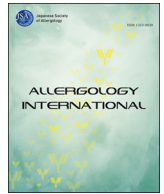




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Invited review article

Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention

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GRADE, Grading of Recommendation

Assessment Development and Evaluation;

HMO, Human milk oligosaccharide;

NCD, non-communicable disease;

RCT, randomized controlled trial;

RR, relative risk; SCORAD, Scoring Atopic

Dermatitis; SCFA, short-chain fatty acid;

Treg, regulatory T-cell; TLR, Toll-like

receptor; WAO, World Allergy Organization

ABSTRACT

Large-scale biodiversity loss and complex changes in social behaviors are altering human microbial ecology. This is increasingly implicated in the global rise in inflammatory diseases, most notably the “allergy epidemic” in very early life. Colonization of human ecological niches, particularly the gastrointestinal tract, is critical for normal local and systemic immune development and regulation. Disturbances in composition, diversity and timing of microbial colonization have been associated with increased allergy risk, indicating the importance of strategies to restore a dysbiotic gut microbiota in the primary prevention of allergic diseases, including the administration of probiotics, prebiotics and synbiotics. Here, we summarize and discuss findings of randomized clinical trials that have examined the effects of these microbiome-related strategies on short and long-term allergy preventative effects – including new guidelines from the World Allergy Organization which now recommend probiotics and prebiotics for allergy prevention under certain conditions. The relatively low quality evidence, limited comparative studies and large heterogeneity between studies, have collectively hampered recommendations on specific probiotic strains, specific timing and specific conditions for the most effective preventive management. At the same time the risk of using available products is low. While further research is needed before specific practice guidelines on supplement probiotics and prebiotics, it is equally important that the underlying dietary and lifestyle factors of dysbiosis are addressed at both the individual and societal levels.

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Introduction

The epidemic rise in allergic diseases and asthma is inexorably linked to complex environmental and modern lifestyle changes. Urbanization and global decline of environmental biodiversity are directly implicated in changes in human commensal microbiota, which are critical for both normal immune maturation and subsequent immune function. While these global effects are likely to vary widely across both macro-scale geographic environments and

micro-scale human microbial habitats, there is growing evidence that ‘dysbiosis’ is a major factor in the global increase in inflammatory non-communicable diseases including allergic disease.^{1–3} The ecological pressures on microbial diversity are multifaceted and reflect changes in individual exposures such as nutritional patterns (increased processed foods, less fresh and fermented foods), sedentary indoor living (vitamin D insufficiency, reduced nature relatedness and exposure to environmental biodiversity) as well as the wider social and economic drivers of ‘dysbiotic drift’.^{4–6}

Thus while it is important to develop strategies for individuals to restore personal biodiversity for disease prevention, as is the subject of this review, it is equally important to address the fundamental drivers of dysbiosis and nutritional supplements must be viewed in this broader ecological context.⁷

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The establishment of commensal microbiota in the gastrointestinal tract is critical to provide the tolerogenic microenvironment necessary for optimal development of both innate and adaptive immunity.⁸ Adverse influences on early colonization may have long-lasting consequences, exemplified by numerous examples of early compositional and functional differences in early life gut microbiota that precede the onset of eczema and asthma.^{9–20} While it has been logical to explore the role of probiotics,²¹ prebiotics²² and combinations of these (synbiotics) (Table 1), to favorably modulate gut microbiota, it is also important that such interventions are considered in tandem with other strategies that address the perinatal practices and environmental factors that are contributing to dysbiosis in the first place. Thus, although this review is concerned with the specific impact of prebiotics and probiotics in the perinatal setting, we underscore that, even if effective, such products are only one aspect of the solutions.

The importance of gut microbiota establishment in the development of oral tolerance and immune competence

The ambient conditions during initial antigen exposure, typically when the gastrointestinal immune system is still immature, are important for the success of oral tolerance. The mechanisms that initiate and maintain tolerance to dietary antigens are still being defined, however early microbial exposure appears essential in promoting an appropriate regulatory milieu during this period of dynamic development (Fig. 1).^{23–25} Delivery method, antibiotic usage, breastfeeding, perinatal environmental factors and numerous factors that influence maternal microbiota in pregnancy and lactation are important in this early colonization processes –

Table 1
Definitions.

