Pre- and probiotics for allergy prevention: time to revisit recommendations?

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Pre- and probiotics for allergy prevention: time to revisit recommendations?

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Abstract

Reduced intensity and diversity of microbial exposure is considered a major factor driving abnormal postnatal immune maturation and increasing allergy prevalence, particularly in more affluent regions. Quantitatively the largest important source of early immune-microbial interaction, the gut microbiota is of particular interest in this context, with variations in composition and diversity in the first months of life associated with subsequent allergy development. Attempting to restore the health consequences of the 'dysbiotic drift' in modern society, interventions modulating gut microbiota for allergy prevention have been evaluated in several randomized placebo controlled trials. In this review, we provide an

overview of these trials and discuss recommendations from international expert bodies regarding prebiotic, probiotic and synbiotic interventions. Recent guidelines from the World Allergy Organization recommend the use of probiotics for the primary prevention of eczema in pregnant and breastfeeding mothers of infants at high risk for developing allergy and in high risk infants. It is however stressed that these recommendations are conditional, based on very low quality evidence and great heterogeneity between studies, which also impedes specific and practical advice to consumers on the most effective regimens. We discuss how the choice of probiotic strains, timing and duration of administration can critically influence the outcome due to different effects on immune modulation and gut microbiota composition. Furthermore, we propose strategies to potentially improve allergy preventive effects and enable future evidence-based implementation.

Introduction

The increasing allergy prevalence in affluent countries has been striking. While this is likely to be multi-factorial, reduced intensity and diversity of microbial stimulation are possible major factors promoting abnormal postnatal immune maturation [1, 2]. In support of this hypothesis, children who later develop allergic disease show differences in the composition and diversity of their gut microbiota during the first months of life compared with those who do not [3-14]. Accordingly, interventions to modulate the gut microbiota have been of key interest as potential allergy preventive strategies, and have now been evaluated in a series of double blind placebo controlled randomised trials [15-17]. Here, we provide an overview of the results of these trials, discuss recent recommendations that have arisen as a result of these microbiota modulating interventions, highlight potential immunomodulatory mechanisms and propose future strategies to confirm and potentially improve allergy preventive effects.

Primary prevention studies using probiotics

Eczema

Several randomized controlled trials (RCTs) have examined the effects of probiotics, defined as “live microorganisms which when ingested in adequate amounts confer a beneficial effect

on the host" [18], for primary prevention of early manifestations of allergic diseases, *e.g.* eczema and IgE-associated eczema [19-34] (Table 1). As shown in Table 1, the probiotic preparations used have generally included strains of lactobacilli and bifidobacteria, either as single strains or in combination. Long-term follow-up data that include respiratory outcomes as well have been reported from some [35-43] but not all studies, as several are still ongoing (Table 1).

Two meta-analyses published in 2015 concluded that there is a benefit of probiotics for primary prevention of eczema, but not for any other allergic manifestations [16, 44]. Zuccotti *et al* [44] included 17 studies (4755 children) in their meta-analysis and found that treatment with probiotics led to a significantly lower relative risk (RR) for eczema compared with placebo (RR 0.78; 95% CI: 0.69-0.89), and that the effect was most pronounced when a combination of probiotic strains was used (RR 0.54; 95% CI: 0.43-0.68). No benefit of probiotics was found for wheeze, asthma or rhinoconjunctivitis. Cuello-Garcia *et al* [16] identified and included 29 studies in their meta-analysis, although some of these were follow-up studies of non-unique populations, and evaluated the effects according to timing and method of probiotic administration. Probiotics were reported to reduce the risk of eczema (follow-up period until 24 months of age) when taken in the last trimester of pregnancy (RR 0.71; 95% CI, 0.60-0.84), when taken by breast-feeding mothers (RR 0.57; 95% CI, 0.47-0.69), or when given to infants and/or mothers (RR, 0.80; 95% CI, 0.68-0.94). However, no significant effect on eczema development was observed when probiotics were administered only to infants (RR, 0.83; 95% CI, 0.58-1.19). Consistent with the meta-analysis of Zuccotti *et al,* [44] no benefit on any other allergic manifestation was reported. The certainty in the evidence when evaluated by the Grading of Recommendation Assessment Development and Evaluation (GRADE) approach was found to be low or very low due to "risk of bias, inconsistency and imprecision of results, and indirectness of available research" [16]. Although the evidence for a combined perinatal intervention appears stronger, it is still open to question when in the gestation period the intervention should be initiated, and for how long it should continue in the postnatal period [17].

