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Probiotics for treatment and primary prevention of allergic diseases and asthma:

looking back and moving forward

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Summary

Microbial ecosystems cover the surface of the human body and it is becoming increasingly clear that our modern environment has profound effects on microbial composition and diversity. A dysbiotic gut microbiota has been associated with allergic diseases and asthma in cross-sectional and observational studies. In an attempt to restore this dysbiosis, probiotics have been evaluated in randomized controlled trials. Here, we review treatment and primary prevention studies, recent meta-analyses, and discuss the current understanding of the role of probiotics in this context. Many meta-analyses have shown a moderate benefit of probiotics for eczema prevention, whereas there is less evidence of a benefit for other allergic manifestations. Because of very low quality evidence and heterogeneity between studies, specific advice on the most effective regimens cannot yet be given – not even for eczema prevention. To be able to adopt results into specific recommendations, international expert organizations stress the need for well-designed studies.

Keywords: diversity; dysbiosis; eczema; gut microbiome; hygiene hypothesis; primary prevention; probiotic

Introduction

Our health depends on the health of our environment, and the biodiversity that sustains all life. The human body is home to many vast and diverse microbial ecosystems, which cover all of our inner and outer surfaces. We are only just beginning to understand how the composition and metabolic function of mutualistic microbes affect almost all aspects of our health – ranging from immune development and barrier defence, to our metabolism, appetite regulation, mood and behaviour. Large-scale disruptions of our natural environment by human activity through pollution, modern agricultural practices, sterile and processed foods, and the other collective effects of human activity, are affecting fragile ecosystems – including the human microbiome. Similarly, changing patterns of human behaviour towards more sedentary indoor lifestyles with progressive disconnection from nature are also contributing to the ‘dysbiotic drift’ which has been implicated in rising rates of allergy, metabolic disease, and mental ill health.

The year 2016 marks 100 years since the death of Elie Metchnikoff, who first pioneered the concept that disruption of intestinal microorganisms contributes to human ill-health, and that restoration of lactic acid bacteria with fermented foods can restore health [1]. Although pioneer researcher Dubos went on to publish dozens of experimental studies implicating the intestinal microbiota in multiple aspects of health [summarized in 2], this work has gone largely overlooked. As early as 1962 Dubos concluded that "*Metchnikoff's concepts of intestinal intoxication may have some factual basis after all*" and that he was "*inclined to believe that the usual intestinal flora is an expression of man's total environment, and that its control may turn out to have as profound effects on the well-being of human infants and adults as it has on the growth of mice and farm animals*" [3]. Although overlooked by history, these concepts are now fundamental in the search for strategies to overcome the growing burden of immune, metabolic, and mental disorders which are inextricably linked to modern environmental changes including declining biodiversity and unhealthy obesogenic dietary

practices [4]. There is now enormous concern that each 'cleaner' generation may be starting life with a smaller endowment of ancient microbes than the last [5]. Given the critical importance of the microbiota in early immune maturation and metabolic programming, this has been emerging as a likely culprit in rising rates of immune dysregulation and the growing propensity for inflammation, [recently reviewed in 6]. The children of today are the third generation to live in the 'age of antibiotics', and the third generation to consume 'fast food' and highly processed foods – a major factor in influencing microbial composition, diversity and metabolic activity [7]. Efforts to restore this remain a challenge when we 'don't even know what we've lost'. Probiotics and prebiotics have been the primary avenues to pursue this, although this has been limited by a still rudimentary understanding of the complexity of the microbiota and how to optimally influence its assembly in early life. Here, we review studies using probiotics in the treatment and primary prevention of allergic diseases and asthma, with an emphasis on human studies.

Gut microbiota development

The microbiota plays a fundamental role in normal immune development and regulation [8]. No animal has evolved independently of microbial symbionts and the neonatal immune system seems to have evolved to require diverse developmental signals from microbes to mature normally [8-10]. The first interactions between the microbiota and the host may occur already *in utero* [11, 12]. Thus, recent evidence contradicts the "sterile womb" paradigm, proposing that the sterile fetus first acquires bacteria when passing the birth canal [11, 12]. Any microbial presence *in utero* has been assumed to be dangerous for the fetus and intrauterine infections can lead to preterm birth [13]. Intracellular bacteria were histologically demonstrated in the basal plate, the peripheral region of the placenta on the maternal side in contact with the uterine wall, at a similar rate in preterm and term pregnancies without overt infection, however [13]. Furthermore, extensive deep sequencing studies recently identified a low abundance but metabolically rich placental microbiome present in normal healthy pregnancies at term [11]. This microbiome was primarily composed of non-pathogenic

commensal microbiota from the Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Tenericutes and Fusobacteria phyla [11]. As compared with the microbiomes from the vagina, gut, skin, nose and mouth, the placental microbiome was characterized by higher abundance of Proteobacteria (with *E. coli* the most commonly detected species) and the unique presence of Tenericutes, including *Mycoplasma* and *Ureaplasma* [11]. Unexpectedly, the placental microbiome was more closely related to the mouth microbiome than the vaginal, gut or other body microbiomes, possibly suggesting that the placental microbiome could be partially established by hematogenous spread of oral microbiota [11, 14].

