Probiotics and Prebiotics: Immunological and Clinical Effects in Allergic Disease

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Abstract

The intestinal microbiota plays an important role in immune development and may play a role in the development of allergic disorders. Manipulation of the intestinal microbiota may therefore offer an approach to the prevention or treatment of allergic diseases. Probiotics and prebiotics, used alone or together (synbiotics), can influence the intestinal microbiota and modulate immune responses in vitro and in vivo. Clinical studies suggest a potential role for selected probiotics (alone or in combination with prebiotics) in the prevention of atopic eczema. A prenatal component of treatment appears important for beneficial effects. Effects are dependent upon the specific bacteria and characteristics of the study population. One study reported beneficial effects for prebiotics in the prevention of eczema in high-risk infants, however, further studies are required to confirm this. The use of probiotics in the treatment of allergic disease is less promising. A Cochrane meta-analysis concluded that probiotics are not effective for the treatment of atopic dermatitis. Probiotic effects in the treatment of asthma and allergic rhinitis are conflicting. Probiotics, prebiotics and synbiotics offer potential treatments for the prevention of atopic eczema; however, there is currently insufficient evidence to recommend their use in clinical practice. Studies to clarify the optimal dose, bacterial species/strains, whether there is added benefit with synbiotics, the optimal timing for intervention, and the patient populations who would benefit most from such therapies are warranted.

Introduction

There has been a rapid rise in the prevalence of allergic and autoimmune disorders in recent decades. This increase is suggested to be due in large part to reduced exposure to microbial stimuli that has come about with a Westernized lifestyle, which includes changes such as smaller family size, improved sanitation, increased use of antibiotics, sterilization of food, and
cesarean delivery. The intestinal microbiota represents the greatest microbial exposure throughout life and is the newborn infant’s first major microbial challenge. It has been shown to play an important role in immune regulation and the induction and/or maintenance of tolerance to environmental and self antigens. Manipulation of the intestinal microbiota may therefore provide one approach to the prevention or treatment of the allergic conditions.

**Importance of the Intestinal Microbiota in Immune Development**

Microbial colonization of the newborn intestine is required for normal immune development, and maintenance of a healthy intestinal microflora is important for the regulation of gut inflammatory responses and oral tolerance [for review see, 1]. An altered intestinal microbiota is associated with an increased risk of developing allergic disease. Infants with eczema have reduced numbers of bifidobacteria and increased numbers of clostridia and staphylococci, and these differences precede the development of allergic disease [2–7]. Children with atopic eczema also have altered bifidobacteria species distribution [8–11] and reduced diversity of early fecal microbiota [12], highlighting the importance of overall diversity of microbial exposure for the regulation of immune homeostasis.

Probiotics and prebiotics have been shown to modulate the composition and/or activity of the intestinal microbiota, which is in turn expected to influence immune responses. The most commonly used probiotic bacteria are bifidobacteria and lactobacilli. The prebiotics that have attracted most interest are the non-digestible oligosaccharides, particularly fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS)/transgalactosylated oligosaccharides (TOS). The beneficial bacteria that serve as targets for prebiotics are predominantly bifidobacteria and lactobacilli. Combinations of probiotics and prebiotics (synbiotics) may offer an optimal means for creating a healthy microbiota that can in turn support the maintenance of immune homeostasis.

**Immunological Effects of Probiotics and Prebiotics**

Both probiotic and commensal bacteria interact with the gut-associated lymphoid tissue via pathogen recognition receptors expressed on gastrointestinal epithelium and dendritic cells (DCs) [for review see, 1]. Interaction of bacterial components with pathogen-recognition receptors on DCs leads to upregulation of cell surface co-stimulatory molecules such as CD80 and CD86, and DC migration to lymph nodes where they activate and influence the differentiation of naïve T cells towards T-regulatory or T-helper (h) cell pathways. DCs that produce interleukin (IL)-12 can promote Th1 responses,
while production of IL-4 preferably promotes Th2 responses, and IL-10 or TGF-β promotes induction of T-regulatory cells. Therefore DCs play a key role in guiding the regulation of immune responsiveness or tolerance.

**Probiotic Effects on Immune Responses**

Specific probiotics can assist in maintaining gut immune homeostasis by enhancing epithelial barrier function, inhibiting pathogen growth, and directly modulating immune responses. As a class of bacteria, probiotics predominantly modulate DC and T-regulatory cell activity, although effects on Th1 or Th2 activity are also observed. Importantly, probiotic effects may vary for different species and even strains of the same species.

