The effects of a synbiotic in infants with atopic dermatitis
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Citation for published version (APA):
van der Aa, L. B. (2010). The effects of a synbiotic in infants with atopic dermatitis
Chapter 1

General introduction and Outline of the thesis
Introduction
Atopic dermatitis (AD) is a chronic, itching, inflammatory skin disease that often presents in infancy (1). The prevalence of AD has risen over the past decades, especially in western societies where it varies in primary school children between 5% and 20% (2). The disease is caused by a combination of genetic and environmental factors. Severe AD in children is often associated with food allergy (3;4). The majority of patients have elevated serum IgE levels and peripheral eosinophilia, although in up to 57% of infants with AD, IgE-sensitization can not (yet) be detected (5).
The disease has a significant impact on children and parents, mainly due to itching, scratching and sleep disruption (6). The prognosis is reasonable with a recovery rate of 40% at age two (7) and 65% in adolescence (8). However, AD can be the starting point of the 'allergic march'; the natural progression of allergic disorders such as asthma and allergic rhinitis. Children with AD have a chance of approximately 40% to develop asthma (7).
Besides avoiding irritants and moisturizing the skin with emollients, local anti-inflammatory treatment with topical corticosteroids is the mainstay treatment for infants with AD. In children with a proven food allergy this is combined with avoidance of the specific allergen. However, flares occur despite treatment and, although rare, topical corticosteroids can have local side effects, such as skin atrophy and telangiectasia, and possibly also systemic side effects, such as growth retardation or osteoporosis. Parents often fear these side effects and this may lead to non-compliance (9).
Innovative prevention and treatment strategies for AD, aiming to manipulate the intestinal flora with pro-, pre- or synbiotics are now focus of interest. This review provides an overview of the theoretical basis for using probiotics and prebiotics in AD and presents the current evidence from randomized controlled trials regarding prevention and treatment of AD in children with pro-, pre- and synbiotics.

Hygiene hypothesis
Several hypotheses have been proposed to explain the rise in allergic disease, including atopic dermatitis. The hygiene hypothesis (10) has gained the most attention. This hypothesis was based on the finding that the prevalence of allergic rhinitis and eczema is inversely related to the number of older children in a household, which lead to the general hypothesis that early childhood infections, caused for example by unhygienic contact with older siblings, could prevent the development of allergic disease (10;11). Later, the T helper 1/ T helper 2 lymphocyte paradigm was added to the theory: It was argued that a lack of early childhood infections results in a decreased Th1 response, which disturbs the Th1/Th2 balance and leads to an abundant Th2 response, causing allergic diseases (12).
However, several facts are not consistent with the hygiene hypothesis as it was first proposed. Although the protective effect of multiple siblings, as well as other "unhygienic" circumstances like exposure to farming or day care (13;14), has been a consistent finding, a number of cross-sectional and prospective studies showed no protective effect of childhood infections (15;16). Moreover, Th2-dominated helminthic infections are not associated with allergy (17) and the coinciding increase in the prevalence of Th1-mediated autoimmune diseases in the Western world does not concur with the Th1/Th2 paradigm.
These inconsistencies have lead to the proposal of a revised hygiene hypothesis, which considers changes in the intestinal colonisation pattern during infancy, also caused by an overly hygienic lifestyle, as an important reason for the increased allergy prevalence and proposes a lack of activity of regulatory T cells (Tregs), causing overactive Th2 as well as Th1 responses, as the underlying mechanism (18-21).

The intestinal microbiota
The human intestines are inhabited by at least 400 different bacterial species, with the greatest density in the large intestine, where concentrations of $10^1$-$10^2$ cells/g of luminal contents can be found (22). Approximately 55% of faecal mass consists of bacteria (23). After birth, bacteria originating from the mother and the environment start colonizing the infant gut. Factors influencing the colonization pattern are mode of delivery (vaginal delivery versus caesarean section), prematurity, hospitalization or antibiotic use after birth, type of feeding (breastfeeding versus formula feeding) and exposure to older siblings (24).
Although some intestinal bacteria are potential pathogens, the relation between the intestinal microbiota and the human host is mostly symbiotic. The microbiota has several important nutritional functions, such as degradation of indigestible dietary carbohydrates, production of short chain fatty acids (SCFA), amino acid synthesis and vitamin synthesis (25). In addition, the intestinal flora appears to be crucial for the development of the mucosal and systemic immune system.