As gut associated bacteria such as *Enterococcus faecium* have been isolated from umbilical cord blood of healthy neonates born by elective Cesarean section [15], it has also been suggested that maternal bacteria may travel to the placenta via the bloodstream after translocation over the gut epithelium [12, 14]. Dendritic cells may facilitate transport of maternal bacteria by taking up luminal bacteria intracellularly and then migrating to lymphoid organs [12]. In murine studies, transmission of orally administered labeled bacterial strains from the mother to her fetus can be demonstrated [15], and pregnancy and lactation is associated with enhanced translocation of gut bacteria to mesenteric lymph nodes [14]. Evidence for microbial maternal transmission is becoming increasingly widespread across the animal kingdom [12]. This may provide the offspring with important microbes at birth, imprinting the offspring microbiota and immune system in preparation for the much larger inoculum transferred during vaginal delivery [10, 12, 16-22] and breastfeeding [10, 12, 14, 17, 23] (Figure 1), and may have shaped the microbiome composition in animal species over evolutionary time. "Heirloom" microbes received from the mother may be uniquely evolved to the offspring's genotype and vertical as compared with horizontal transmission may increase the chance for optimal mutualism [12]. Thus, Caesarean section delivery, which is performed with increasing rates worldwide and may increase the risk for development of allergy and other immune mediated diseases [10], potentially disrupts the opportunities for the microbiota to be transferred from a mother to her baby to provide a genetically tailored microbiota and

optimal mutualism [12]. The potential for vertical transmission of ancestral microorganisms to the next generation is also decreased, particularly when horizontal transmission is also diminished by factors such as antibiotics, antibacterial agents and decreased family size [5]. Delivery by Caesarean section has been associated with persistent changes in the gut microbiota of children followed up to one [17, 18] and two [20] years of age. The disruptions in infant gut microbial ecology caused by Caesarean section delivery include a reduced abundance of the genus *Bacteroides* [16-21] and a decreased diversity of the Bacteroidetes phylum [20]. A reduced abundance and diversity of bifidobacteria has also been observed in children delivered by Caesarean section in some studies [18, 19, 24]. Furthermore, increased occurrence of colonization with *Clostridium difficile*, an opportunistic pathogen, expanding when gut microbiota niches are vacant, has been reported among infants born by Caesarean section [21, 22].

After birth, the early colonizers are accompanied by externally acquired species as the infant gut microbiota diversity increases with age [17, 18, 20, 25]. During this early phase, the gut microbiota composition is highly dynamic within and between infants [20, 25, 26]. As the neonatal gut initially contains oxygen, a large proportion of the early colonizers are aerotolerant facultative bacteria, including *Enterobacter*, *Lactobacillus*, *Staphylococcus* and *Streptococcus* genera [26]. When the bacteria consume the oxygen in the intestine, strict anaerobes, including *Bifidobacteria*, *Bacteroides*, *Eubacteria* and *Clostridia*, begin to dominate at one week of age [18, 26].

Apart from the delivery mode, another strong influence shaping the developing infant gut microbiota relates to nutritional factors in early life. Breastfeeding provides a secondary route of maternal microbial transmission [12, 14, 17, 23]. Gut microbiota diversity increases following weaning and introduction of solid food, with enhanced colonization of butyrate producers, including *Bacteroides* and certain *Clostridium* species [18]. The maternal oral microbiota may have represented another source of vertical microbial transmission during

evolution, e.g. by the mother chewing up solid foods and then feeding the resulting mash to the infant. In summary, vertical transmission of microbial symbionts from mother to offspring occurring through a variety of pre-, peri- and postnatal routes may crucially influence infant microbiota and immune development (Figure 1).

Immune system maturation parallels the gut microbiota development

Interactions occurring between the microbiota and the host during critical time windows of developmental plasticity may exert particularly long lasting effects [8, 10]. Thus, several immunological abnormalities observed in germ free mice can only be reversed if the animals are colonized with symbiotic microbes during the perinatal period, whereas later colonization fails to reverse homeostasis. Normalization of excessive allergen induced Th2 and IgE responses in germ free mice seems to require a critical colonization time window, both after oral [27, 28] and airway [29] exposure to allergens. Furthermore, reversal of Th2 cytokine dependent oxazolone-induced colitis in mice via regulation of iNKT cell homeostasis is only obtained after neonatal colonization [29] or even maternal colonization throughout gestation [30], with similar findings observed for intestinal proinflammatory responses [31, 32] and LPS-induced proinflammatory responses in mesenteric lymph node cells [33].