Atopy, food allergy and respiratory allergic disease

In a recent meta-analysis of 17 trials (2947 infants) [45], pooled analysis indicated that a combined pre- and postnatal probiotic treatment reduced the risk of (any) sensitization (RR 0.78; 95% CI 0.66–0.92), especially when administered prenatally to the pregnant mother and postnatally to the infant (RR 0.71; 95% CI 0.57–0.89); and also the risk of food sensitization (RR 0.77; 95% CI 0.61–0.98). Prenatal or postnatal probiotic administration

alone did not influence the risk of sensitization. The authors concluded that there is still need for studies assessing the effects of probiotics for prevention of food allergy using objective evaluations, *i.e.* food challenges [45]. This was also identified by the Prevention Taskforce for the European Academy of Allergy and Clinical Immunology's (EAACI) Guidelines for Food Allergy and Anaphylaxis that concluded that the current available evidence does not support the use of probiotics for food allergy prevention [46]. Similarly, for respiratory allergies, the evidence remains low. In a meta-analysis of 9 trials (3257 children) the RR of diagnosed asthma in children randomized to receive probiotics was 0.99 (95% CI 0.81 -1.21) and the RR of incident wheeze was 0.97 (95%CI 0.87- 1.09), based on 9 trials (1949 children) [47]. Collectively, the current available evidence does not support a role for probiotics for prevention of other allergic manifestations than eczema. The evidence does not exclude such as possibility either, however [16, 17], as the majority of studies has not been adequately powered to examine the effects of less prevalent allergic manifestations (*e.g.* asthma and food allergy). To summarize, more RCTs are needed to examine the role of probiotics for primary prevention of atopy, food allergy and respiratory allergies.

Primary prevention studies using prebiotics

Prebiotics have been defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [48]. Human milk is plentiful of human milk oligosaccharides that serve as substrates for specific microbes and shape infant gut microbiota establishment [49]. Consequently, galactooligosaccharides and/or fructooligosaccharides have been added to infant formula to try to mimic the effects of HMOs when breastfeeding is not feasible. In the most recent systematic review of prebiotics for allergy prevention [48], meta-analysis of five studies (1313 infants) found no significant difference in eczema (RR: 0.57, 95 % CI: 0.30-1.08); whereas meta-analysis of the two studies (249 infants) that reported early respiratory outcomes found a reduction in infant asthma or recurrent wheeze (RR: 0.37, 95 % CI: 0.17-0.80) in prebiotic-treated infants. One single study assessed the risk of developing food allergy and reported a reduction (R: 0.28, 95 % CI 0.08-1.00) by prebiotics [50].

The first RCT to examine the effects of prebiotics for allergy prevention included non-exclusively breastfed infants at high risk of allergic disease (based on parental family history) [51]. Infants were assigned to an extensively hydrolysed formula with (or without) prebiotics (90% short-chain galactooligosaccharides (scGOS) and 10% long-chain fructooligosaccharides (lcFOS)), which approximates to the proportions of these

oligosaccharides in human milk. Partial breastfeeding was allowed until 6 weeks of age. There was a significant decrease in the cumulative incidence of eczema at six months of age in the prebiotic compared with the placebo group (9.8% versus 23.1%) [51] and the benefit was sustained at two and five years of age [52, 53], although limited by a high drop-out rate at the latter ages. Ivakhnenko *et al* [54], also found reduced cumulative incidence of eczema at 18 months of age in an open RCT of non-breastfed children fed standard formula with scGOS/lcFOS compared with standard formula without any addition. In a double-blind RCT including children at low risk of atopy (based on the absence of allergic heredity), there was a transient benefit of prebiotics (nonhydrolyzed cow's milk–based formula with scGOS and lcFOS and long-chain fructo-OS, ratio 9:1, plus specific pectin-derived acidic oligosaccharides) on eczema in the first year of life [55], but this was not sustained at preschool age [56]. The authors concluded that although prebiotics transiently prevented early eczema in this non-breastfed low atopy risk population, the number needed to treat to prevent 1 case of eczema was 25 infants. Thus, recommendations need to weigh the cost, effort and burden of these interventions against transient benefits [56]. Collectively, more carefully conducted RCTs in both high and low atopy risk populations are needed before firm conclusions on the effectiveness of prebiotics for allergy prevention in formula-fed infants can be drawn.