The intestinal microbiota and the immune system
The largest mass of lymphoid tissue of the body is found in the gastrointestinal tract and is called the gut-associated lymphoid tissue (GALT). The GALT interacts with intestinal bacteria, which are sampled by dendritic cells and intestinal epithelial cells, through two classes of pattern recognition receptors, Toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD) molecules (26;27).
The intestinal microbiota appears to be important for the development of the GALT. Studies have shown that mice without microbiota, i.e. germ-free mice, have an underdeveloped GALT with low numbers of αβ- intestinal intraepithelial lymphocytes (28), hypoplastic Peyrer’s patches containing few germinal centres and greatly reduced numbers of IgA-producing plasma cells and lamina propria CD4+ T cells (29).
Furthermore, the intestinal microbiota also seems to be involved in oral tolerance induction. Oral tolerance is the establishment of peripheral tolerance to a specific antigen after ingestion of that antigen. A pivotal role in the induction and maintenance of peripheral tolerance is played by Tregs. The importance of the intestinal microbiota in tolerance induction was demonstrated by Sudo and coworkers, who showed that germ-free mice do not develop oral tolerance after ingestion of ovalbumin and maintain a Th2 response with IgE production (30). Inoculation with
**Chapter 16**

The term or synbiotics. This can be done with probiotics, prebiotics, and even prevented by manipulating the microbiota. This can be done with probiotics, prebiotics, or probiotics. Prebiotics are nondigestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of a limited number of bacterial species in the colon (63). A food ingredient must fulfil three criteria to be considered a prebiotic: it should not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract, it has to be a selective substrate for beneficial commensal bacteria in the colon, for example bifidobacteria, and it must be able to alter the intestinal microbiota towards a healthier composition (64). Breast milk contains natural prebiotics, human milk oligosaccharides, which could explain the bifidobacteria-dominated microbiota seen in breast fed infants (65). In formula fed infants a similar effect can be realized with a prebiotic mixture of 90% galacto-oligosaccharides (GOS) and 10% fructo-oligosaccharides (FOS), which stimulates the growth of bifidobacteria (66;67) and, to a lesser extent, lactobacilli (68). Prebiotics are generally considered to be safe, as they are naturally present in several kinds of food. The main side effects of over-consumption in humans are flatulence, bloating and diarrhoea (69). Increased intestinal tumour formation in mice fed inulin has been reported (70), but on the opposite, reduction of colon tumours in mice, has also been described (71).

**Immunomodulatory effects of prebiotics**

Several animal studies demonstrated effects of prebiotics on the immune system. Elevation of the number of bifidobacteria. Recently, a Dutch multicenter trial reported an increased mortality risk in adults with severe acute pancreatitis who received probiotic prophylaxis (44). However, in children with AD no adverse events are reported.

**Immunomodulatory effects of probiotics**

Many different effects of probiotics have been described in animal, human and in vitro studies, most of which are strain-specific. In general, effects include stabilizing of intestinal barrier function (45;46), stimulation of intestinal IgA-production (47) and, most importantly, modulation of cytokine production. In vitro studies show that probiotics are able to down-regulate Th2 cytokine production by stimulation of Th1 cytokines, such as IL-12 and IFN-γ (48) or regulatory cytokines, such as IL-10. The latter is accomplished by either increasing production of these cytokines by antigen presenting cells or by driving the development of IL-10 or TGF-β producing regulatory T cells (49-51).

However, ex vivo immunomodulatory effects of probiotics in children with AD are inconclusive (table 1). There are studies that show up-regulation of IL-10 (52-54) or IFN-γ (55;56), although often without accompanying down-regulation of Th2 cytokines, but several studies do not show any effect on cytokine production (57-62). Also, one study showed down regulation of the Th2 cytokine IL-5, combined with down-regulation of IL-10 and TGF-β instead of the expected up-regulation of these cytokines (59). These conflicting outcomes could be caused by the use of different probiotic strains and different ways of stimulating cytokine production.

**Prebiotics**

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**Immunomodulatory effects of prebiotics**

Several animal studies demonstrated effects of prebiotics on the immune system. Elevation of the
Table 1. Effects of probiotics on cytokine responses in children with AD