While it is not clear if a similar critical colonization time window for optimal immune maturation exists in humans, early life events occurring during critical periods of immune development can have long-term impact on development of immune mediated diseases [9, 10, 34]. Even prenatal exposures may shape immune developmental trajectories. For example, maternal exposure to a traditional farm environment during pregnancy protects against allergic sensitization and disease, whereas exposures during infancy alone have weaker or no effect at all [35]. Furthermore, a combined prenatal and postnatal supplementation seems to be important for the preventive effect of probiotics on infant eczema [10, 34, 36-41], (Table 1). It is thus becoming increasingly evident that the maternal microbial environment during pregnancy is important in childhood immune programming [9,

10, 14, 34]. The close immunological interaction between the mother and her offspring during pregnancy [9, 42] provides enormous opportunities for the maternal microbial environment to influence the immune development of her offspring, potentially *via* epigenetic effects [9, 10, 14, 42]. Starting probiotic supplementation already from the second trimester of pregnancy, when circulating fetal T cells have developed, may have a more powerful allergy preventive effect than observed in previous studies where probiotics were administered from the third trimester [10, 34, 36-41]. Furthermore, the impact on maturation of innate and adaptive immune regulatory responses [43, 44] may be more long lasting with a prolonged prenatal probiotic intervention strategy, combined with postnatal supplementation.

The Th2-skewed state of the neonatal immune system [45] is likely a consequence of the intrauterine immune milieu during pregnancy [9]. This neonatal Th2-skewing is even more marked in infants later developing allergic disease [45, 46], supporting that the prenatal immune environment can influence allergy development [9, 42]. The neonatal Th2-bias should then develop toward a more balanced immune phenotype, including maturation of Th1-like responses [45] as well as appropriate development of regulatory T cell [10] and protective mucosal IgA responses [47]. A failure of Th2-silencing during maturation of the immune system may underlie development of Th2-mediated allergic disease [45]. Appropriate microbial stimulation, both pre- and postnatally, may be required to avoid this pathophysiological process [10, 14, 34]. In this respect, the gut microbiota is quantitatively the most important source of microbial stimulation and may provide a primary signal for the maturation of a balanced postnatal innate [48] and adaptive immune system [10].

The gut microbiota differs in composition and diversity during the first months of life in children who later do or do not develop eczema [21, 22, 24, 48, 49] and asthma [50, 51], although no specific microbes with consistently harmful or allergy protective roles have yet been identified. Early establishment of a diverse gut microbiota, with repeated exposure to new bacterial antigens, may be more important than the distribution of specific microbial

species in shaping a normal immune mucosal and systemic maturation [10]. In this regard, perinatal probiotic interventions have generally not affected gut microbiota diversity [25, 49, 52], although the impact of prebiotic and combined probiotic and prebiotic (synbiotic) preventive interventions, which are more likely to affect diversity due to their growth promoting effects on a large variety of bacteria [6], has not been evaluated so far.

Probiotics for treatment of allergic disease and asthma

Eczema

Although the concept of probiotics is not novel [1], a new era of probiotic research targeting infants with food allergy and eczema was initiated in the 1990's. Early studies reported a preliminary benefit of feeding extensively hydrolysed whey formula with added *Lactobacillus rhamnosus* (*L. rhamnosus*) GG [53] and *L. rhamnosus* GG plus *Bifidobacterium lactis* (*B. lactis*) [54] on recovery of infant eczema and markers of intestinal inflammation. This was followed by clinical trials evaluating the effect of probiotics on established eczema in infants, children and adults. In the Cochrane review from 2008 [55], involving 781 infants and children (12 trials), the authors concluded that probiotics are not effective for eczema treatment. In a more recent meta-analysis [56], involving 1599 participants (infants, children and adults from 25 trials), the Scoring Atopic Dermatitis (SCORAD) values favoring probiotic treatment over the control were overall (mean -4.51, 95% CI -6.78 to -2.24), in children 1 to 18 years old (-5.74, 95% CI -7.27 to -4.20), and in adults (-8.26, 95% CI -13.28 to -3.25). In infants < 1 year old, there was no benefit of probiotics. This meta-analysis included studies using synbiotics, *i.e.* probiotics with added prebiotics, but found no evidence of a differential effect of probiotics and synbiotics. However, probiotic treatment with a combination of different bacterial species, or lactobacilli, was superior to treatment with bifidobacteria alone. The authors concluded that probiotics could be considered for eczema treatment in children and adults, despite heterogeneity of studies. As the doses used ranged from 0.3 to 20 billion colony-forming units, this could have contributed to the heterogeneity between studies. The various probiotic products used contained many different strains and the authors decided not

to apply uniform dose criteria. Instead they underscored the need for comparative studies identifying the most effective strains, and optimization of dosing regimens in future studies, including a direct “*head to head comparison study*” to identify the most effective dose [56]. Since probiotic treatment studies have been small and heterogeneous, the generalizability of results has been questioned [57]. Based on the available evidence, expert bodies do not recommend probiotics for treatment of established eczema. An alternative future option might be topical bacteriotherapy as there is preliminary evidence of a benefit in the treatment of eczema. As this is outside the scope of the current review, we recommend a recent, comprehensive summary by Biedermann *et al* [58].