Probiotics	“live microorganisms, which when administered in adequate amounts confer a health benefit on the host” ²¹
Prebiotics	“substrate that is selectively utilized by host microorganisms conferring a health benefit” ²²
Synbiotics	The combination of probiotics and prebiotics

many of these now implicated in altered patterns of early colonization which may predispose to allergy and possible other early onset NCDs.³ For example, reduced gut microbial diversity and an elevated *Enterobacteriaceae/Bacteroidaceae* ratio in early life appears associated with an increased risk of developing food sensitization and atopic eczema.^{13,14,26,27} Moreover, gut microbiota composition at age 3–6 months was recently associated with milk allergy resolution at 8 years of age.²⁸

New insights into how the gut microbiota influences food allergy have been provided by experimental animal models, clearly demonstrating that absence of microbiota during a short time interval in early life can result in defects in immune regulation.²⁹ While this extreme microbial depletion does not resemble the more subtle disruptions observed in humans, it nonetheless indicates that the high microbial content in the gut is crucial for sustaining the homeostatic host-microbiome relationship and preventing intestinal inflammation and allergies by inducing mucosal IgA and regulatory T-cell (Treg) responses.^{8,25,29,30} The main mechanisms of induction of oral tolerance are mediated by Foxp3+ Treg, known to mediate suppressive reactions thus avoiding excessive immune activation.²⁴ IgA, as the most abundant mucosal immunoglobulin isotype, is important in the establishment of composition of intestinal microbiota³¹ and may reinforce oral tolerance by strengthening the mucosal barrier function.³² In line with this hypothesis, aberrant IgA responses to the gut microbiota during infancy were recently observed to precede allergy development during the first seven years of life.³³

The lack of intestinal microbiota in germ free mice is associated with a Th2-skewed immune response, with enhanced IgE responses to food antigens, and/or a defect in mounting proper regulatory T-cell responses.^{8,34,35} Thus, an early exposure to microbial symbionts occurring during certain time windows of developmental plasticity, potentially also during the prenatal period (either directly or indirectly through maternal immunomodulation), might be beneficial in preventing development of Th2-mediated allergic disease.³⁶ Preliminary evidence that reduced fecal diversity of Bacteroidetes in pregnancy is associated with increased risk of atopic eczema in their young children gives further support for a

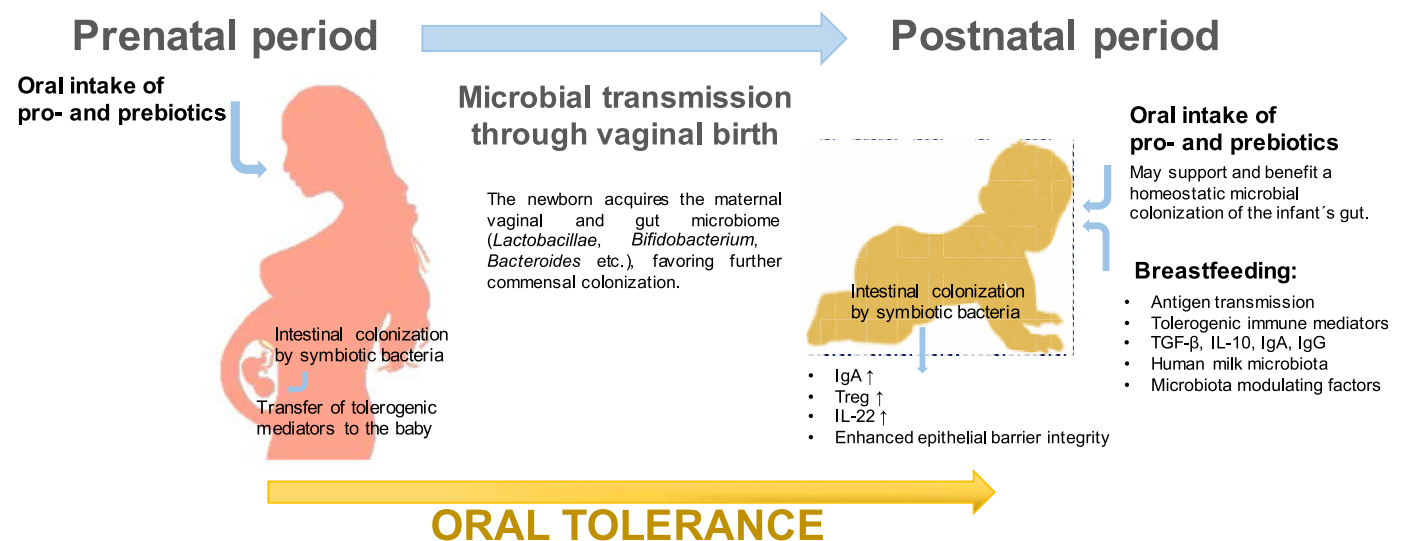


Fig. 1. Possible prenatal and postnatal mechanisms for induction of oral tolerance. Pro- and prebiotics administration during pregnancy can influence mother's gut microbiome potentially resulting in transmission of tolerogenic mediators (such as regulatory cytokines, antibodies and growth factors) through the placenta instructing foetal immune system development. Following vaginal delivery, the newborn's gut acquires the maternal vaginal (including *Lactobacillae* and *Bifidobacterium*) and gut (*Bacteroides*) microbiome favouring initial microbial colonization. Postnatally, oral administration of pro- and prebiotics together with breastfeeding and high-fibre diet might support ongoing intestinal colonization by symbiotic bacteria (including *Clostridium* spp. and *Bacteroides fragilis*) sustaining the homeostatic host-microbiome relationship. This might in turn prevent intestinal inflammation and decrease susceptibility to food allergies by inducing mucosal IgA and regulatory T-cell (Treg) responses.