<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic</th>
<th>Dose (cfu)</th>
<th>Number of subjects</th>
<th>Age at inclusion (mean)</th>
<th>Treatment period</th>
<th>Cytokines</th>
<th>Immunomodulatory effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Majamaa 1997</td>
<td>LGG ATCC 53103</td>
<td>5 x 10⁹ cfu/g formula</td>
<td>27 (AD and cow's milk allergy)</td>
<td>2.5-15.7 months (range)</td>
<td>4 weeks</td>
<td>PBMCs (stimulus conA and cow's milk protein): no difference in IL-4, IFN-γ and TNF-α between the probiotic and the placebo group</td>
<td>No effect</td>
</tr>
<tr>
<td>Pessi 2000</td>
<td>LGG ATCC 53103</td>
<td>2 x 10⁸</td>
<td>9</td>
<td>21 months</td>
<td>4 weeks</td>
<td>Serum: IL-10 at 8 weeks, no effect on IL-6, IL-12, IFN-γ and TNF-α</td>
<td>Up-regulation regulatory cytokine, down regulation Th1 and Th2 cytokines (no placebo group)</td>
</tr>
<tr>
<td>Rosenfeldt 2003</td>
<td>L. rhamnosus</td>
<td>2 x 10⁹</td>
<td>43</td>
<td>5.2 years</td>
<td>6 weeks</td>
<td>PBMCs (stimulus LPS-PHA): no significant changes in IL-2, IL-4, IL-10 and IFN-γ</td>
<td>No effect</td>
</tr>
<tr>
<td>Pohjrerui 2004</td>
<td>LGG ATCC 53103 or Mixture a</td>
<td>2 x 10⁹</td>
<td>62, of which 38 IgE-associated AD</td>
<td>1.4-11.5 months (range)</td>
<td>4 weeks</td>
<td>PBMCs (stimulus: anti-CD3/anti-CD28): IFN-γ in LGG group only in IgE-associated AD. No effect on IL-4, IL-5 and IL-12 and in mix group</td>
<td>Up-regulation Th1 cytokine</td>
</tr>
<tr>
<td>Viljanen 2005</td>
<td>LGG ATCC 53103 or Mixture a</td>
<td>2 x 10⁹</td>
<td>121, of which 69 IgE-associated AD</td>
<td>1.4-11.5 months (range)</td>
<td>4 weeks</td>
<td>Plasma: IL-10 in mix group, IL-6 in LGG group only in IgE-associated AD. No effect on IL-4, TGF-β and IFN-γ</td>
<td>Up-regulation regulatory cytokine and proinflammatory cytokine</td>
</tr>
<tr>
<td>Prescott 2005</td>
<td>L. fermentum VRI-003 PCC</td>
<td>2 x 10⁹</td>
<td>53</td>
<td>10.9 months</td>
<td>8 weeks</td>
<td>PBMCs stimulated with PHA and SEB: IFN-γ response, stimulated with heat-killed bacteria: TNF-α response and with ovalbumin: IL-13 response in probiotic group</td>
<td>Up-regulation pro-inflammatory cytokines and down regulation TH2 cytokine</td>
</tr>
<tr>
<td>Brouwer 2006</td>
<td>L. rhamnosus or LGG</td>
<td>3 x 10⁹ (cfu/g powder)</td>
<td>23</td>
<td>5.2 months</td>
<td>12 weeks</td>
<td>PBMCs (stimulatus conA and anti-CD3/anti-CD28): no effect on IL-4, IL-5 and IFN-γ</td>
<td>No effect</td>
</tr>
<tr>
<td>Taylor 2006</td>
<td>L. acidophilus LAVRI-A1</td>
<td>3 x 10⁹</td>
<td>118 infants with allergic mother</td>
<td>Prevention study: Infants: 6 months</td>
<td></td>
<td>PBMCs stimulated with SEB: IL-5 and TGF-β, tetanus toxoid: IL-10, and house dust mite: frequent IL-10 and TNF-α responses in probiotic group</td>
<td>Downregulation of Th2, regulatory and proinflammatory cytokines No increase in thymus derived regulatory T cells</td>
</tr>
<tr>
<td>Flinterman 2007</td>
<td>Mixture a</td>
<td>1 x 10⁹</td>
<td>13 (AD and cow’s milk allergy)</td>
<td>1.7 years</td>
<td>3 months</td>
<td>PBMCs unstimulated and stimulated with peanut extract or anti-CD3: IL-10 and IL-6 and TNF-α in probiotic group, but non-significant compared to placebo (p=0.063)</td>
<td>Non-significant downregulation of proinflammatory and immunosuppressive cytokines</td>
</tr>
<tr>
<td>Kopp 2007</td>
<td>LGG ATCC 53103</td>
<td>5 x 10⁹</td>
<td>68 infants with atopic family history</td>
<td>Prevention study: Mothers: 4-6 weeks before delivery Infants: 6 months</td>
<td></td>
<td>CBMCs and PBMCs stimulated with PHA, LGG or b-lactoglobulin: no difference in IL-10, IL-13 and IFN-γ between the 2 groups</td>
<td>No effect</td>
</tr>
<tr>
<td>Marschán 2008</td>
<td>Mixture a</td>
<td></td>
<td>98 infants with atopic family history</td>
<td>Prevention study: Mothers: 2-4 weeks before delivery Infants: 6 months</td>
<td></td>
<td>Plasma: IL-10 in synbiotic group IL-2, IL-4, IL-6, IFN-γ and TNF-α values below detection limit in both groups</td>
<td>Up-regulation of immunosuppressive cytokine</td>
</tr>
</tbody>
</table>