**Regulatory Responses**

Both bifidobacteria and lactobacillus species and strains can promote regulatory responses, but may do so by different mechanisms. Bifidobacteria species induce production of the regulatory cytokine IL-10 by both myeloid DCs and plasmacytoid DCs, downregulate expression of co-stimulatory molecules CD80 and CD40 on DCs, and may induce IL-10-dependent reductions in IFN-γ production [13]. In contrast, specific lactobacillus species and strains induce T-regulatory cells by generating semi-mature DCs with increased expression of co-stimulatory molecules and low production of proinflammatory cytokines, but downregulate or have no effect upon IL-10 production [13]. In another study, various lactic acid bacteria strains, particularly *Lactobacillus paracasei* NCC2461, strongly inhibited CD4+ T-cell proliferation and production of Th1 (IFN-γ) and Th2 (IL-4, IL-5) cytokines, and induced production of the regulatory cytokines IL-10 and TGF-β in a dose-dependent manner [14]. Similarly, *Lactobacillus rhamnosus* induced T-cell hyporesponsiveness to CD3/CD28 stimulation and reduced cytokine production of IL-2, IL-4 and IL-10, both in vitro and following in vivo administration [15]. In vivo studies in animal models and human clinical trials confirm these tolerogenic effects of probiotics, particularly lactobacillus species and strains. Mice treated with a probiotic mix experienced less severe colitis, and this protective effect was mediated by increased IL-10 production and increased numbers of TGF-β+ regulatory CD4+ T cells [16]. Children with atopic dermatitis treated with *L. rhamnosus* GG (LGG) had increased serum IL-10 and IL-10 production by peripheral blood mononuclear cells ex vivo [17]. Treatment of pregnant and breastfeeding mothers with LGG led to increased TGF-β2 in breast milk that corresponded with a reduced risk of eczema in their infants [18]. While these findings suggest a general ability for bifidobacteria and lactobacillus strains to induce regulatory responses, there remains variation in species/strain-specific effects and some bifidobacteria and lactobacillus strains can have inhibitory or opposing effects on regulatory function. For example, *Lactobacillus*...
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*reuteri* specifically inhibits *Lactobacillus casei*-induced DC cytokine production and upregulation of co-stimulatory markers; and *Bifidobacterium lactis* downregulates TGF-β production [19].

**T-Helper Responses**

Effects of probiotics on T-helper responses are varied and may reflect direct targeting of T-helper function or indirect effects via regulatory T cells. Effects are highly species- and strain-dependent. Some bacterial species/strains have been shown to stimulate Th1 cytokine production while others have induced Th2 responses or mixed Th1/Th2 response. LGG promotes Th1 responses with increased IFN-γ and IL-12 production, no change in IL-4 and a modest increase in IL-10 production by peripheral blood mononuclear cells in vitro [20]. Similar Th1-promoting effects have been demonstrated in vivo with increased production of IFN-γ in infants with cow’s milk allergy and eczema treated with LGG [21]. Interestingly, in the same study, a mixture of 4 probiotics that included LGG had no effect on IFN-γ and increased IL-4 production [21], highlighting the different and potentially antagonistic effects of individual bacterial species/strains on immune responses. Different bifidobacteria may also induce distinct and even opposing responses [22]. In animal models of autoimmune arthritis, some probiotic bacteria inhibit Th1 responses providing beneficial effects, while others aggravate disease by inducing Th1 cytokine responses [23].

Of relevance to the induction of oral tolerance, probiotic bacteria can enhance IgA immune responses to both oral and parenteral antigens. Administration of LGG in conjunction with oral rotavirus vaccine increased rotavirus IgA seroconversion [24]. Infants who received cow’s milk formula supplemented with probiotics (LGG and *B. lactis* Bb-12) had higher numbers of cow’s milk-specific IgA-secreting cells than placebo-treated infants [21]. Administration of LGG to children with acute rotavirus diarrhea resulted in an increased percentage of children who developed rotavirus-specific IgA antibody-secreting cells as compared to placebo [25]. Probiotic supplementation in infants can enhance IgA responses to parenteral vaccines [26]. Strain specificity can again be demonstrated – a study of lactobacillus strains showed that LGG enhanced IgA responses against rotavirus while different strains of the same species did not [27].

**Prebiotic Effects on Immune Responses**

Prebiotics may modulate immune responses either directly or indirectly through their actions on resident beneficial bacteria such as bifidobacteria and lactobacilli. Metabolism of GOS and FOS can lead to production of short-chain fatty acids, which have direct immunomodulatory properties. For example, GOS and FOS fermentation can lead to the production of butyrate which
stimulates apoptosis, suppresses cytokine-induced and constitutive expression of the transcription factor NF-κB, and enhances immune system reactivity [for review see, 28]. Prebiotics can modulate immune responses in vivo, via direct actions or indirectly via modulation of the microbiota. In animal studies, FOS (alone or with lactulose) increased intestinal IgA production, mesenteric lymph nodes and Peyer’s patches, and altered cytokine production and lymphocyte numbers in the spleen and intestinal mucosa [29–31]. GOS, raffinose and alginate-based oligosaccharides enhanced IL-12 production [32, 33] and inhibited Th2 responses [32–34] in mice. Consistent with these effects, GOS, GOS/FOS and raffinose inhibited airway inflammation, airway hyperresponsiveness and Th2 cytokine production in animal models of asthma [34, 35]; and GOS/FOS (but not FOS/inulin) enhanced delayed type hypersensitivity responses to influenza vaccination in mice [36]. In some studies, these beneficial effects on murine allergic airway disease and vaccine responses were independent of microbiota changes [34, 36]. Synbiotic combinations may provide similar or enhanced effects predicted from the combined actions of chosen probiotic(s) and prebiotic(s). The capacity to modulate immune responses has been confirmed for various symbiotic combinations [37]. However, as for the probiotic bacteria, effects of prebiotics may vary with different preparations [34, 36]; therefore selection of synbiotic combinations must be based upon demonstrated effects of the combined preparation.