role of maternal microbiota in this process.¹⁷ In addition to induction of Treg and IgA, the maintenance of oral tolerance to dietary antigens requires protective epithelial barrier integrity that may be enhanced by a class of mucosal associated commensal anaerobes, such as *Clostridium* spp.^{29,30,37,38} *Bacteroides fragilis*, with the immunomodulatory molecule polysaccharide A,³⁹ and *Clostridium* spp., belonging to clusters IV and XIV^{37,38} are potent inducers of Foxp3+ Treg differentiation, thereby favouring mucosal tolerance. Commensal anaerobes from the Clostridia class have additionally shown to be important for programming physical adaptation of intestinal epithelial cells to the continuous exposure to the wide range of dietary and microbial antigens present in the intestinal lumen. Studies in mice have shown that this adaptation is acquired by innate lymphoid cells producing IL-22 that controls enterocyte proliferation, the production of mucus and the secretion of antimicrobial peptides as well as reducing serum peanut allergen levels after oral exposure.^{30,40}

Deficiency in genetic elements that are coding for microbial sensors in mice, such as Toll like receptor 4 (TLR4) and CD14 (enhancing the detection of bacterial LPS by TLR4) also increases susceptibility to food allergies.^{41,42} Food allergy-prone mice, with a gain-of-function mutation in the IL-4 receptor, exhibit an altered gut microbiota signature, reflected by changes in the abundance of bacterial families *Lachnospiraceae* and *Lactobacillaceae*.⁴³ In this food allergy model, transplanted healthy infant microbiota, comprising mainly of *Bifidobacterium* and *Bacteroides*, had a protective role on sensitization and cow's milk allergy development in mice despite altered T-cell responses in the ileum. This could be due to the capability of the above-mentioned bacteria to restore Treg cell responses over time in the ileum.^{35,43}

Other indigenous commensal bacteria can stimulate innate signalling pathways in the host by direct cell-to-cell interactions or through secretion of short chain fatty acids (SCFA), regulated by high-fiber diet. Studies have shown that bacterially produced SCFA can regulate both the proportions and functional properties of intestinal Treg cells^{44–46} and inhibit pro-inflammatory responses by intestinal macrophages.^{44,47} Intervention studies, elucidating the effect of high-fiber prebiotics in modulating the intestinal human microbiota for prevention or treatment of food allergy is a logical strategy that should be assessed in future research.

Primary prevention studies using probiotics, prebiotics and synbiotics

The first studies targeting the gut microbiota for allergy prevention focused on the potential benefits of probiotics, with more recent studies examining prebiotics and synbiotics in this context.

Probiotics and eczema

The preventative effects of probiotics have been evaluated in randomized controlled trials (RCTs)^{48–63} (Table 2) and the majority used eczema or IgE-associated eczema as the primary outcome. Most studies used single strains of lactobacilli and bifidobacteria, or their combination (Table 2). Long-term follow-up data is available for less than half of these studies^{64–74} while many others are still under way (Table 2). The most recent meta-analyses reported a benefit of probiotics for primary prevention of eczema, but no significant effects on any other allergic outcomes.^{75,76} In the meta-analysis of Zuccotti et al.,⁷⁶ which included 17 studies (4755 children), there was a significantly lower relative risk (RR) for eczema in those treated with probiotics compared with placebo (RR 0.78; 95% CI: 0.69–0.89). Notably, the benefit was most evident when a mix of probiotic strains was administered (RR; 0.54 95% CI, 0.43–0.68). Cuello-Garcia et al.⁷⁵ included 29 studies in their meta-

analysis, though some of these were reports from the same study population but at different occasions of follow-up. In their meta-analysis, they also examined the effects of both timing of probiotics and the method of administration. There was a benefit of probiotics for eczema reduction (follow-up period until 2 years of age) when administered in the last trimester of pregnancy (RR 0.71; 95% CI, 0.60–0.84), when administered during breast-feeding (RR 0.57; 95% CI, 0.47–0.69), or when administered to infants and/or mothers (RR, 0.80; 95% CI, 0.68–0.94). No significant effect on eczema was reported when probiotics were administered solely to the infant (RR, 0.83; 95% CI, 0.58–1.19). The authors assessed the certainty in the evidence based on the Grading of Recommendation Assessment Development and Evaluation (GRADE) approach, and found it to be low or very low due to “risk of bias, inconsistency and imprecision of results, and indirectness of available research”.⁷⁵

Peldan et al.⁷³ recently reported long-term follow up data on eczema from the largest probiotic study conducted to date, results that were not available for inclusion in the 2015 meta-analyses.^{75,76} They found the life-time prevalence of eczema until 10 years of age to be reduced in the probiotic group that received a combination of 4 probiotic strains (Table 2) (35.2% vs 41.7%, adjusted OR: 0.74; 95% CI: 0.55–1.00). While their previous reports^{54,66} included clinical examinations including Scoring Atopic Dermatitis (SCORAD) assessment, the long-term follow-up data relied solely on questionnaire –reported data, which could be a limitation. Nevertheless, the study gives further support for long-term preventative effects on eczema of specific probiotics.

