined.
Reference List


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