Host-Specific Factors May Modify Probiotic and Prebiotic Effects in Vivo

Immune effects in vivo can be modified by host-specific factors that may be (a) intrinsic (e.g. age, genetic polymorphisms affecting immune response genes, disease status) or (b) extrinsic environmental factors (e.g. farm exposure). The newborn microbiota changes rapidly in the first weeks of life and at the time of weaning, and does not reflect adult patterns until 2 years age. In contrast, the species composition during adulthood appears to be very stable for a given individual over time and probiotic supplementation in adults generally does not result in persistent changes in bacterial strain composition. Infants may therefore be more amenable to manipulation by probiotic or prebiotic supplementation than adults, as illustrated by findings from clinical studies in humans [38, 39]. A recent study found that CD14/-1721 polymorphisms can modify the protective effects of farm milk ingestion on the development of atopic dermatitis, highlighting the important contribution of genetic factors in the regulation of immune responses [40]. The presence of allergic disease and/or the presence of allergic sensitization status in the host have been reported to influence the response to probiotics. LGG administration, along with milk, was associated with immunostimulatory effects in healthy hosts, but anti-inflammatory effects in milk-hypersensitive infants.
In children with eczema, the presence of allergic sensitization [42–44] and greater severity of disease [39, 43] correlated with stronger therapeutic response to probiotic treatment. Therefore, although selected probiotics and prebiotics may have allergy-averting immune effects in vitro and in animal models of disease, it is not certain that administration in humans will confer beneficial effects on clinical disease in specific patient populations, and evaluation in clinical trials is required.

Clinical Studies of Probiotics and Prebiotics in Allergic Disorders

Prevention of Allergic Disease

Probiotics

At the time of writing, 8 randomized double-blind placebo-controlled studies of various probiotic bacteria (used alone or in combination with other probiotics, and in one study also prebiotics) for the prevention of allergic disease have been completed [45–52] (table 1). Five of the 8 randomized controlled trials (RCTs) evaluated combined prenatal (last 2–6 weeks of pregnancy) and postnatal (6–24 months) probiotic treatment in high-risk infants with a family history of allergic disease. These 5 studies evaluated 5 probiotic [45–47, 52] and 1 synbiotic [48] treatments. Four of the 6 treatments resulted in significantly less atopic eczema (OR 0.51–0.74) [45, 48, 52] or IgE-associated atopic eczema (RR 0.4–0.66) [46, 48, 52] at 2 years. In the first randomized controlled prevention study reported, protective effects persisted to 7 years of age (RR 0.64) [53, 54]. Interestingly, subgroup analysis of outcomes from this study at 2 years showed that the reduction in risk of eczema was greatest in infants who were breastfed for at least 3 months [18]. In these infants (n = 57), there was a 68% reduction in the risk of chronic relapsing atopic eczema at 2 years with LGG treatment [18], compared with a 34% relative risk reduction for infants who were not breastfed for this time. This suggests that administration of LGG to mothers together with exclusive breastfeeding for the first 3 months of life may enhance the protective effects of LGG, possibly related to increased breast milk TGF-β2 levels [18]. Sensitization was not changed in the majority of studies; although treatment with L. reuteri was associated with a trend towards reduced atopic sensitization which reached significance in infants with an allergic mother [46]. Wickens et al. [52] evaluated 2 different probiotic treatments compared with placebo and reported that L. rhamnosus HN001 had beneficial effects on eczema but Bifidobacterium animalis subsp lactis HN019 did not; emphasizing the species-specific effects of probiotic bacteria. Kopp et al. [47] evaluated prenatal and postnatal LGG treatment using the same dose and a similar protocol to the original Kalliomäki et al. study but found no effects on eczema. The discrepancy between the
Kalliomäki and Kopp studies [45, 47, 53, 54] may reflect intrinsic or environmental differences in the study populations. A study of nutritional interventions during pregnancy in healthy mothers (provision of nutritional advice and food products with or without probiotic supplementation vs. placebo) reported allergic outcomes in a subset of infants at 12 months (a subgroup of subjects who had been assigned to receive nutritional advice with or without probiotic) [55]; however, this study was not originally designed as a RCT to evaluate the effect of probiotic supplementation for the prevention of allergic disease and the allergic disease risk of infants was varied and details were not provided. In this study, combined LGG and *B. lactis* Bb12 supplementation to mothers during pregnancy and lactation was found to reduce sensitization in a subgroup of infants whose mothers had atopic sensitization (26 vs. 50%, OR 0.34, p = 0.023), but not in the whole population, and no effects on eczema at 1 year were observed [55].