Effects of probiotics beyond the skin-atopy and food allergy

As discussed, most studies were designed to evaluate effects of probiotics on early manifestations of allergic disease, e.g. eczema. Most studies also included measures of sensitization and in a meta-analysis from 2016, which included 17 trials (2947 infants),⁷⁷ pooled analysis showed that the combination of pre- and post-natal probiotic treatment decreased the risk of “any” sensitization (RR 0.78; 95% CI 0.66–0.92). This was most evident when probiotics were given in pregnancy to the mother and then to the infant postnatally (RR 0.71; 95% CI 0.57–0.89). A combined prenatal plus postnatal probiotic administration also reduced the risk of food sensitization (RR 0.77; 95% CI 0.61–0.98). In their conclusion, the authors underscored a call for studies examining the effects of probiotics for prevention of food allergy and that the studies should use objective clinical assessments, such as food challenges.⁷⁷

Probiotics and respiratory allergies

There is very little evidence for a role of probiotics in the prevention of respiratory allergies including asthma, wheeze and rhinitis. In the meta-analysis by Azad et al.⁷⁸ which included 9 trials (3257 children) the RR of asthma in children treated with probiotics was 0.99 (95% CI 0.81–1.21) and the RR of wheeze was 0.97 (95% CI 0.87–1.09), (9 trials, 1949 children). Similarly, in the follow-up study by Peldan et al.,⁷³ there was no benefit of probiotic treatment on parental questionnaire-reported asthma until 10 years of age. In another recent report, there was no preventative effect of probiotics on asthma in follow-up (ranging from 5 years to 10–15 years of follow-up) of a pooled population of probiotic RCTs⁷⁴ (Table 2). Intriguingly, the recent study by Peldan et al.⁷³ found questionnaire-reported allergic rhinoconjunctivitis to be more prevalent in children at 5–10 years of age who had previously received a probiotic combination compared with placebo. A similar finding of increased allergic rhinitis in probiotic-treated children was reported previously by Kalliomäki and coworkers.^{65,79} (Table 2). As it is difficult to distinguish between allergic and viral

Table 2
Overview of conducted randomized controlled trials of probiotics for allergy prevention.

Study population and probiotic(s)	Effect on eczema	Effect on sensitization	Effect on respiratory outcomes	Effect on objective lung function measures
Administration to mother only				
Huurre et al, 2008⁵⁷ Maternal allergic disease <i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb-12 1×10^{10} CFU daily from first trimester and then to breastfeeding mother until cessation of exclusive breastfeeding	No Long term outcomes not reported	Not reported	No benefit when this study was pooled in a combined long-term analysis (74)	Not reported
Dotterud et al, 2010⁴⁹ and Simpson et al, 2015⁶⁴ Unselected cohort - about 2/3 with family history of allergic disease <i>L. rhamnosus</i> GG, <i>L. acidophilus</i> LA5, and <i>B. lactis</i> Bb-12 (5×10^{10} CFU of each daily) from 36 weeks gestation and then to breastfeeding mother for 3 months	Reduced cumulative incidence of eczema at 2 and 6 years	No	No	Not reported
Boyle et al, 2011⁴⁸ Any first degree relative with allergic disease <i>L. rhamnosus</i> GG 1.8×10^{10} CFU daily from 36 weeks gestation until delivery - no postnatal administration to mother	No at 12 months Long term outcomes not reported	No	No	Not reported
Rautava et al, 2012⁵⁰ Maternal allergic disease <i>L. rhamnosus</i> LPR and <i>B. longum</i> BL999 or <i>L. paracasei</i> and <i>B. longum</i> BL9 – each probiotic at a daily dose of 1×10^9 CFU from two months before delivery and during two months to breastfeeding mother	Reduction of eczema at 2 years in both probiotic groups Long term outcomes not reported	No	No benefit when this study was pooled in a combined long-term analysis (74)	Not reported
Administration to mother and/or infant				
Kalliomäki et al, 2001⁵² and Kalliomäki et al, 2007⁶⁵ Any first degree relative with allergic disease <i>L. rhamnosus</i> GG 1×10^{10} CFU daily given to mothers 2–4 weeks before delivery and then to breastfeeding mothers or directly to infant, for 6 months	Reduction of eczema at 2 years which remained at 7 years	No	No benefit when this study was pooled in a combined long-term analysis (74)	No Reported allergic rhinitis more common in the probiotic group at 7 years of age
Abrahamsson et al, 2007⁵⁵ and Abrahamsson et al, 2013⁶⁷ Any first degree relative with allergic disease <i>L. reuteri</i> 1×10^9 CFU daily 2–4 weeks before delivery and then to infant for 12 months	No reduction of eczema, but reduction of IgE-associated eczema in the probiotic group at 2 years No difference between the two groups at 7 years follow up	No	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 7 years
Kukkonen et al, 2007⁵⁴ and Kuitunen et al, 2009⁶⁶, Peldan et al, 2017⁷³ Any first degree relative with allergic disease Mix of <i>L. rhamnosus</i> GG and LC705 (both 5×10^9) and <i>B. breve</i> Bb99 and <i>Propionibacterium freudenreichii</i> ssp. <i>shermani</i> JS (both 2×10^9) plus prebiotic galactooligosaccharides; given twice daily to mother 2–4 weeks before delivery and then to infant for 6 months	Eczema reduction in the probiotic group at 2 years and 10 years No eczema reduction at five years	No	No	No differences in FeNO levels between the groups at 5 years in a randomized subpopulation Reported allergic rhinoconjunctivitis increased in the probiotic group at 5–10 years
Kopp et al, 2008⁵³ Any first degree relative with allergic disease <i>L. rhamnosus</i> GG 1×10^{10} CFU daily given to mothers 4–6 weeks before delivery and then to	No at 2 years Long term outcomes not reported	No	No	Not reported