Primary prevention studies using synbiotics

Although less studied, synbiotics (a combination of prebiotics and probiotics) have also been examined for the prevention of eczema [25, 57]. In a recent meta-analysis of synbiotics [58], the pooled relative risk ratio (RR) of eczema for synbiotic treatment versus placebo was 0.44 (95% CI, 0.11-1.83) (2 studies, 1320 children). This meta-analysis included the Kukkonen 'synbiotic' study [25] (Table 1) that has also been included in most meta-analyses of 'probiotics' for primary prevention of allergic diseases. The review concluded that there is still need for studies to assess the effects of synbiotics for primary prevention of eczema [58] and obviously, this includes the need to assess the effect on other allergic outcomes as well.

Challenges when evaluating and comparing pre- and probiotics for allergy prevention

As identified in many reviews and opinion papers, the lack of harmonisation of probiotic primary prevention studies hampers direct comparison. It also remains to be determined which preventive strategy is most effective, including the optimal strains, dosages, timing and duration. As discussed by Cuello-Garcia *et al* [16], there is still call for well-designed and executed RCTs to examine the effects of probiotics in the prevention of *all* allergic diseases,

as well as potential adverse effects, to reduce the overall risk of bias. Compared with primary prevention studies using probiotics, there are still relatively few published studies using prebiotics specifically for allergy prevention, although the nutritional benefit of prebiotics has been examined in other studies. Still, lack of harmonisation is apparent in existing prebiotic studies as well. Collectively, there is a call for uniform clinical outcome assessments and harmonisation of protocols in future prebiotic and probiotic studies.

Recent recommendations regarding probiotics and prebiotics for eczema prevention

International expert bodies including EAACI, the American Academy of Pediatrics, European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), National Institute of Allergy and Infectious Diseases (NIAID) and Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) [46, 59-62] do not generally recommend probiotics for allergy prevention at this time. However, recent GRADE based guidelines from the World Allergy Organization (WAO) concluded that, when taking into account all the critical outcomes, there is a likely net advantage of probiotics, resulting primarily from eczema prevention [50]. However, there was a lack of evidence that probiotics prevented any other allergic conditions. As discussed, these findings are consistent with recent meta-analyses [16, 44, 45, 47]. In otherwise healthy individuals, the WAO guideline panel suggested considering using probiotics in pregnant women, during breastfeeding, and in infancy if the child is at high risk of developing allergic disease – where this risk is defined by family history of allergic disease in a first-degree relative. In their report, the WAO guideline panel also stressed that the recommendations are conditional, and based on very low quality evidence due to the great heterogeneity between studies [50]. The heterogeneity between studies also makes it difficult to translate these recommendations into practical advice regarding specific strains, optimal dosages and treatment timing and duration [50]. Choice of strains, treatment duration and timing can have different effects on vertical transmission, immune modulation and gut microbiota composition, as discussed in more detail below, thus critically influencing the preventive outcome.

Even more recently, the WAO guideline panel suggested using prebiotic supplementation in not-exclusively breastfed infants for allergy prevention and not using prebiotic supplementation in exclusively breastfed infants, also based on GRADE evidence decision frameworks [48]. Again, the panel stressed that the recommendations are conditional and based on very low certainty of the evidence.

Microbial transmission from mother to offspring and possible varying capacity for vertical transmission between probiotic strains

The importance of a combined prenatal and postnatal supplementation for the preventive effect of probiotics on infant eczema suggests that the maternal microbial environment during pregnancy is involved in shaping childhood immune maturation [1, 17, 63-65]. In support of this, maternal exposure to a traditional farm environment during pregnancy confers stronger protection against allergic sensitisation and disease than postnatal exposure alone [66]. The mechanisms by which prenatal exposures influence immune developmental trajectories need to be clarified, but are the likely result of the close immunological interaction between mother and foetus during pregnancy [17, 63-65, 67]. Recently, direct presentation of maternal bacterial components to the foetus has been recognised as a potential route for immune imprinting [17, 65, 67, 68], which may in some way prepare for the much larger inoculum transferred during vaginal delivery [10, 69-74] and breastfeeding [69, 73, 75, 76].