*Lactobacillus rhamnosus GG, Lactobacillus, LGG ATCC 53103 5 x 10⁸ cfu, L. rhamnosus LC705 5 x 10⁸ cfu, Bifidobacterium breve Bbi99 2 x 10⁹ cfu, Propionibacterium freudenreichii sp shermanii JS 2 x 10⁹ cfu, L. acidophilus, L. casei, Lactococcus lactis, B. infantis, B. lactis and B. longum, in case of breast feeding mothers took the probiotics, peripheral blood mononuclear cells, phytohemagglutinin mitogen, Staphylococcus antigen, cord blood mononuclear cells.
total cell number in Peyer’s patches (72), increased natural killer cells and peritoneal macrophage phagocytic activity (73), increased production of IgA, IL-10 (74), IFN-γ (75) and TGF-β (76) have all been described. A human study in which FOS was given to 10 patients with Crohn’s disease reported an increase in the number of IL-10 positive dendritic cells (77).

There are at least two possible underlying mechanisms that can elicit the immunological effects of prebiotics. First, most prebiotics stimulate the growth and/or activity of lactic acid bacteria, such as bifidobacteria or lactobacilli, which have immunomodulatory qualities as described above. Second, fermentation of prebiotics by lactic acid bacteria enhances SCFA and lactate production (63). SCFAs primarily act as energy substrate for colonocytes and several other cells in the human body (78). The three main SCFAs are acetate, propionate and butyrate. In vitro, acetate and propionate are significantly increased after prebiotic fermentation (79). It has been shown that these two SCFAs stimulate IFN-γ and IL-10 production (80). Receptors for SCFAs have been identified on leukocytes (81), which could explain their immunological effect but more studies are needed to elucidate this pathway.

Probiotics and prevention of atopic dermatitis

To identify all randomized controlled trials (RCTs) regarding prevention or treatment of AD in children with probiotics, prebiotics and combinations of both, i.e. synbiotics, the databases of Pubmed, Embase and Cochrane up to February 2008 were searched. The following keywords were used: (probiotics OR prebiotics OR synbiotics) AND (atopic dermatitis OR atopic eczema OR atopic eczema OR food allergy). Only randomized controlled trials considering children were included in this review. Reference lists of the found articles were checked for additional randomized studies.

Probiotics have investigated the effect of probiotics on the prevention of AD in infants. In five trials the preventive effect on the development of AD in infants was investigated. In these trials probiotics (different Lactobacillus species and one Bifidobacterium strain) were given to infants with a high risk of developing allergy, starting immediately after birth. In four of these trials mothers also received probiotics during the last weeks of pregnancy.

The first study reported a 50% reduction of the incidence of AD in the probiotic group compared to the placebo group at the age of 2 years (82). This effect was still evident at the age of 4 and 7 years (83;84). No effect on the incidence of other allergic diseases or sensitization was found. In the study of Abrahamsson and co-workers no reduction of the incidence of AD was found, but subgroup analysis revealed a significant reduction in IgE-associated AD (85). Again, no effect on the incidence of other allergic diseases or sensitization was found. Recently, Wickens et al showed that Lactobacillus rhamnosus, but not Bifidobacterium animalis, significantly reduced the incidence of AD with almost 50% compared to placebo, without any effect on sensitization (86).

In contrast, two prevention studies did not show a reduced AD incidence (87;88). Moreover, one of these studies found an increased sensitization rate for at least one out of ten food- or aeroallergens and an increased rate of skin prick test positive (food and/or inhalant allergens)AD in the probiotic group (87).

There are several possible explanations for these inconclusive results. First, different Lactobacillus species were used. Probiotic effects are strain specific, therefore not all probiotic strains might be good candidates for AD prevention. Second, probiotic effects are probably dose-dependent and there was considerable variation in the daily dosage that was given in these studies. A third explanation could be differences in study design, such as number of participants, atopic risk of participants, maternal supplementation during pregnancy, supplementation directly to infants or via breastfeeding and the supplementation period.