Table 2 (continued)

Study population and probiotic(s)	Effect on eczema	Effect on sensitization	Effect on respiratory outcomes	Effect on objective lung function measures
breastfeeding mother for 3 months or to infant for 6 months Wickens et al, 2008⁵⁶ and Wickens et al, 2013⁶⁸ Any first degree relative with allergic disease <i>L. rhamnosus</i> HN001 or <i>B. lactis</i> HN019 1×10^{10} CFU daily from 2 to 5 weeks before delivery and then to infant directly for 2 years Niers et al, 2009⁶⁸ and Gorissen et al, 2014⁷² Allergic disease of either parent and in at least one sibling <i>Lactococcus lactis</i> W58, <i>B. lactis</i> W52 and <i>B. bifidum</i> W23 1×10^9 CFU each daily six weeks before delivery and then directly to infant for 12 months Kim et al, 2010⁵⁹ Any first degree relative with allergic disease <i>B. bifidum</i> BGN4, <i>B. lactis</i> AD011, and <i>L. acidophilus</i> AD031 (1.6×10^9 CFU of each daily) 4–8 weeks before delivery, 3 months to breastfeeding mother and then to infant from 4 to 6 months Ou et al, 2012⁵¹ Maternal allergic disease <i>L. rhamnosus</i> GG 1×10^{10} CFU daily from second trimester and then 6 months to mother if breastfeeding or directly to infant Allen et al, 2014⁶⁰ Any first degree relative with allergic disease <i>L. salivaris</i> CUL61, <i>L. paracasei</i> CUL08, <i>B. animalis</i> ssp <i>lactis</i> CUL34 and <i>B. bifidum</i> CUL20, 10^{10} CFU daily in total starting 2–4 weeks before delivery and then to the infant for six months	Eczema reduction in the <i>L. rhamnosus</i> group at 2 years which remained until 6 years No benefit of <i>B. lactis</i> Reduced cumulative incidence of eczema in the first three months of life No difference at 6 years Reduced cumulative incidence and prevalence of eczema at 12 months Long term outcomes not reported No Long term outcomes not reported No reduction of eczema, but a reduction of IgE-associated eczema at 2 years of age in the probiotic group	Lower cumulative sensitisation in the group receiving <i>L. rhamnosus</i> at 6 years No benefit of <i>B. lactis</i> No Not reported No Reduced cumulative frequency of sensitization at 2 years in the probiotic group	No No No No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 6 years Not reported Not reported Not reported Not reported
Administration to infant				
Taylor et al, 2007⁶¹ and Jensen et al, 2012⁶⁹ Maternal allergic disease <i>L. acidophilus</i> (LAVRI-A1) 3×10^8 CFU given within 48 h, and then for six months, directly to infant Soh et al, 2009⁶² and Loo et al, 2014⁷⁰ Any first degree relative with allergic disease, <i>L. rhamnosus</i> LPR 1×10^9 CFU and <i>B. longum</i> (BL999) 6×10^8 CFU daily to infant (in infant formula) for 6 months West et al, 2009⁶³ West et al, 2013⁷¹ Mixed (2/3 with at least one first grade relative with allergic disease) <i>L. paracasei</i> ssp <i>paracasei</i> F19 1×10^9 CFU daily to infant (in infant cereal) during weaning from 4 to 13 months	No reduction at 1 year nor at the 5 year follow-up No reduction at 2 or 5 years Reduced cumulative incidence of eczema at 13 months No difference at 8 years	No Sensitisation more common in the probiotic group at 1 year, but not at the later follow-ups No No	No No No	Not reported Not reported No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 8 years