Food allergy

Apart from the early studies assessing the effects of probiotics in infants and children with eczema and food allergy, recent studies are scarcer. Hol and coworkers examined the effect of probiotics (*L. casei* CRL431 plus *B. lactis* Bb-12) added to extensively hydrolyzed casein-based formula (EHCF) on tolerance acquisition in cow's milk allergic infants [59]. They randomly assigned infants to intake of EHCF with probiotics or EHCF alone. At 6 and 12 months of age, infants underwent a double-blind placebo-controlled food challenge to assess tolerance to cow's milk. The cumulative percentage of tolerance was high in both groups, 77% in the probiotic and 81% in the placebo group, with no statistically significant difference between the groups. Probiotic intake was accompanied by slightly lower frequencies of CD3⁺ T cells and CD3⁺CD4⁺ T helper cells at 12 months compared with EHCF without added probiotics, indicating immunomodulatory effects of this probiotic combination. Conversely, Berni Canani *et al* [60], reported a faster recovery when treating cow's milk allergic infants with EHCF with added *L. rhamnosus* GG for six months, compared with EHCF alone. Both of these randomized controlled trials (RCTs) included infants with IgE-mediated and non-IgE-mediated cow's milk allergy, and in the latter, the benefit of EHCF with added LGG was more pronounced in non-IgE-mediated cow's milk allergy [60]. Non-IgE-mediated food allergies are complex disorders and little is yet known about their pathophysiology [61]. Consequently,

future studies are needed to examine the mechanistic effects of probiotics in both IgE-mediated and non-IgE-mediated cow's milk allergy.

Studies assessing probiotics for treatment of food allergy beyond cow's milk allergy are even scarcer. Notably, in a recent study by Tang and coworkers [62], a combination of peanut and probiotic (*L. rhamnosus*) oral immunotherapy (OIT) induced desensitization in 89.7% of the subjects compared with 7.1% in the placebo arm (who received a similar product with neither peanut nor probiotic) ($p < 0.001$). Although promising, a three-arm design is necessary to disentangle if the benefit of the combined peanut and probiotic therapy is superior to peanut OIT alone.

Allergic rhinitis and asthma

Probiotics have been examined in the treatment of allergic rhinitis in RCTs and randomized crossover studies. In a recent systematic review and meta-analysis 1919 patients (23 trials) were included [63]. A variety of outcome measures were used in these trials, e.g. Rhinitis Quality of Life Scores and Rhinitis Total Symptom Scores, but also total and allergen-specific IgE concentrations. Of the 23 studies included, 17 reported a clinical benefit of probiotics in at least one outcome measure. Meta-analysis showed that the overall quality of life scores were improved in those treated with probiotics. No benefit of probiotic treatment was shown for the other outcomes. The authors identified that studies were heterogeneous in terms of the probiotic(s) used, study populations and outcomes measures. They concluded that probiotics may be beneficial for improving quality of life and symptoms in patients with allergic rhinitis, but stressed the need for well-designed studies before general recommendations can be given [63]. Studies have also assessed the effect of probiotics for asthma treatment and in a meta-analysis including four RCTs, there was no benefit [64]. Collectively, the available evidence does not support the use of probiotics for treatment of established allergic diseases or asthma.

Probiotics for primary prevention of allergic diseases and asthma

Eczema

As shown in Table 1, probiotics have been examined as a preventive measure in several RCTs [36-41, 65-74]. All of these studies assessed the preventive effects on eczema in infancy and early childhood. Follow-up data on eczema and respiratory allergic diseases at 4 years of age [75, 76] and in children ≥ 5 years have been reported [77-85] (Table 1), however, several of the studies are still ongoing and follow-up data are not yet available. Probiotics have been administered in the form of drops, in sachets and in infant foods. Most studies have used a combined perinatal administration, with the probiotic given to the mother in pregnancy and then postnatally either to the mother while breast-feeding or to the infant directly (Table 1). Consistent with previous meta-analyses, two very recent meta-analyses concluded that there is a benefit of probiotics for primary prevention of eczema [86, 87]. Cuello-Garica *et al* concluded that probiotic use by pregnant or breast-feeding mothers and/or when given to infants reduced the risk of eczema in infants albeit the certainty of evidence is low due to risk of bias, inconsistency and imprecision of estimated effect [86]. Although the evidence for a combined perinatal intervention is stronger, studies initiating the probiotic intervention postnatally are scarce. It also remains unanswered when in the gestation period the intervention should be initiated, and for how long it should continue in the postnatal period. The authors further stressed the need for well-designed trials and suggested that a direct comparison of different probiotics could be useful [86].