In contrast to the promising outcomes reported for prenatal/postnatal interventions, studies that evaluated postnatal (without prenatal) pro-

| Table 1. | Probiotic prevention studies: effects on eczema, IgE-associated eczema and sensitization |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Subjects at follow-up, n (age) | Eczema | IgE-associated eczema | Sensitization |
| Prenatal/postnatal | | | | |
| LGG [51, 59, 60] | 115 (7 years) | RR 0.64* | NA | RR 0.92 |
| 107 (4 years) | RR 0.57* | NA | RR 1.1 |
| 132 (2 years) | RR 0.51* | NA | RR 1.3 |
| LGG [53] | 94 (2 years) | RR 0.96 | NA | RR 0.70 |
| Probiotic mix plus prebiotic [54] | 925 (2 years) | RR 0.74* | RR 0.66* | RR 0.86 |
| L. *reuteri* [52] | 188 (2 years) | RR 1.06 | RR 0.40* | RR 0.53 |
| 302 (2 years) | RR 0.51* | RR 0.51* | RR 0.74 |
| L. *rhamnosus* HN001 [58] | 294 (2 years) | RR 0.9 | RR 0.69 | RR 0.82 |
| B. *animalis* subsp *lactis* HN019 [58] | | | | |
| Postnatal | | | | |
| LGG + Bb12 [55] | 72 (1 year) | RR 0.62 | NA | RR 0.83 |
| L. *acidophilus* | 175 (1 year) | RR 1.10 | RR 1.87* | RR 1.63* |
| LAVRI-A1 [57] | | | | |
| BL999+ L. *rhamnosus* (LPR) [56] | 235 (1 year) | RR 0.94 | RR 1.17 | RR 1.28 |
| NA = Not assessed. | | | | |
| * p < 0.05. | | | | |
biotic treatment found no beneficial effects on development of eczema, IgE-associated eczema or sensitization at 12 months [49–51]. Indeed, *L. acidophilus* LAVRI-A1 was instead found to be associated with an increased risk of both IgE-associated eczema (RR 1.87) and atopic sensitization (RR 1.63) [51]. Two of these studies involved high-risk infants with a family history of allergic disease [50, 51], while 1 study included formula-fed infants regardless of allergic disease family history [49]. These findings strongly suggest that probiotic supplementation during the prenatal period is most important for beneficial effects.

Other allergic disease outcomes (recurrent wheeze, asthma, allergic rhinitis, food allergy) were assessed in 4 studies and a concerning trend to increased airway allergic disease was noted in 3 of these. Although Kalliomäki et al. [53] reported reduced exhaled nitric oxide production at 4 years suggesting reduced airway inflammation, a trend for increased asthma was noted at 7 years [54]. Taylor et al. [51] reported a similar trend to increased wheeze and asthma at 2 years and Kopp et al. [47] reported an almost 3-fold increased risk of recurrent wheezing bronchitis. Kukkonen et al. [48] found no difference in the cumulative incidence of all allergic diseases or IgE-associated allergic diseases. Additional data on asthma and allergic rhinitis outcomes will become available as further follow-up analyses are performed in ongoing prevention studies.

Current studies suggest a potential role for probiotics or synbiotics in the prevention of eczema, however there is no evidence of benefit for the prevention of other allergic conditions. A Cochrane meta-analysis of the first 5 prevention studies (excluding the 3 most recent trials) concluded that although a reduction in eczema was observed, further studies are required to determine whether the findings for eczema are reproducible [56]. Prenatal treatment appears necessary for protective effects, highlighting the importance of prenatal influences on the development of infant immune responses. It is not known whether ongoing postnatal therapy is also important, and results from an ongoing study of prenatal (without postnatal) LGG for the prevention of eczema will provide insight on this issue [Probiotic Eczema Prevention Study registered with Cochrane Skin Group www.nottingham.ac.uk/ongoingskintrials Trial No. 36]. For future studies, careful selection of probiotic bacteria will be important, which may be aided by in vitro, preclinical and pilot studies.

**Prebiotics**

There have been 2 studies of prebiotics for the prevention of eczema. Arslanoglu et al. [57] and Moro et al. [58] evaluated GOS/FOS versus placebo supplemented into extensively hydrolyzed formula for 6 months for the prevention of eczema in high-risk infants with parental history of allergy. There was a significantly reduced risk of eczema (RR 0.42) at 6 months, and a significantly reduced risk of eczema (RR 0.49), recurrent wheeze (RR 0.37), and allergic urticaria (RR 0.15) at 2 years [57, 58]. Ziegler et al. [59] compared 2
prebiotic supplements (4 g/l polydextrose/GOS 50:50; 8 g/l polydextrose/GOS/lactulose 50:33:17) with control formula in healthy term infants not selected on the basis of family history of allergy, and reported no effect on eczema with polydextrose/GOS/lactulose and an increased rate of eczema with polydextrose/GOS; however no information was provided regarding the criteria for diagnosis of eczema or when this outcome was assessed. Further studies are required to confirm the findings of Moro et al. [58].