Modified from: West CE. Probiotics for allergy prevention. Beneficial Microbes 2016; 7:171–9.

rhinitis, particularly when relying on parent-reported data, a causal relationship between probiotics and increased allergic rhinitis/rhinoconjunctivitis cannot be concluded.

In all, the current collected evidence does not support the use of probiotics for primary prevention of allergic outcomes other than eczema. On the other hand, there is insufficient evidence to exclude this possibility either, as most conducted RCTs have not been adequately powered to examine the effects of less prevalent allergic outcomes e.g. asthma and food allergy.⁸⁰ For these reasons, there is still need for carefully designed and adequately powered RCTs to examine the effects of probiotics for prevention of food allergy and respiratory allergies.

Prebiotics, synbiotics and allergic outcomes

Human milk oligosaccharides (HMOs) are the third largest fraction in human milk and they serve as substrates for specific microbes to modulate infant gut microbiota composition.⁸¹ They are sometimes referred to as “natural prebiotics” and until very recently, they have not been commercially available. No studies to date have been designed to assess the allergy-preventive effects of HMOs. Although much less structurally diverse than HMOs, specific prebiotics e.g. galactooligosaccharides and/or fructooligosaccharides have been added to infant formula and examined as an allergy-preventive measure. In a systematic review of prebiotics for allergy prevention from 2016⁸² meta-analysis (5 studies, 1313 infants) reported no difference in eczema (RR: 0.57, 95% CI: 0.30–1.08). The two studies (249 infants) that reported early respiratory outcomes were included in a meta-analysis that found reduced infant asthma or recurrent wheeze (RR: 0.37, 95% CI: 0.17–0.80) in infants that had received prebiotics. Only one study examined the risk of food allergy and found a reduced risk (RR: 0.28, 95% CI 0.08–1.00) in prebiotic-treated infants.⁸²

The effects of synbiotics for eczema prevention have been assessed in two RCTs.^{54,83} Meta-analysis of these studies (2 studies, 1320 children) reported the pooled relative risk ratio (RR) to be 0.44 (95% CI, 0.11 to 1.83; $P = 0.26$).⁸⁴ In their meta-analysis, the Kukkonen “synbiotic” study⁵⁴ was included. This study has also been included in many meta-analyses of probiotics.⁸⁰ Collectively, there is need for well-designed studies assessing the effects of both prebiotics and synbiotics for allergy prevention.

Effects on immunological outcomes

Pre- and postnatal probiotic supplementation has been hypothesized to provide microbial stimuli aiding the maturation of well-regulated innate and adaptive immune responses during infancy that protect from early infection, while minimizing inappropriate inflammatory responses to allergens.^{80,85,86} While infants have capacity to initiate Th1 responses, this appears to be relatively suppressed under the influence of the Th-2 promoting hormonal milieu of pregnancy^{87–92} which reflects the close immunological interaction between the mother and her offspring during pregnancy.^{93–96} In some studies this neonatal Th2-skewing is even more marked in infants who later develop allergy^{90,96–98} and there is evidence that these children fail to attenuate their perinatal propensity for Th2 responses as their immune system matures.^{90,91,95,99–102} Appropriate development of regulatory T cell responses^{91,103} and maturation of Th1-like responses^{90,104} are characteristic as the more mature immune phenotype develops during childhood. Also, establishment of an adequate mucosal barrier function, e.g. by increasing secretory IgA production during infancy, seems important to counteract allergic responses.^{29,33,105–107} It is logical to propose that pre- and postnatal probiotic supplementation could be

one strategy to shape appropriate development of systemic and mucosal immunity when these signal are otherwise deficient.