Probiotics and treatment of atopic dermatitis

RCTs exploring the role of probiotics, mainly lactobacilli, in the treatment of AD in children also have conflicting results (table 3). Four studies had positive outcomes. The first study showed a significant reduction of SCORAD (SCORing Atopic Dermatitis (89)) score in the probiotic group, but unfortunately the study had few participants and no between-group analysis was performed (62). The second study was also small and the included children had relatively mild AD. Although there was a significant reduction in SCORAD score after two months of probiotic treatment compared to placebo, this difference disappeared after 6 months of treatment, as SCORAD score was 0 by that time in all groups (90). The study of Kirjavainen et al (91) was designed to assess the effect of heat-inactivated probiotics. Although this study was terminated early due to gastrointestinal adverse events in the heat-inactivated group and therefore had few participants, it did show that SCORAD score decreased significantly more in the viable probiotic group than in the placebo group. The fourth study, of Weston et al, had more participants, with more severe AD (92). A significant reduction of SCORAD score was found in the probiotic group and not in the placebo group, but this difference was not significant in between-group analysis.

Three other studies showed no effect on AD in general, but did find a significant, although modest, positive effect on SCORAD score in children with IgE-associated AD (61;93;94). This suggests that possibly only children who at a young age already have evidence of IgE sensitization (positive skin prick test and/or elevated total or specific IgE levels) benefit from probiotics. Children in two of these studies (61;94) had a mean age at inclusion of 4 to 5 years, which was considerably older than the age at inclusion in other treatment studies. In the third study (93) children were younger but were only treated for a period of 4 weeks. These factors possibly reduced the beneficial effect of probiotics.

Finally, three studies didn’t show any effect of probiotics on the severity of AD (60;95;96). In the study of Brouwer et al (60) groups were small, since three groups were formed (two probiotic groups and one placebo group) of the 50 children that were included in total. However, the two other studies (95;96) were well-designed, with more participants, and did not show any effect on AD or on IgE-associated AD.
<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic</th>
<th>Dose</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Treatment period</th>
<th>Follow-up period</th>
<th>Level of evidence</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalliomaki 2001 (82)</td>
<td>LGG⁵</td>
<td>1x10⁹ cfu</td>
<td>RDBPCT</td>
<td>132, mother, father or sibling with atopic disease</td>
<td>Mothers: 2-4 weeks before delivery Infants: 6 months⁴</td>
<td>7 years</td>
<td>A2</td>
<td>Reduction of AD incidence at age 2 (23% vs. 46%, p 0.008), reduction still significant at age 4 and 7. No reduction other allergic diseases or sensitization.</td>
</tr>
<tr>
<td>Abrahamsson 2007 (85)</td>
<td>L.⁴ reuteri (ATCC 55730)</td>
<td>1x10⁹ cfu</td>
<td>RDBPCT</td>
<td>188, mother, father or sibling with atopic disease</td>
<td>Mothers: week 36 until delivery Infants: 12 months</td>
<td>2 years</td>
<td>A2</td>
<td>No reduction of eczema incidence (probiotics: 36%, placebo: 34%). Less IgE-associated eczema at age 2 (8% vs. 20%, p 0.02). No reduction other allergic diseases or sensitization. If allergic mother: significantly less IgE-associated AD and less sensitization at age 6-24 months.</td>
</tr>
<tr>
<td>Taylor 2007 (87)</td>
<td>L. acidophilus (LAVRI-A1)</td>
<td>3x10⁸ cfu</td>
<td>RDBPCT</td>
<td>178, atopic mother</td>
<td>Infants: 6 months</td>
<td>1 year</td>
<td>A2</td>
<td>No prevention of AD (probiotics: 26%, placebo: 23%) Probiotic group: higher sensitization rate (40% vs.24%, p0.03) and more IgE-associated AD (26% vs.14%, p0.045) at age 12 months</td>
</tr>
<tr>
<td>Kopp 2008 (88)</td>
<td>LGG (ATCC 53103)</td>
<td>1x10⁹ cfu</td>
<td>RDBPCT</td>
<td>94, mother, father or sibling with atopic disease</td>
<td>Mothers: 4-6 weeks before delivery Infants: 6 months⁴</td>
<td>2 years</td>
<td>A2</td>
<td>No prevention of AD (probiotics: 28%, placebo: 27%). No reduction in sensitization to inhalant allergens (food allergens were not determined)</td>
</tr>
<tr>
<td>Wickens 2008 (83)</td>
<td>L. rhamnosus (HN001) or Bifidobacterium animalis subsp lactis (HN 019)</td>
<td>6x10⁹ cfu 9x10⁹ cfu</td>
<td>RDBPCT</td>
<td>474, parental atopic disease</td>
<td>Mothers: week 35 until baby was 6 months (in case of BF) Infants: 2 years</td>
<td>2 years</td>
<td>A2</td>
<td>Reduction of AD (HR 0.51, 95% CI 0.30-0.85, p 0.01) and IgE-associated AD (HR 0.51, 95% CI 0.27-0.97, p 0.04) incidence in <em>Lactobacillus</em> group compared to placebo. <em>Bifidobacterium</em> group: no effect. Both groups: no effect on sensitization.</td>
</tr>
<tr>
<td>Moro 2006 (97)</td>
<td>Prebiotics</td>
<td>0.8 g/100 ml formula</td>
<td>RDBPCT</td>
<td>206, parental atopic disease</td>
<td>Infants: 6 months</td>
<td>6 months</td>
<td>B⁸</td>
<td>Reduction of AD incidence at age 6 months (10% vs.23%, p0.01), reduction still significant at age 2. Less recurrent wheezing and allergic urticaria at age 2 (7.6% vs.20.6% and 1.5% vs.10.3%, p&lt;0.05)</td>
</tr>
<tr>
<td>Kukkonen 2007 (101)</td>
<td>Mixture+ prebiotics (synbiotics)</td>
<td>c</td>
<td>RDBPCT</td>
<td>925, parental atopic disease</td>
<td>Mothers: 2-4 weeks before delivery Infants: 6 months</td>
<td>2 years</td>
<td>A2</td>
<td>Reduction of AD (OR 0.74, 95% CI 0.55-0.98, p0.035) and IgE-associated AD (OR 0.66, 95% CI 0.46-0.95, p0.025) incidence at age 2 No reduction other allergic diseases or sensitization</td>
</tr>
</tbody>
</table>