Food allergy and respiratory allergic disease

The benefits for probiotics in the prevention of other allergic conditions remain low [87, 88]. The Prevention Taskforce for the European Academy of Allergy and Clinical Immunology's (EAACI) Guidelines for Food allergy and Anaphylaxis has concluded that current evidence is lacking to support their administration to prevent food allergy [89] and in a recent meta-analysis there was no evidence to support a benefit of perinatal probiotic treatment on doctor diagnosed asthma or childhood wheeze [90]. On the other hand, Cuello-Garcia *et al*

concluded that even though the currently available evidence does not support that probiotics reduce the risk of other allergic manifestations than eczema, the evidence does not exclude such as possibility either [86].

Tailoring probiotic intervention for specific groups

There are also certain high-risk groups that may benefit from probiotic supplementation. In the largest RCT for allergy prevention, supplementation of pregnant women (whose fetuses were at high family risk for atopy) with a lactobacillus and bifidobacterial combination and of their newborns with the same probiotics plus a prebiotic for 6 months (Table 1), Kuitunen *et al* found less IgE-associated eczema and food sensitization at age 2 but not at age 5 [39, 81]. However, Cesarean delivered children (n=149) who were treated with probiotics had significantly fewer IgE-associated allergic diseases at age 5, particularly eczema (15.7 % vs 30.4%; Odds Ratio, 0.43; 95% CI, 0.19 – 0.95) and food sensitization (10.0% vs 25.3%; Odds Ratio, 0.33; 95% CI, 0.12 – 0.85) compared with Cesarean delivered children who received placebo. These associations were not adjusted for any factors such as extent of breastfeeding, siblingship or pets in the home. The Cesarean section rate was 16.7% in this study. No results were reported for vaginally delivered children.

In a recent publication from the population-based MoBa (Norwegian Mother and Child Cohort Study) observational cohort study, the effectiveness of lactobacillus-bifidobacterial milk and yogurt (Biola, Cultura) product consumption was evaluated in 35,000 vaginally delivered infants in Norway [91]. Questionnaire-reported maternal milk and yogurt consumption during pregnancy at 18, 22 and 30 weeks of gestation and postnatally at 6, 18, and 36 months, in conjunction with the administration of the same probiotics to infants between 6-18 months of age, significantly reduced the likelihood of eczema at 6 months (adjusted Odds Ratio, 0.94; 95%CI: 0.89-0.99) and rhinoconjunctivitis (adjusted Odds Ratio, 0.87; 95%CI: 0.78-0.98) at 18-36 months. These associations were independent of breast-feeding status; maternal history of allergy, age, smoking during pregnancy, pre-pregnancy

BMI, dietary fiber intake and energy intake; parity; infant's sex and mode of delivery. A reduction in allergic disease outcomes with probiotic treatment was not observed in 5,557 Cesarean-delivered infants, comprising 15% of the cohort. Even though this is still a large sample size, it cannot be precluded that loss of statistical power contributed to this finding. Also inconsistent with the Kuitunen *et al* high risk study [81] were results related to family history of atopy. One quarter of women had self-reported atopy in the MoBa cohort [91]. No reduction in atopic disease after probiotic treatment was seen in their infants, in contrast to statistical significance achieved among infants with a negative family history.

Several cohort studies have confirmed that relative to vaginal delivery, Cesarean section delays post birth increases in the abundance and diversity of bifidobacteria, and reduces maternal-infant sharing of bifidobacterial species [18, 19, 24], a phenomenon which appears to be remedied by infant probiotic treatment in the Kuitunen study [81]. Indeed, bifidobacterial diversity was also observed to be lower among cesarean-delivered infants who developed eczema in the Hong *et al* study, yet it did not differ between vaginally born infants who did and did not develop eczema [24]. The nature of gut dysbiosis that predicts atopic disease remains to be determined. In the interim, it is conceivable that probiotic-induced alterations to infant gut microbial composition, directly and indirectly through breast milk [92] enhance colonization resistance to microbes such as *C. difficile*, and this change is more beneficial to the infant after Cesarean delivery. Colonization of the infant gut with *C. difficile* is becoming increasingly more prevalent than it was in the 1980s [26], especially following Cesarean delivery [16]. Associations between Cesarean induced dysbiosis of the gut and atopic disease have been found to be mediated by *C. difficile* colonization [21, 22].