This adds to the increasing evidence that the first interactions between the microbiota and the host are initiated *in utero,* contrary to assumptions of a “sterile womb” paradigm in which the first acquisition of bacteria occurs at birth [69, 77, 78]. Any microbial presence *in utero* has been assumed to be dangerous for the foetus, based on intrauterine infections as a risk factor for preterm birth [79]. However, intracellular bacteria have been histologically demonstrated at a similar rate in the basal plate (the peripheral region of the placenta on the maternal side in contact with the uterine wall) in preterm and term pregnancies without overt infection [79]. Furthermore, bacterial DNA has been detected in placenta [78, 80, 81], amniotic fluid [78, 81], umbilical cord [82] and meconium [78, 83, 84] after ‘sterile’ term elective caesarean section deliveries. Finally, a low abundance but metabolically rich placental microbiome was identified in normal healthy pregnancies at term by extensive deep sequencing [77]. Importantly, data obtained by 16S rRNA gene sequencing only demonstrates the presence of microbial DNA, without direct evidence of viable bacteria. Nonetheless, the presence of microbial DNA in the intrauterine compartment suggests that the fetus may be in direct contact with microbial components during gestation [65]. Similarities between the placental and oral microbiome composition [77] have led to speculation that the placental microbiome is partially established by haematogenous spread of oral microbiota [65, 77]. Microbiota sampling and characterization from the same pregnant women at multiple sites would give important information to address this further.

Another hypothesis is that maternal bacteria may reach the placenta via the bloodstream after dendritic cell facilitated translocation over the gut epithelium [65, 69]. An experimental mouse study using labelled *Enterococcus faecium* demonstrated transfer of maternal bacteria to foetuses *in utero via* the gastrointestinal tract [82], and enhanced translocation of gut bacteria to mesenteric lymph nodes has been demonstrated during pregnancy and lactation [65, 85]. In support of an entero-mammary-pathway, maternal intestinal microbes have been detected in immune cells circulating in peripheral blood and in breast milk in both lactating mice and humans [85]. Furthermore, the probiotic bacterium *Lactobacillus reuteri* could be detected in colostrum after administration from gestational week 36 to delivery in mothers participating in an allergy intervention study [75]. It would be highly interesting to investigate whether probiotic bacterial components may be transferred from the mother to her foetus *in utero* after maternal supplementation in future human intervention studies.

Vertical transmission of maternal vaginal and gut microbes to the neonate occurs during vaginal delivery [10, 69-74]. Caesarean section (CS) delivery, which is performed with increasing rates worldwide and may increase the risk for development of allergy and other immune mediated diseases [1], thus disrupts the opportunities for the microbiota to be transferred from a mother to her baby [10, 69-74]. Vaginally delivered infants, but not infants born by CS, share a significantly higher proportion of gut microbiota 16S rRNA gene sequences with their own mother than with other mothers during the first year of life [71, 72]. The importance of maternal gut derived bacteria in early infant gut colonization is also supported by the findings of a recent one month-follow up study, where CS delivered neonates were inoculated with maternal vaginal microbes [86]. Thus, the gut microbiota of the infants was not influenced by the ”vaginal seeding” to the same extent as their skin and oral microbiota, as maternal gut derived bacteria, which are specialized to thrive in this niche, expanded in the stool samples of vaginally delivered but not inoculated CS delivered neonates [86].

It needs to be established how probiotics are transferred from mother to offspring when the mother is supplemented during pregnancy and lactation [87]. A recent study suggested that the capacity for vertical transmission may vary between different probiotic strains. Mothers were supplemented with a mixture of three probiotic strains; *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp *lactis* Bb-12 and *Lactobacillus acidophilus* La-5 from 36 weeks gestation and during breastfeeding for three months [88]. Only *Lactobacillus rhamnosus* GG and not the other probiotic strains were detected in infant stool samples during the first three months of life, however [88]. The influence of mode of delivery would have been interesting to address, but this information was unfortunately not available.

Further studies on the complex interactions between the maternal and offspring microbiome and immunity are needed to identify strategies to avert the allergy epidemic.

What are the immune modulating effects of probiotics?