⁵*Lactobacillus rhamnosus GG, ⁴Lactobacillus reuteri, ⁶LGG 5x10⁹ cfu, ⁷L. rhamnosus LC705 3x10⁹ cfu, ⁸Rifidobacterium breve Bb09 2x10⁹ cfu, ⁹Propionibacterium JS 2x10⁹ cfu, ¹randomized, double-blind, placebo-controlled trial, ²in case of breast feeding mothers took the probiotics, ³Level of evidence according to the criteria of the Dutch Institute for Healthcare Improvement (CBO) : A2=RCT of good quality, B=RCT of less quality, reason: <80% follow-up, ⁴hazard ratio, ⁵odds ratio
<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic</th>
<th>Dose</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Age at inclusion (mean)</th>
<th>Mean SCORAD at inclusion</th>
<th>Treatment period</th>
<th>Level of evidence</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Majamaa 1997 (62)</td>
<td>LGG*(ATCC 53103)</td>
<td>5 x 10^8 cfu/g formula</td>
<td>RDBPCT^c</td>
<td>27</td>
<td>2.5-15.7 months</td>
<td>23.4</td>
<td>4 weeks</td>
<td>B^f</td>
<td>Significant reduction of SCORAD in probiotic (-11 points) and not in placebo group (-2 points). No between-group analyses performed, follow-up after 2 months: no differences between the groups.</td>
</tr>
<tr>
<td>Isolauri 2000 (90)</td>
<td>LGG (ATCC 53103) or B.lactis Bb-12^e</td>
<td>3 x 10^8 cfu/g or 1 x 10^9 cfu/g</td>
<td>RDBPCT</td>
<td>27</td>
<td>4.6 months</td>
<td>16</td>
<td>6 months</td>
<td>B^f</td>
<td>Significant reduction of SCORAD in both probiotic groups compared to placebo (p 0.02), study was prematurely terminated due to adverse gastrointestinal symptoms in the heat-inactivated LGG group.</td>
</tr>
<tr>
<td>Kirjavainen 2003 (90)</td>
<td>viable LGG (ATCC 53103) or heat-inactivated LGG</td>
<td>1 x 10^8 cfu/g formula</td>
<td>RDBPCT</td>
<td>35</td>
<td>5.5 months</td>
<td>mean: 7.5 weeks (range 0.4-45.3)</td>
<td>16</td>
<td>B^f</td>
<td>Significant reduction of SCORAD in viable LGG group compared to placebo in all groups.</td>
</tr>
<tr>
<td>Rosenveldt 2003 (61)</td>
<td>L. rhamnosus (19070-2)^c and L. reuteri (DSM 12246)</td>
<td>2dd 1 x 10^9 cfu</td>
<td>RDBPC cross-over study</td>
<td>43</td>
<td>5.2 years</td>
<td>35</td>
<td>6 weeks</td>
<td>B^f</td>
<td>Reduction of SCORAD only in IgE-associated AD (-2.4 points vs +3.2, p 0.04)</td>
</tr>
<tr>
<td>Weston 2005 (92)</td>
<td>L. fermentum (VRI-003 PCC)</td>
<td>2dd 1 x 10^10 cfu</td>
<td>RDBPCT</td>
<td>53</td>
<td>10.9 months</td>
<td>42.4</td>
<td>8 weeks</td>
<td>A2</td>
<td>Significant reduction of SCORAD in probiotic group (-18) and not in placebo group (-10) at 8 wks. No significant difference in between-group analyses.</td>
</tr>
<tr>
<td>Viljanen 2005 (93)</td>
<td>LGG (ATCC 53103) or Mixture^d</td>
<td>2dd 5 x 10^8 cfu</td>
<td>RDBPCT</td>
<td>230</td>
<td>6.4 months</td>
<td>32.6</td>
<td>4 weeks</td>
<td>A2</td>
<td>LGG: reduction of SCORAD only in IgE-associated AD (-26 vs -20, p 0.04) Mixture: no effect.</td>
</tr>
<tr>
<td>Sistek 2006 (94)</td>
<td>L. rhamnosus and B. lactis</td>
<td>2 x 10^10 cfu/g</td>
<td>RDBPCT</td>
<td>59</td>
<td>4.1 years</td>
<td>30.6</td>
<td>12 weeks</td>
<td>A2</td>
<td>Marginal effect in food sensitized children: SCORAD ratio 0.73 (95% CI 0.54-1.00, P=0.047)</td>
</tr>
<tr>
<td>Brouwer 2006 (60)</td>
<td>L. rhamnosus or LGG</td>
<td>3 x 10^8 cfu/g powder</td>
<td>RDBPCT</td>
<td>50</td>
<td>5.2 months</td>
<td>18.7</td>
<td>12 weeks</td>
<td>A2</td>
<td>No effect on SCORAD or sensitization in either probiotic group</td>
</tr>
<tr>
<td>Folster-Holst 2006 (93)</td>
<td>LGG</td>
<td>2dd 5 x 10^8 cfu</td>
<td>RDBPCT</td>
<td>47</td>
<td>18.8 months</td>
<td>42.3</td>
<td>8 weeks</td>
<td>A2</td>
<td>No effect on SCORAD in AD or in IgE-associated AD</td>
</tr>
<tr>
<td>Grüber 2007 (105)</td>
<td>LGG</td>
<td>2dd 5 x 10^9 cfu</td>
<td>RDBPCT</td>
<td>102</td>
<td>7.4 months</td>
<td>24.1</td>
<td>12 weeks</td>
<td>A2</td>
<td>No effect on SCORAD in AD or in IgE-associated AD or on sensitization</td>
</tr>
<tr>
<td>Passeron 2006 (102)</td>
<td>probiotics: L. rhamnosus (Lc35) and prebiotics (placebo group: only prebiotics)</td>
<td>3dd 1.2 x 10^10 cfu</td>
<td>RDBT</td>
<td>39</td>
<td>5.9 years</td>
<td>39.7</td>
<td>12 weeks</td>
<td>A2</td>
<td>No difference in SCORAD between the 2 groups</td>
</tr>
</tbody>
</table>