As nicely shown by Mastromarino *et al* [23], inconsistencies in the effectiveness of maternal probiotic treatment in preventing allergic disease in offspring may be attributed to birth method since Cesarean delivery may alter the systemic effects of probiotics in the mother. In their study of probiotic treatment between 36 weeks of gestation and 1 month after birth,

elevations in colostrum and mature breast milk of lactobacilli and bifidobacteria were reported after the probiotic treatment of women who delivered vaginally (n=46) but not among women who had delivered by cesarean (n=20). What then, could explain conflicting results in probiotic effectiveness within cesarean-delivered infants? In both the Kuitunen and Bertelsen studies [81, 91] mothers and infants received probiotic formulations prenatally and following delivery for approximately 6 months. Differences in probiotic dose, duration or species strain, co-administration of prebiotics (Kuitunen study), lack of randomization and blinding (Bertelsen study), age and method of atopic disease evaluation are candidate explanations, as are sample size, family history of atopy, duration of breastfeeding, extent of antibiotic use, and other confounding factors which can bias observational cohorts and randomized controlled trials. But type of Cesarean section also has a potential role to play. Newborns are not exposed to maternal vaginal microbes such as lactobacilli, during elective Cesarean but this exposure may occur during emergency Cesarean after attempted vaginal birth. Hence, the early gut microbiota profiles of infants delivered by emergency Cesarean are found to differ from those following elective Cesarean, independent of breastfeeding status [17]. In neither the Kuitunen nor Bertelsen studies were separate results presented for elective versus emergency cesarean or the percentage contribution for each type reported within the cesarean groups [81, 91]. Further in Norway, women undergoing elective cesarean delivery were not pre-treated with antibiotics at the time of the MOBA cohort study [93]. Data from the clinical follow-up at school age of the Kuitunen study [81], will provide more insight into the effects of perinatal probiotic treatment but also on long-term outcomes. Ultimately, new intervention studies designed to evaluate if stronger long term effects of probiotic supplementation are observed in children delivered by cesarean section than in vaginally delivered children would further clarify these issues.

Current recommendations

In 2015 the World Allergy Organization applied the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach to the evidence in this field [94], which

balances desirable and undesirable consequences, the quality of the evidence, and consumer preferences and values [95]. Consistent with other meta-analyses [86, 87] and considering all critical outcomes they concluded that there is a net benefit from using probiotics (in pregnancy, lactation and in infancy) resulting from the prevention of eczema when there is considered to be a high-risk of allergy (based on a history of allergy in the immediate family). Based on this the WAO guideline panel suggested using probiotics in pregnant and lactating women, and in infants when there is high risk of allergy in the children. This recommendation places a relatively high value on prevention of eczema in children, and a relatively lower value on avoiding possible adverse effects [94]. The panel also recognized that the recommendation was supported by very low quality evidence, and there was a lack of evidence that probiotics prevented any other allergy. Although the strain of probiotic is likely to be important, there is insufficient evidence to recommend specific strains at specific times, or for specific durations. For this reason, it has been difficult to translate the WAO recommendations into practice guidelines [96, 97]. In our own attempt to do this, we highlighted to consumers and practitioners that *'the great heterogeneity of the studies makes it difficult to advise on specifics regarding therapy (eg, strains, dose, timing, and duration)'* [96]. Based on the paucity of evidence to address these questions, we were unable to recommend giving probiotics during pregnancy, lactation and infancy for the prevention of eczema. However, we also recognize that *'the data suggest that there is a modest preventive effect of probiotics on the development of eczema, but not other atopic diseases, in at-risk infants (defined as presence of a biologic parent or sibling with asthma, allergic rhinitis, eczema, or food allergy)'* [96]. Based on these potential benefits, in our experience some parents are still interested in using probiotics, particularly as though risk of adverse effects is low. In that situation scenario, we emphasize that documented benefits appear limited to eczema, that the evidence is weak, and that any risk reduction is likely to be small.

So what do we say in our own personal clinical practice when confronted with this question? Our approach is to emphasize that currently the best way to improve our healthy gut microbiome is a healthy balanced diet - rich in fiber and fresh fruit and vegetables – particularly in pregnancy, lactation and infancy, but ideally at every stage of life. This is likely to have many other benefits for the mother, and lasting effects on the child, including healthy dietary patterns and future taste preferences. It is currently difficult to recommend specific commercial products for ‘gut health’, and a whole food approach is likely to have more general health and societal benefits. For respiratory outcomes, we also support the conclusions emanating from the 2013 National Heart Lung and Blood Institute (NHLBI) workshop on the primary prevention of chronic lung diseases, suggesting that potential universal lung health strategies to prevent chronic lung disease could include prevention of preterm delivery, reduction of exposure of the fetus and young infant to environmental pollutants and tobacco smoke, reduction of psychosocial stress and prevention of maternal and child obesity [98].

Conclusion

With increasing evidence that our modern environment has detrimental effects on the human gut microbiota and its diversity, there has been intense interest in the promotion and restoration of gut microbial composition using probiotic interventions. While meta-analyses have shown a moderate effect of probiotics for eczema prevention, there is less evidence of an effect for the treatment and prevention of other allergic manifestations. Because of very low quality evidence and heterogeneity between studies, specific advice on probiotic regimens cannot yet be given for any allergic condition. Looking back, we can see that the still rudimentary understanding of the complexity of gut microbiota- host interactions has made it difficult to design the most effective interventions. Moving forward, we see the need for multidisciplinary collaborations in large networks [6, 10]. We anticipate that powerful sequencing techniques and bioinformatics tools will aid in deciphering the mechanistic effects of probiotics, however, to be able to deliver specific clinical guidelines, we need

adequately powered and prospective clinical trials, using uniform strategies and clinical outcome measures [6, 10, 96-97].