Breast milk composition may be affected by probiotics

Probiotics may affect the composition of breast milk since nutritional, metabolic and immunological processes in the gut could be reflected in the mammary gland and milk *via* the entero-mammary pathway [69]. In addition to providing nutrients for growth and development, breast milk also contains many important immunological components. In several probiotic intervention studies, the influence of supplementation on the immune profile of breast milk has been investigated (Supplementary Table 1). In 3 month samples transforming growth factor-β2 (TGF-β2) was increased in breast milk from mothers receiving *L. rhamnosus* GG compared with placebo [89]. Another study found that colostrum TGF-β2 levels were higher in individuals treated with *L. rhamnosus* GG and *B. lactis* Bb than with placebo but no other mediators measured were affected by supplementation [28]. In contrast, TGF-β2 levels in colostrum were decreased after supplementation with *L. reuteri* compared with placebo and also associated with less likelihood to become sensitized during their first two years in life [90]. The same study found increased levels of interleukin-10 (IL-10) in colostrum of probiotic treated mothers [90]. Increased IL-10 levels and reduced levels of immunoglobulin A (IgA) to casein were observed in 3 month milk samples after supplementation with a mix of *L. rhamnosus* GG and LC705 and *B. breve* Bb99 and *Proprionibacterium freudenreichii* ssp. *shermani JS* plus prebiotic galactooligosaccharides in another cohort, while total IgA levels and IgA levels to cow milk (CM), beta-lactoglobulin (BLG) and ovalbumin (OVA) in colostrum were similar [91]. In this study, human neutrophil alpha-defensins (HNP1-3), human β-defensin 2 (HBD2) or sCD14 levels were not affected by the synbiotic treatment [92]. In contrast, another study found lower milk levels of sCD14 at day 7 and total IgA at day 28 in *L. rhamnosus* GG compared with placebo treated participants, while TGF-β1 levels were not affected by the intervention [19]. However, colostrum TGF-β1 levels were increased after *B. lactis* supplementation in another study, with a similar tendency after *L. rhamnosus* supplementation [93].

Increased colostrum IgA levels were observed after both *B. lactis* and *L. rhamnosus* administration [93]. In conclusion, supplementation has not consistently affected breast milk TGF-β1, TGF-β2 and IgA levels and immunomodulatory effects likely vary between strains.

Probiotic supplementation may induce some peripheral tolerance

Several theories have been proposed regarding the effect of probiotic supplementation on peripheral immune responses, including enhanced immune maturation, increased T helper 1 (Th1) associated immunity, but also induction of T regulatory cells (Tregs) and increased tolerance. Studies have collected both cord and peripheral blood mononuclear cells and by various measures tried to elucidate the effect of supplementation on peripheral immunity (Supplementary Table 1)**.** However, prenatal *L. rhamnosus* GG supplementation did not influence dendritic cell (DC) and Treg phenotype and numbers [94]. In the same cohort, no differences in cytokine production after stimulation of CBMC with Toll Like receptor (TLR) ligands were observed [19]. Another study investigated the effect of *L. rhamnosus* GG stimulation on cord blood mononuclear cells (CBMC) and found that stimulation resulted in enhanced release of IL-10 and interferon-γ (IFN-γ) but independently of probiotic supplementation [95]. After pre- and postnatal *L. reuteri* supplementation, reduced allergen responsiveness was observed during the first two years of life in the probiotic compared with the placebo group, *i.e.* reduced cat allergen induced levels of IL-5 and IL-13 at 6 months, IFN-γ at 24 months, IL-10 at birth and 12 months [96]. Furthermore, probiotic supplementation was associated with reduced CCL22 levels after birch stimulation at 24 months [96]. Also, in the same cohort, probiotic supplementation was associated with reduced Lipoteichoic acid (LTA) induced C-C Motif Chemokine Ligand (CCL4), C-X-C Motif Chemokine Ligand (CXCL8), IL-1β and IL-6 levels [97]. Reduced anti-CD2/CD28 induced IL-5 and IL-13 levels in whole blood cultures was noted at 3 months of age after pre- and postnatal supplementation with a mixture of *B. bifidum*, *B. lactis* and *L. lactis* as compared with placebo [29]. The same pattern with reduced responses to polyclonal stimuli with Staphylococcal Enterotoxin B (SEB) (lower IL-5 and TGF-β levels) and house dust mite (HDM) allergens (lower tumour necrosis factor (TNF) and IL-10) at 6 months

were found after postnatal *L. acidophilus* as compared with placebo administration [98], while responses to TLR2 and TLR4 [99] and Treg frequencies were not affected by the intervention [100]. Feeding *L. paracasei* ssp *paracasei F19* during weaning was associated with a higher ratio of anti-CD3/CD28 induced IFN-γ/IL-4 [34] and IFN-γ/IL-2 mRNA [101] at 13 months of age.

Collectively, probiotic supplementation during pregnancy and/or infancy may be associated with reduced cytokine responses to certain stimuli. All studies have slightly different designs and time points for sample collection, however, in addition to the variation in probiotic strains and treatment duration.