**Treatment of Allergic Disease**

**Probiotics and Eczema**

Most studies assessing probiotic effects in the treatment of allergic disease have focused on atopic eczema with or without associated food allergy in infants and children and have administered lactobacillus species or bifidobacteria species either alone or in combination with other probiotics (table 2). Early studies in small numbers of infants and children with eczema reported improvement in eczema (SCORAD or symptoms) following treatment with LGG, *B. lactis* Bb-12, or *Bifidobacterium breve* [61–63]. In the studies by Isolauri et al. [62] and Majamaa et al. [63], probiotic treatment was associated with a more rapid improvement in eczema as compared to placebo, however by 2 months in the Majamaa study and 6 months in the Isolauri study, SCORAD was equivalent in the treatment and placebo groups. A study of *Lactobacillus fermentum* VR1–003PCC in children with eczema also demonstrated improvements in eczema severity and extent [39]. In contrast, other more recent and larger clinical trials have failed to confirm beneficial effects of probiotics for the treatment of eczema as assessed by SCORAD [42–44, 64–66]. In 2 negative studies, subgroup analysis revealed improvements in SCORAD with probiotic treatment for a subgroup of children with IgE-associated eczema [42, 44], and beneficial effects were further increased when infants who had received antibiotics during the study were also excluded from the analysis [44]. Clinical effects were associated with low-grade inflammation as evidenced by moderate increases in CRP and IL-6, which may in turn suppress inflammation by inducing IL-10 and IgA production and inhibiting production of other inflammatory factors (TNF-α, chemokines, IFN-γ) [67]. It has been suggested that beneficial effects of probiotics in the treatment of eczema may be limited to those infants with food allergy [43, 63], however only 1 RCT has separately examined SCORAD outcomes in infants with eczema and proven cow’s milk allergy and found no treatment effects in this subset of infants [44]. One study comparing a symbiotic to a prebiotic reported improved SCORAD with both interventions, however there was no placebo group so it is not possible to determine if there were significant beneficial effects [68].

Two meta-analyses of studies evaluating the use of probiotics for the treatment of atopic eczema have recently been completed [69, 70] and both have
Table 2. Randomized controlled trials evaluating the use of probiotics for the treatment of eczema

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>Participants</th>
<th>Interventions</th>
<th>Outcomes</th>
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</table>
| Brouwer et al. [64]        | 3-month parallel group RCT   | 50 formula-fed infants with eczema and CMA(?)      | Test: eHF with *L. rhamnosus* or LGG at 5 × 10⁹ CFU/100 ml  
Control: eHF without probiotic | SCORAD                        |
| Fölster-Holst et al. [65]  | 8-week parallel group RCT    | 53 children with eczema                           | Test: 2 × 10¹⁰ CFU/day LGG  
Control: microcrystalline cellulose | Global assessment;  
QoL score; SCORAD; medication use |
| Gruber et al. [66]         | 12-week parallel group RCT   | 106 infants with eczema SCORAD 15–40               | Test: 1 × 10¹⁰ CFU/day LGG  
Control: placebo capsule | SCORAD; medication use |
| Hattori et al. [61]        | 3-month parallel group RCT   | 17 children with eczema, CMA(?) and low fecal *Bifidobacterium* level | Test: eHF with prebiotic and *B. breve* M16-V at 5–15 × 10⁹ CFU/day  
Control: eHF with prebiotic and no probiotic | Eczema severity scoring scale |
| Isolauri et al. [62]       | Parallel group RCT duration(?) | 27 breastfed infants with eczema                  | Test: eHF with Bb-12 at 1 × 10⁹ CFU/g or LGG at 3 × 10⁸ CFU/g  
Control: eHF without probiotic | SCORAD                        |
| Kirjavainen [71]           | Parallel group RCT duration(?) | 35 infants with eczema and CMA(?)                 | Test 1: eHF with live LGG at 3 × 10¹⁰ CFU/kg/day  
Test 2: eHF with heat-inactivated LGG  
Control: eHF without probiotic | SCORAD                        |
| Majamaa et al. [63]        | 1-month parallel group RCT   | 31 children with eczema and CMA(?)                | Test: eHF with LGG at 5 × 10⁸ CFU/g  
Control: eHF without probiotic | SCORAD                        |
| Passeron et al. [68]       | 3-month parallel group RCT   | 48 children with eczema SCORAD >14                | Test: prebiotic powder with *L. rhamnosus* Lcr35 at 3.6 × 10⁹ CFU/day  
Control: prebiotic powder alone | Global assessment SCORAD       |
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Intervention</th>
<th>Outcome Measures</th>
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<tbody>
<tr>
<td>Rosenfeldt et al. [42]</td>
<td>6-week cross-over RCT</td>
<td>58 children with eczema</td>
<td>Test: <em>L. rhamnosus</em> 19070-2 and <em>L. reuteri</em> DSM12246 at 2 × 10^{10} CFU/day</td>
<td>Global assessment SCORAD; medication use</td>
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<td>Control: skim milk powder + dextrose</td>
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<tr>
<td>Sistek et al. [43]</td>
<td>12-week parallel group RCT</td>
<td>60 children with eczema, atopy, SCORAD &gt;10</td>
<td>Test: <em>L. rhamnosus</em> and <em>B. lactis</em> at 2 × 10^{10} CFU/day</td>
<td>SCORAD</td>
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<td></td>
<td></td>
<td></td>
<td>Control: microcrystalline cellulose</td>
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<tr>
<td>Viljanen et al. [44]</td>
<td>4-week parallel group RCT</td>
<td>252 infants with eczema and CMA(?)</td>
<td>Test: cow’s milk elimination, eHF and LGG at 10^{10} CFU/day or probiotic mix</td>
<td>SCORAD</td>
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<td>Control: cow’s milk elimination and eHF</td>
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<tr>
<td>Weston et al. [39]</td>
<td>8-week parallel group RCT</td>
<td>56 children with eczema; modified SCORAD ≥ 25</td>
<td>Test: <em>L. fermentum</em> VR1-003PCC 2 × 10^{8} CFU/day</td>
<td>Global assessment; QoL score; SCORAD; Medication use</td>
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<td></td>
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<td>Control: maltodextrin placebo</td>
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RTC = Randomized controlled trial; CMA = cow’s milk allergy; eHF = extensively hydrolyzed formula; LGG = *Lactobacillus rhamnosus* GG; CFU = colony-forming units; SCORAD = scoring atopic dermatitis.
concluded that there is insufficient evidence to support the use of probiotics for the treatment of eczema. The Cochrane meta-analysis included 12 studies of probiotics in the treatment of eczema (described above and summarized in table 2). It found no significant reduction in eczema symptoms with probiotic treatment compared with placebo (mean difference 0.90 points on a 20-point visual analog scale 95% CI –1.04, 2.84), and no significant difference in investigator-rated eczema severity between probiotic and placebo treatments (588 participants) [70]. Significant heterogeneity was noted between studies, which may be explained by the use of different probiotic strains. Subgroup analysis by eczema severity or presence of atopy did not identify a population with different treatment outcomes.