Expert commentary

Probiotics are “live micro-organisms, which when administered in adequate amounts, confer a health benefit on the host [99]. Typically, strains of lactobacilli and bifidobacteria, or their combinations, have been evaluated for the treatment or prevention of manifestations of allergic disease in clinical trials. Meta-analyses show a benefit of probiotics for eczema prevention [86, 87], but there is limited evidence of an effect in the treatment and prevention of other allergic manifestations. In 2015 the World Allergy Organization (WAO) launched guidelines on the use of probiotics as a preventive strategy to reduce allergic diseases [94]. Considering all critical outcomes, they concluded that there is a net benefit from using probiotics (in pregnancy, lactation and in infancy) resulting from the prevention of eczema when there is considered to be a high-risk of allergy (a history of allergy in the immediate family). The WAO guideline panel thus suggested using probiotics in pregnant and lactating women, and in infants when there is high risk of allergy in the children. However, the panel stressed that the recommendation was supported by very low quality evidence, and that there was a lack of evidence that probiotics prevented any other allergy. Although the strain of probiotic is likely to be important, there is insufficient evidence to recommend specific strains at specific times, or for specific durations. For this reason, it has been difficult to translate the WAO recommendations into practice guidelines [96, 97]. Consequently, specific recommendations on probiotic regimens cannot yet be given.

Five-year view

Probiotics remain a promising strategy to influence early gut colonization, gut integrity and immune response patterns in allergic disease. We anticipate metagenomic, metaproteomic and metabolomic studies examining the impact of probiotic supplementation to shed light on the mechanistic and functional aspects of probiotic interventions. As long-term follow data on

already initiated cohorts using probiotics for the primary prevention of allergic disease are limited, we await the results of currently ongoing clinical trials with interest. The use of synbiotics is another emerging option, which is anticipated to have more global effects on intestinal colonization. The results of ongoing clinical trials examining the effects of synbiotics in the treatment and prevention of allergic disease will provide more insight.

Key issues

- A dysbiotic gut microbiota is associated with increased risk of developing allergic diseases and asthma in cross-sectional and observational studies, but a clear cause-effect relationship has not been demonstrated.
- Animal models provide evidence that intestinal colonization is key to normal development of immune functions and regulation.
- The WHO/FAO definition of probiotics is “Live micro-organisms, which when administered in adequate amounts, confer a health benefit on the host” [1].
- Probiotics have immunomodulating effects and these effects are considered strain-specific.
- The most commonly used probiotics for prevention and treatment of allergic diseases and asthma are strains of lactobacilli and bifidobacteria, and their combinations.
- Meta-analyses have shown a moderate benefit of probiotics for eczema prevention, but there is little evidence of a treatment or preventive effect for other allergic manifestations.
- Very low quality evidence and heterogeneity between studies have precluded translation of the results from clinical trials and meta-analyses into clear clinical recommendations.
- There is still need for well-designed, prospective clinical trials both in the treatment and prevention of allergic diseases and asthma.

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Reference annotations

* Of interest

** Of considerable interest

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colonized hosts were protected against experimental iNKT cell-mediated, oxazolone-induced colitis. When germ free pregnant dams were cohoused with *B. fragilis* monocolonized mice before delivery, the offspring's colonic iNKT cell numbers were not normalized to the level observed when germ free dams were mated with *B. fragilis* monocolonized mice, although at time of delivery, the mother was heavily colonized with *B. fragilis* bacteria. Thus, presence of *B. fragilis* during a prolonged prenatal period seemed to be required for achieving iNKT cell homeostasis.

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Figure 1:

Transmission of microbes from the mother to the fetus during pregnancy may provide the offspring with important microbes at birth, imprinting the microbiota and immune system in preparation for the much larger inoculum transferred during vaginal delivery and breastfeeding. "Heirloom" microbes received from the mother may be uniquely evolved to the offspring's genotype, increasing the chance for optimal mutualism.