Immune modulatory effects of probiotic administration have been characterized during infancy in several of the randomized double blind placebo-controlled allergy prevention trials. In line with Th1 maturation promoting effects, a higher ratio of anti-CD3/CD28 induced IFN- γ /IL-4 mRNA expression was observed at 13 months of age after feeding *Lactobacillus paracasei* ssp *paracasei* F19, as compared with placebo, during weaning.⁶³ Moreover, pre- and postnatal supplementation with a mixture of *Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Lactococcus lactis* was associated with reduced levels of the Th2 cytokines IL-5 and IL-13 at 3 months of age after anti-CD2/CD28 stimulation of whole blood cultures.⁵⁸ Lower cat allergen induced IL-5 and IL-13 production by peripheral blood mononuclear cells has also been observed in 6-month-olds after pre- and postnatal *Lactobacillus reuteri* supplementation compared with a placebo group, although these children subsequently also showed reduced cat allergen induced IFN- γ levels at 2 years of age.¹⁰⁸ In the same cohort, detection of *L. reuteri* in fecal samples, collected during the first week, was associated with lower circulating levels of the Th2-associated chemokines CCL22 and CCL17 and higher Th1-associated CXCL11 levels at 6 months.⁹⁰ However, comparison of chemokine levels were not significantly different, suggesting potential dose-dependent immune modulatory effects depending on variations in colonization.⁹⁰ Interestingly, in a placebo-controlled intervention trial comparing two different probiotic strains, *Lactobacillus rhamnosus* supplementation 2–5 weeks before delivery was associated with increased cord blood IFN- γ levels whereas *B. lactis* supplementation was not.¹⁰⁹ Furthermore, only the *L. rhamnosus* strain (and not the *B. lactis* strain) showed eczema preventative effects.⁵⁶ Thus, immune modulatory effects vary with dose, strain and also treatment duration and timing. This may also explain why Th1 maturation promoting effects by probiotics have not been consistently observed in all studies.^{110–112}

Beneficial effects of probiotics may also be mediated through improved mucosal barrier function and integrity – potentially through effects on IL-22, which induces epithelial cell proliferation and enhancing production of mucus and antimicrobial peptides.¹¹³ In a recent study increased proportions of IL-22 producing Th cells were observed in 3-month-old infants after maternal pre- and postnatal supplementation with a mixture of *L. rhamnosus*, *Lactobacillus acidophilus* and *B. lactis* compared with the placebo group.¹¹² Even though these children had similar proportions of PMA/ionomycin-induced Th1, Th2 and Th17 cells, changes in barrier function could be of biological significance in reducing mucosal inflammation and the risk of sensitization.

Secretory IgA also plays an important role in enhancing mucosal barrier function.^{31,114} Pre- and postnatal supplementation with a mix of *L. rhamnosus* GG and LC705 and *B. breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermani* JS plus prebiotic galactooligosaccharides tended to be associated with increased fecal IgA levels at 3 months of age¹⁰⁶ and increased plasma IgA levels were observed at 6 months of age in the active compared with the placebo group.¹¹⁵ Increased colostrum IgA levels were observed after *L. rhamnosus* as well as *B. lactis* administration, in comparison with placebo treatment,¹⁰⁹ while IgA levels in colostrum were not affected by probiotic treatment in other allergy intervention trials.^{116,117}

Regulatory T cells can induce IgA isotype switching via secretion of IL-10 and TGF- β .¹¹⁴ Defining regulatory T cells *in vitro* can be difficult, however,^{118,119} and clear effects of probiotic treatment on circulating regulatory T cell populations in infants have not been observed.^{111,112,120} However, reduced responsiveness to allergens¹⁰⁸ and TLR2 ligands¹²¹ *in vitro* after pre- and postnatal *L. reuteri* supplementation may indirectly indicate enhancement of regulatory

responses. Further studies are required to delineate the effects of pre- and postnatal probiotic supplementation on development of systemic and mucosal immunity.

Effects on gut microbiota

Pre- and postnatal probiotic supplementation has been hypothesized to beneficially affect gut microbiota composition.^{3,122} When collating the findings from the different randomized double blind placebo controlled allergy prevention trials, it is important to recognize the impact of varying methodologies used to characterize the gut microbiota composition.^{3,123,124} Findings from traditional culture based methods are difficult to compare to studies based on next generation sequencing. However, even within next generation sequencing studies, there is another layer of variability introduced by the choice of sequencing platform, DNA extraction, primer design, sequencing depth, data processing etc which may also hamper comparability.^{3,123,124}

While there is some evidence for a bifidogenic effect of probiotic supplementation in allergy prevention trials,^{125,126} this has not been consistently observed.^{58,61} Generally, more global gut microbiota diversity promoting effects have not been observed in the early in life probiotic allergy prevention trials, using next generation sequencing methodologies after pre- and postnatal supplementation with *L. reuteri*,¹³ *L. rhamnosus*, *L. acidophilus* and *B. lactis*¹²⁷ or *B. bifidum*, *B. lactis* and *L. lactis*.¹²⁸ There is insufficient data using next generation sequencing methodologies in prebiotic studies for allergy prevention in the perinatal period.