Probiotics and Food Allergy

Although there are mechanistic data to suggest that probiotics may play a role in the treatment of food allergy by promoting gut barrier integrity, suppressing intestinal inflammatory response, and inducing IgA production and tolerogenic immune responses (see above) [1], there is currently no evidence that probiotics are effective for induction of tolerance in the clinical setting. In a recent randomized double-blind placebo-controlled study of 119 infants with challenge-confirmed cow’s milk allergy, probiotic supplementation with Lactobacillus casei CRL431 and B. lactis Bb-12 for 12 months had no effect on acquisition of tolerance to cow’s milk [72]. In a small mechanistic study of children <3 years with sensitization to egg, peanut or cow’s milk and clinical symptoms, oral administration of a probiotic mix comprising predominantly lactobacillus and bifidobacteria species for 3 months failed to influence sensitization (skin prick test size or allergen-specific IgE levels) or ex vivo immune responses [73]. Of note, in contrast to other studies that demonstrated similar in vivo and in vitro effects for individual probiotic bacteria (see section above: Immune effects of probiotics and prebiotics), the probiotic mix in this study induced opposite effects in vitro as compared to in vivo [73].

Probiotics and Allergic Rhinitis or Asthma

Double-blind placebo-controlled studies of probiotic treatment for allergic rhinitis and asthma provide conflicting results. Many studies have included mixed patient populations with allergic rhinitis or asthma rather than either condition alone. Double-blind placebo-controlled studies in adolescents with allergic rhinitis reported improvements in quality of life scores and rescue antihistamine use following 3–4 weeks treatment with L. paracasei 33 or Bifidobacterium clausii [74–76]. Studies in adults with Japanese cedar pollen-induced seasonal allergic rhinitis treated with Bifidobacterium longum BB536 or L. casei strain Shirota failed to demonstrate significant beneficial effects [77, 78] with only limited improvement in eye symptoms in one study [77]. Studies involving mixed patient populations – children aged 2–5 years
with asthma and/or allergic rhinitis treated with *L. casei* [79], and adults and children with asthma/allergic rhinitis/oral allergy syndrome treated with LGG – reported no beneficial effects [80]. One study in adult patients with asthma failed to demonstrate beneficial effects following treatment with *L. acidophilus* [81]. In summary, although studies in adolescents with allergic rhinitis appear promising, there is currently insufficient evidence to suggest a role for probiotics in the treatment of allergic rhinitis. There is no evidence to support a role for probiotic treatment in asthma.

**Conclusions**

Probiotics and prebiotics offer an approach to modulation of the intestinal microbiota that may in turn provide beneficial effects for the prevention or treatment of allergic disease. Studies suggest a promising role for probiotics, prebiotics or synbiotics in the prevention of eczema. However, it remains uncertain whether this beneficial effect will extend to the prevention of other allergic conditions such as food allergy, asthma or allergic rhinitis, and follow up of subjects from prevention studies will be of interest. Further studies are required to clarify the optimal dose, timing, and combination of agents, as well as the optimal patient populations to achieve beneficial effects. In contrast to the findings from prevention studies, studies of probiotics for the treatment of allergic disease have been disappointing. Two recent meta-analyses have concluded that there is insufficient evidence to support a role for probiotics in the treatment of eczema. Probiotic effects in the treatment of allergic rhinitis and asthma are similarly conflicting, although further studies are warranted to clarify potential effects in children with allergic rhinitis.