Table 1:

Study population Probiotic(s) and dose (References)	Effect on eczema	Effect on sensitization	Effect on respiratory allergic disease	Effect on objective lung function measures
<i>MATERNAL ADMINISTRATION ONLY</i>				
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 Boyle et al, 2011 (68)	No at 1 year Long term outcomes not reported	No	No	Not reported
Unselected- 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 Dotterud et al, 2010 (37) and Simpson et al, 2015 (83)	Reduced cumulative incidence of eczema at 2 and 6 years	No	No	Not reported
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 Rautava et al, 2012 (40)	Reduction of eczema at 2 years in both probiotic groups Long term outcomes not reported	No	Not reported	Not reported
<i>PERINATAL ADMINISTRATION TO MOTHER AND/OR CHILD</i>				
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 mother or directly to infant for 6	Reduction of eczema at 2 years which remained at 4 and 7 years	No	No	No

months Kalliomäki et al, 2001 (38), Kalliomäki et al 2003 (75) and Kalliomäki et al, 2007 (80)				
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 breastfeeding mother for 3 months or to infant for 6 months Kopp et al, 2008 (70)	No at 2 years Long term outcomes not reported	No	No	Not reported
Any first degree relative with allergic disease Mix of <i>L. rhamnosus</i> GG and LC705 (both 5×10^9) and <i>B.</i> <i>breve</i> Bb99 and <i>Propionibacterium</i> <i>freudenreichii</i> ssp. <i>shermani</i> JS (both 2×10^9) plus prebiotic galacto-oligosaccharides; given twice daily to mother 2-4 weeks before delivery and then to infant for 6 months Kukkonen et al, 2007 (39) and Kuitunen et al, 2009 (81)	Eczema reduction in the probiotic group at 2 years No eczema reduction at 5 years	No	No	No differences in FeNO levels between the groups at 5 years in a randomized subpopulation.
Any first degree relative with allergic disease <i>L. reuteri</i> 1×10^8 CFU daily 2- 4 weeks before delivery and then to infant for 12 months Abrahamsson et al, 2007 (36) and Abrahamsson et al, 2013 (77)	No reduction of eczema, but reduction of IgE- associated eczema in the probiotic group at 2 years No difference between the groups at the 7-year follow up	No	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 7 years
Any first degree relative with allergic disease <i>L. rhamnosus</i> HN001 or <i>Bifidobacterium lactis</i> (<i>B.</i> <i>lactis</i>) HN019 1×10^{10} CFU daily from 2-5 weeks before	Eczema reduction in the <i>L. rhamnosus</i> group at 2 years which remained	Yes, lower cumulative prevalence of sensitization in the group receiving <i>L.</i> <i>rhamnosus</i> at	Reduction of allergic rhinitis at 4 years with both probiotics.	No differences between the groups when evaluated by spirometry reversibility test and

delivery and then to infant directly for 2 years Wickens et al, 2008 (41), Wickens et al 2012 (76) and Wickens et al, 2013 (85)	until 6 years No benefit of <i>B. lactis</i>	6 years No benefit of <i>B. lactis</i>	No difference between the groups in wheeze or asthma	FeNO levels at 6 years
Maternal allergic disease in ~80% of the study population <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 Huurre et al, 2008 (74)	No Long term outcomes not reported	No, but reduced sensitization in infants of sensitized mothers at 1 year in a subgroup analysis.	Not reported	Not reported
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 Niers et al, 2009 (71) and Gorissen et al, 2014 (78)	Reduced cumulative incidence of eczema in the first three months of life No difference between the groups at the 6-year follow up	No	No	Not reported
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 Kim et al, 2010 (69)	Reduced cumulative incidence and prevalence of eczema at 12 months Long term outcomes not reported	Not reported	Not reported	Not reported
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	No Long term outcomes not reported	No	No	Not reported

Ou et al, 2012 (72)				
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 the to infant for six months Allen et al, 2014 (67)	No reduction of eczema, but a reduction of IgE-associated eczema at 2 years in the probiotic group Long term outcomes not reported	Not reported	No	Not reported
POSTNATAL ADMINISTRATION				
Maternal allergic disease <i>L. acidophilus</i> (LAVRI-A1) 3 x 10 ⁸ CFU given within 48 hours, and then for six months, directly to infant Taylor et al, 2007 (66) and Jensen et al, 2012 (79)	No reduction at 1 year nor at the 5 year follow-up	No Sensitization more common in the probiotic group at 1 year, but not at the later follow-ups	No	Not reported
Any first degree relative with allergic disease <i>L. rhamnosus</i> LPR 1 x 10 ⁹ CFU and <i>B. longum</i> (BL999) 6 x 10 ⁸ CFU daily to infant (in infant formula) for 6 months Soh et al, 2009 (65) and Loo et al, 2014 (82)	No reduction at 2 or 5 years	No	No	Not reported
Mixed (2/3 with at least one first grade relative with allergic disease) <i>L. paracasei</i> ssp <i>paracasei</i> F19 1 x 10 ⁹ CFU daily to infant (in infant cereal) during weaning from 4-13 months West et al, 2009 (73) and West et al, 2013 (84)	Reduced cumulative incidence of eczema at 13 months No difference between the groups at the 8-year follow up	No	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 8 years

CFU- colony-forming units; FeNO- fractional exhaled nitric oxide; IgE- Immunoglobulin E

The table was modified from West C.E. Probiotics for allergy prevention. *Benef Microbes*, in press (ref 97).