In several studies the specific probiotic strains used in supplement studies have been detected in feces only during the administration period, but not after the intervention,^{56,71,127–129} suggesting only transient colonization. Long-term follow up of gut microbiota development has been performed only in one allergy prevention study so far.¹²⁸ In that study, gut microbiota development was followed to the age of six years, and only minor and short term differences were observed between the probiotic and placebo groups, as determined using 16S–23S rDNA interspace region based profiling.¹²⁸ Another important aspect that only has been evaluated in one study so far,¹³⁰ is whether the eczema preventative effects of the probiotic supplementation are dependent on the intrinsic gut microbiota composition in early infancy. Interestingly, high abundance of *Bifidobacterium dentium* in infant fecal samples collected at 10 days of age was associated with a lack of eczema preventive efficacy by maternal pre- and postnatal supplementation with a mixture of *L. rhamnosus*, *L. acidophilus* and *B. lactis*.¹³⁰

To summarize, while probiotic strains may be transiently detected during the supplementation period in most studies, clear gut microbial diversity promoting effects early in life have not been observed. This does not exclude changes in the metabolic activity of resident microbiota¹³¹ and/or other biologically relevant effects on host immune interactions. Effects on gut microbiota composition are likely to vary with dose, strain and also treatment duration and timing, in line with the reported differences for immunomodulatory and clinical outcomes. Long-term effects of pre- and postnatal probiotic supplementation on gut microbiota development need to be investigated in further studies.

Challenges and current recommendations

We recently discussed the challenges when analyzing the results of conducted studies and meta-analyses in this research area, and these include large heterogeneity and lack of harmonization in both probiotic and prebiotic studies.⁸⁰ Many international expert organizations including the American Academy of Pediatrics,¹³² National Institute of Allergy and Infectious Diseases,¹³³ European

Academy of Allergy and Clinical Immunology,¹³⁴ European Society for Paediatric Gastroenterology, Hepatology and Nutrition¹³⁵ and Food and Agriculture Organization of the United Nations/World Health Organization¹³⁶ do not advocate the use of probiotics or prebiotics for primary prevention of allergic disease.⁸⁰ On the other hand, the GRADE-based guidelines from the World Allergy Organization (WAO) recommend probiotics for the primary prevention of eczema in pregnancy and during breastfeeding when there is high risk of allergic disease (based on a positive family history) and in high risk infants.¹³⁷ WAO also recommends prebiotics in the primary prevention of allergy in non-exclusively breastfed infants.⁸² In accordance with recent meta-analyses and other expert bodies, the WAO guideline panel recognized that the recommendations on both probiotics and prebiotics are conditional and based on very low quality evidence. In their document, conditional recommendations refer to the notion that most patients may want to follow the proposed recommendation, whereas other may not.^{80,82,137} Clinicians need to help families in making decisions according to their preferences.⁸⁰ To date, these general recommendations cannot yet be translated into specific practice recommendations on the most effective strains, dosages or optimal duration of treatment. Collectively, if the families choose to use prebiotics or probiotics, they are unlikely to cause harm,^{135,138} also in a long-term perspective^{66–68,70,74,139,140} however, families should be aware that the beneficial effects are limited and do not include all allergic outcomes.⁸⁰

Summary and perspectives

Recent meta-analyses demonstrate a preventative effect of probiotics on eczema but not any other allergic manifestations. In their GRADE-based recommendation the WAO guideline panel suggests using probiotics in pregnant and breastfeeding women and in infants when there is high risk of allergy based on allergic heredity. Compared with probiotic studies, prebiotic studies for allergy prevention are fewer. Meta-analyses report a benefit of prebiotics on early respiratory outcomes and food allergy and WAO now recommends prebiotics in non-exclusively breastfed infants. To replicate the encouraging results we propose collaborative interdisciplinary multicentre studies with harmonized protocols and outcomes.^{3,8,80,141} Ultimately, such efforts could lead to specific practice guidelines on probiotics, prebiotics and synbiotics in the prevention of allergic diseases and asthma- and possibly other conditions.

In the meantime, this should not delay interim advice to both practitioners and the public about the importance of the early colonization including the role of healthy nutrition, breastfeeding, introduction of complementary foods and nature-relatedness. Nor should it be a substitute for addressing the other economic, environmental, societal and lifestyle factors which are contributing to dysbiotic drift at both an individual and a planetary level. Diseases of 'dysbiosis' (which literally translates to 'life in distress') can be viewed as a human barometer of social and ecological dysbiosis on a global scale.⁶ This underscores the inexorable links between human and environmental health and our responsibility to address these wider issues in the quest to improve human health. Dysbiosis cannot be achieved without addressing these up-stream drivers.⁶

Conflict of interest

CEW has received honoraria from Abbott Nutrition, Nutricia/Danone, Thermo Fisher Scientific and Nestlé Nutrition, and receives royalties from UpToDate. SLP has received honoraria from Abbott Nutrition, Nutricia/Danone, Nestlé Nutrition and Health World, consultancy fees from Bayer Pharmaceuticals and royalties from UpToDate. MCJ has received funding and honoraria for lectures from BioGaia AB, consultant fees and travel support from Nutricia/Danone. MD declares no conflict of interest.

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