**References**

Tang


Discussion

Dr. Makrides: Could you comment on some of the data with regard to probiotics, for example, where the mechanistic data do not quite match up with the clinical data? One of the really uncomfortable things that we all find difficult to addressing is actually the quality of the studies and what influence that has. Could you comment on study quality and how that might actually influence the outcome of the studies especially in terms of the quality of randomization, the degree of blinding of the outcome assessment and issues of attrition? All of those things can introduce quite significant biases that will change the study outcome, and I would really welcome your comments about how study quality comes into that mix as well.

Dr. Tang: Of course the quality of a study in terms of randomization, blinding, outcome assessment and loss to follow-up is very important when determining the strength of findings and this can assist us in our consideration of the conflicting data. We have to have very carefully designed studies that are randomized with well-defined outcome measures outlined at the outset, and minimize post hoc analysis. Beyond this, the selection of subjects must also be done carefully. There is much variability in the diagnostic criteria that have been used. The ability to do a SCORAD varies hugely between different individuals. There are all these different aspects, but in particular a study needs a good team in order to maintain high levels of participation retention because that is probably one of the most significant biases that can be introduced.

Dr. Brandtzaeg: It seems that everybody working with probiotics is forgetting that the administered bacteria are actually received in an environment where there is a very high concentration of secretory IgA antibodies. We know that the antibody repertoire varies very much with the geographic location of the mother or where the child is growing up, and we know that secretory IgA antibodies can cause effects on how the bacteria in the gut, or wherever on the mucous membranes, are contained or controlled – whether they are subjected to a better uptake or rejection or whatever. This may influence what is seen with probiotics, so to me it’s not a surprise that we have these geographic differences. I would also like to say to Dr. Salminen that microarray or cultivation is just a snapshot of the microbiota. Secretory IgA antibodies can cause antigenic drifts; so the bacterial phenotypes can change the next day or the next week. Therefore it is not a stable microbiota. I wonder about all the data we have now because they are actually not easy to understand.
Dr. Tang: You made a really valid point. The intestinal environment is not necessarily receptive to administered probiotic bacteria and also the resident microbiota will be influenced by geographically determined intestinal factors. Another issue is that it is surprising that introduction of one bacteria can do anything at all. Of course, if you introduce a bacteria into the newborn intestine, such pioneer bacteria can have important effects in determining the subsequent colonization and may therefore have an impact on the development of allergic disease, but introduction of a probiotic bacteria in later life would not really be expected to have a major effect on the treatment of allergic disease given that the microbiota is already well established and, you are absolutely correct, they have to compete against so many adverse factors in order to adhere and induce any signaling.

Dr. Brandtzaeg: That is not exactly what I said; the secretory IgA antibodies can actually also promote the induction of an immune response in the breastfed baby. So we only see one side of a partnership, bacteria against antibodies which might or might not be helpful.

Dr. Thornton: My first comment relates to what you said about doing clinical trials and the opportunity to do status of immune function. I think that is an excellent idea but caution has to be taken in that people shouldn’t try to second guess what the outcome of the clinical trial is going to be and therefore do immune tests as they go along. Perhaps the best way is to encourage people to have really good archives of material that they can then use retrospectively based on the clinical outcomes. They can then go back a couple of years later based on what has been published in the interim and what they actually find in their clinical trial, and then look at what immune functions are most relevant to their findings. We are already getting ourselves into a mess by just sticking to Th1, Th2 really.

More recently we have data coming from probiotic- and placebo-fed infants, but lots of the studies on choosing probiotics based on immunomodulatory effects are not done in the gut, which is the ideal place and of course in humans it is very challenging, they are actually generally done using adult peripheral blood, and I think we never really look at it at the right ontogenic stage. Again blood is still not the ideal setting but at least using blood from neonates and young children might be a better way of assessing a probiotic before we take it to a clinical trial.

Dr. Tang: That’s exactly right, we know that immune responses in the infant are so very different from those in the adult and I think it would certainly be of great assistance to be able to understand what the probiotic bacteria might be doing in a relevant setting such as the neonate or infant. It would also be ideal to select probiotic bacteria based upon an understanding of effects on local tissue immune cells, but it is very difficult to achieve that.

Dr. Mack: With regard to the data you presented about the serious consequences of asthma and with the information we heard earlier about the epidemic of celiac disease when babies did not receive gluten, I was wondering if you would like to comment on the safety of using these high-dose probiotic preparations in very young infants and especially those that are not breastfeeding?

Dr. Tang: Of course we know that probiotics are generally considered to be safe and they are used in the food industry without regulatory requirements. We recently reviewed the adverse effects of probiotic use [1] and found that by and large probiotics were safe in the absence of certain risk factors. The risk factors for probiotic sepsis that were identified include: inflammation of the gut, bypassing the gastric processes with a gastrostomy or jejunostomy, or the presence of a foreign body such as an indwelling catheter. So a number of different factors can increase the risk of probiotic-associated sepsis and in some cases death. But probiotics have been given to babies with HIV and to premature infants without any problems. Nevertheless, when
we performed the meta-analysis of studies evaluating the use of probiotics for the treatment of eczema, there were a number of quite significant adverse events and one death that were considered to be due to probiotic sepsis. You quite rightly pointed out that we need to proceed with some caution and that is why I think at this point in time we can’t be recommending the use of probiotics in a clinical setting, it must be done within the context of a clinical trial. Dr. Makrides’ comment about well-designed, well-run, high-quality trials is very important, and Dr. Thornton’s comment also about not second guessing what we are looking for is important for keeping an open mind about the possibility of adverse effects. I am often asked what is stopping us from just giving probiotics for either prevention or treatment of allergic disease as they are fairly safe. I think the issue is that there is still so much we don’t understand – we don’t know which bacteria, what dose, and in some instances, as we have seen in the prevention studies, there can be an increased risk of allergic disease and sensitization [2]. We really shouldn’t be recommending it at all in a clinical setting until we better understand what we are actually doing.