aLactobacillus rhamnosus GG, bBifidobacterium, cLactobacillus, d2dd LGG 5x10^8 cfu, eL. rhamnosus LC705 5x10^8 cfu, fB. Bb12 2x10^10 cfu, Propionibacterium freudenreichii ssp. shermanii JS 2x10^10 cfu, grandomized, double-blind, placebo-controlled trial, level of evidence according to the criteria of the Dutch Institute for Healthcare Improvement (CBO): A2=RCT of good quality, B=RCT of less quality, reason: few participants, hreason: cross-over study
Prebiotics in the prevention and treatment of atopic dermatitis

One double-blind RCT has been performed to investigate the preventive effect of prebiotics in the development of AD (97) (table 2). In this study 259 high risk infants were enrolled. They received a hydrolysed protein formula with either GOS/ FOS mixture or maltodextrine (placebo) for 6 months. The incidence of AD, diagnosed according to clinical criteria, during the study period was significantly lower in the intervention group than in the placebo group (9.8 % compared to 23.1%). At age 2, the cumulative incidence of AD was still significantly reduced in the prebiotic group, as were the cumulative incidence of recurrent wheezing and allergic urticaria (98). Faecal bifidobacteria counts were significantly higher in the intervention group. Although these results seem promising, a limitation of the study was the relatively large percentage (more than 20%) of infants that were lost to follow-up during the intervention period. Up until now there are no studies that explore the role of prebiotics in the treatment of AD.

Symbiotics

Combinations of pro- and prebiotics are called symbiotics. This term should be used to describe products in which the prebiotic compound selectively stimulates the probiotic compound, thus creating a synergetic effect (99). Theoretically, optimal symbiotic preparations can be expected to obtain better results in AD prevention or treatment than either pro- or prebiotics alone. This was confirmed by an animal study that shows that severity of AD lesions and total immunoglobulin E levels were significantly reduced in mice fed Lactobacillus casei subsp. casei together with its prebiotic, dextran, compared to placebo. This effect was also seen in mice that were fed either the probiotic or the prebiotic compound alone, but to a lesser extent than when the two compounds were given together (100).

A large human prevention trial showed that a preparation of 4 probiotic strains combined with prebiotics significantly reduced the incidence of eczema (26% versus 32%, p 0.04) and IgE-associated eczema (12% versus 18%, p 0.03) in high risk children compared to placebo (101) (table 2).

Only one clinical trial investigated the efficacy of symbiotics as treatment for AD (102) (table 3). Thirty-nine children, with a mean age of 6 years, were included and randomized to receive either symbiotics or prebiotics. A significant improvement of AD was found in both study groups, but symbiotics did not appear to be superior to prebiotics alone. However, the study design, without a placebo group, makes it impossible to draw conclusions whether symbiotics and prebiotics improve AD since the improvement in both groups may also be due to the natural course of the disease.

Moreover, the number of participants was small and they were school aged children. It needs to be considered that manipulating the intestinal microbiota probably has more effect in early infancy, when immune programming is initiated (103). To further explore the role of symbiotics in the treatment of AD, it is necessary to conduct larger, well-designed, randomized placebo-controlled trials in a young age group, preferably infants.

Aim of the thesis

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

Outline of the thesis

In Chapter 2 of this thesis we present the results of a randomized controlled multi-centre trial that we performed to evaluate the effects of an infant formula with an added symbiotic, a combination of Bifidobacterium breve M-16V and a specific mixture of 90% short chain galactooligosaccharides and 10% long chain fructooligosaccharides (Immunofortis®), on the severity of atopic dermatitis in infants. Additionally, we investigated the effect of this symbiotic on topical corticosteroid usage, serum total and specific IgE (against inhalant- and foodallergens), serum eosinophil count and on the composition and metabolic activity of the intestinal microbiota.

In Chapter 3 we investigate the immunological effects of this symbiotic on plasma levels of IL-5, IgG1, IgG4 and the AD-associated chemokines cutaneous T cell-attracting chemokine (CTACK) and thymus and activation-regulated chemokine (TARC), on ex vivo cytokine responses of peripheral mononuclear blood cells (PBMCs) to non-specific and allergen-specific stimuli, and on circulating regulatory T cell percentages in these infants.

In Chapter 4, we investigate the differences in atopic marks (eosinophilic granulocytes, IL-5, IgG1, IgG4, CTACK and TARC), ex vivo cytokine responses of PBMCs, and circulating regulatory T cell percentages between infants with IgE-associated atopic dermatitis and non IgE-associated atopic dermatitis.

In Chapter 5, we present the results of a one-year follow-up study of the infants that participated in the trial. The aim of this follow-up study was to determine the prevalence of asthma-like symptoms, use of asthma medication and sensitization against aeroallergens in the infants that had received the symbiotic and the infants that had received placebo.

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 and Experimental Allergy (104).
Chapter 7 gives an overview of the current European feeding recommendations regarding breastfeeding, cow’s milk formula, complimentary foods and probiotics/prebiotics, for allergy prevention in infants. This review was part of the EuroPrevall project, a European multicentre research project on the prevalence and causes of food allergy in different countries across Europe, in which the department of Pediatric Respiratory Medicine and Allergy of the Emma Children’s Hospital AMC participates.

In Chapter 8 the results presented in this thesis are summarized and discussed against the background of the international literature. Moreover, concluding remarks and suggestions for further research are made.

Reference List
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Chapter 30


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General introduction and Outline of the thesis