Dr. Conway: We recognized and discussed today that the gut microflora varies enormously and prebiotics often function by enhancing the indigenous organisms that are there. If the population varies so much, are we in fact better looking at putting them together, the symbiotic concept?

Dr. Tang: I think the symbiotic approach is very sensible because, as you have pointed out, delivering a prebiotic presupposes that you have the favorable bacteria already present. However, that may not be the case or they may not be present in sufficient numbers. It also presupposes that only bacteria are going to increase in numbers. Similarly, for a probiotic, I think it is astounding that we can achieve significant effects by delivering one type of bacteria, and perhaps a smarter approach would be to use a combination of a probiotic and prebiotic, in other words, providing favorable bacteria together with the right fertilizer that is going to allow the bacteria to take hold and expand. Indeed a study by Kukkonen et al. [3] evaluating a symbiotic for the prevention of allergic disease in high-risk infants found a significant protective effect against eczema at 12 months.

Dr. Isolauri: I think indeed that it’s challenging to try to make a review and combine studies in different parts of the world, in different populations, on different diets and different allergic manifestations. I want to make a comment on both prevention and treatment. First treatment. If you look at the publications it appears that the effect of probiotics is indeed in atopic eczema patients with food allergy or sensitization. Considering the comment Dr. Brandtzaeg made earlier on how gut IgA and the barrier are affected in food challenges and food allergy, it makes a sense to target treatment in an eczema patient. Concerning sensitization, a more recent study using a combination of B. lactis and LGG had a positive effect on sensitization [4].

Dr. Stanley: These are comments and questions related to stool analysis and how that relates to the action part of the gut where we think the probiotics are working, which is far away. Are they the same and how do we get bits of the gut where the interesting organisms are without slicing out little bits of gut? I was thinking about the frequency of stools in different age group babies. When they are little they produce lots of frequent stools and I wonder if that’s actually fresher? As they get bigger the stools become less frequent, and then I wonder about the stools actually being representative of the organisms from the same part? Does the change in frequency of the stool represent an alteration in the amount of probiotics in that child’s gut, and is that the mechanism of slowing down of the stools as the oligosaccharides are being metabolized? When people analyze the content of the stools, do they take into consideration the frequency of stools, in other words are they rapid-transit stools or are they slow-transit stools?
Tang

Dr. Tang: The issue is that what we see from the stool may not be representative of what is actually going on in the more proximal intestine. Certainly, there are lots of bacteria that we never sample and never really see. With respect to motility, the presence of a prebiotic would potentially modulate the frequency and consistency of stool.

Dr. Stanley: Does the stool frequency or the rapid transit of the stool represent the fact that there are different bacteria present in that infant’s gut? As the child acquires more probiotic organisms, is that the mechanism for the slowing down of the stool? Is the early frequent stool more closely related to upper or mid gut bacteria than the later infrequent stool that an infant produces?

Dr. Salminen: Of course there is the situation that stool frequency is influenced by the collective composition of the microbiota in adults and you can alter it by different probiotics. We have looked at samples from upper parts of the adult intestine but unfortunately we cannot do that in infants. With the help of the study that Dr. Isolauri designed, we can see more bifidobacteria in the gut epithelium than we can in the stool sample, but the composition of the stool sample certainly reflects on what we see in the epithelium. In adults, whether there is a large or a small number of bowel movements, there doesn’t appear to be too much difference, but again with the infant gut it is a much more simple situation and it may be reflective there. But I would like to touch on the other point and I would like to challenge you, Dr. Tang, on the safety issue because I personally try to focus on bacteria that are safe. I also personally try to focus on prebiotics that are safe, and there are not too many of those. So in terms of looking at the situation in the infant, it is totally different from looking at all of us in this room for instance, we can reasonably safely have all sorts of things, probiotics and prebiotics, without having a significant detrimental effect. But in infants we have to look at those prebiotics with a safety record, where there has been safety assessment from different regulatory authorities, and also in the clinical setting. I don’t see problems, you challenged that probiotic.

Dr. Tang: I agree with you, I think we can regard them as safe but just because they are safe does not mean we can make recommendations for use in clinical practice in the absence of strong evidence of a beneficial effect. Also, although they are mostly safe, they are not 100% safe, there is a potential for problems in the setting of risk factors such as intestinal inflammation. So my comments are directed more to highlighting the point that we should wait for better evidence before we recommend their use widely.

References