Immune deviation in vivo as measured by circulating immunoglobulin, cytokine and chemokine levels

Circulating chemokine and cytokine levels may reflect immune deviation *in vivo*. Probiotic supplementation has shown minor effects on these mediators (Supplementary Table 1). In an intervention trial using two strains, *L. rhamnosus* but not B. *lactis* supplementation was associated with increased cord blood IFN-γ levels as compared with placebo [93]. Pre- and postnatal synbiotic administration led to elevated C-reactive protein (CRP), total IgA, total IgE and IL-10 levels at 6 months [102], suggestive of a low-grade inflammation. Total IgE levels at 13 months were not affected by feeding *L. paracasei* ssp *paracasei F19* during weaning, however [34]. In another intervention study, detection of *L. reuteri* in faeces, collected during the first week, was associated with lower levels of the Th2-associated chemokines CCL22 and CCL17 and higher Th1-associated CXCL11 levels at 6 months, while the levels were not significantly different in the probiotic *vs* placebo group [103].

To summarise, consistent effects on infant immune deviation *in vivo* by probiotic supplementation have not yet been observed, possibly due to strain specific effects.

Effects on antibody titres to vaccines

As the immunomodulatory mechanisms behind probiotic supplementation are still unclear, effects on immune responsiveness to vaccines in probiotic supplemented infants can provide further clues and are also of importance from a safety point of view. Supplementation postnatally with *L. rhamnosus* LPR and *B. longum* was found to enhance Hepatitis B (HepB) surface antibody responses at 12 months in subjects receiving monovalent doses of HepB vaccine at birth, 1 month and a DTPa–HepB combination vaccine at 6 months, but not those who received 3 monovalent doses [104]. Supplementation with *L. paracasei* ssp *paracas*ei F19 (LF19) during weaning increased the capacity to mount responses to vaccine protein antigens, but not a polysaccharide antigen [105]. More specifically, antibody concentrations to *Haemophilus influenzae* type b (Hib) capsular polysaccharide (HibPS), diphtheria toxin (D) and tetanus toxoid (T) before and after the second and third doses were measured. LF19 enhanced antibody concentrations to D and T, especially in infants breastfed less than 6 months. Conversely, breastfeeding duration influenced the anti-HibPS concentrations, with no effect by LF19 [105]. In another intervention study using a mix of *L. rhamnosus* GG and LC705 and *B. breve* Bb99 and *Proprionibacterium freudenreichii* ssp*. shermani JS* plus prebiotic galactooligosaccharides, infants were immunized with a DTwP (diphtheria, tetanus and whole cell pertussis) and with a Hib polysaccharide. In the probiotic group, protective Hib antibody concentrations occurred more frequently at 6 months, while diphtheria and tetanus, IgG titers were comparable in the different groups [106]. Thus, while there is some evidence that probiotic supplementation may enhance antibody responses to certain vaccine antigens, the specific effects seem to vary between strains.

Epigenetic modulation after probiotic interventions

Epigenetic modifications can alter the DNA sequence without heritable changes and have been shown to be important in perinatal immune programming. The effects of pre- and postnatal probiotic supplementation may thus be mediated by epigenetic mechanisms [17]. No published studies so far have investigated the effect of probiotic supplementation on epigenetic regulation in infants, and it would be interesting to see studies reporting the epigenetic effects of intervention.

Genetic influences on clinical outcomes

Genetic predisposition may affect the outcome of intervention trials, since eczema prevalence for example are different in various regions were studies have been conducted [107]. One study found that 26 TLR Single nucleotide polymorphisms (SNPs) interacted with *L. rhamnosus* resulting in a reduced risk of eczema, while only two interacted with *B. lactis* resulting in a reduced risk of eczema, eczema severity or atopy [108]. Another study from the same cohort found that infants carrying an eczema susceptibility genetic variant (among 33 eczema susceptibility SNPs in eleven genes) were less likely to develop eczema if they had been randomised to the *L. rhamnosus* group compared to placebo. *B. lactis* were also capable to protect against the effect of some SNPs [109]. Genetic effects on clinical outcomes have not been reported in other intervention studies.

Safety reports

There have been discussions about the safety of using live bacteria in intervention trials including pregnant and lactating mothers as well as neonates and infants. No severe adverse events have been reported in allergy prevention trials, although on rare occasions sepsis has been observed in high-risk immunocompromised patients [110]. Intake of lactobacilli and bifidobacteria during pregnancy had no effect on the incidence of caesarean section, birth weight, or gestational age in a pooled analysis of several different studies [111]. In addition, several studies have evaluated the effect of supplementation on height and weight development in children, after follow up for 4 to 8 years [36-38, 40, 112, 113]. Administration of *L. reuteri* [37], *L. paracasei* ssp *paracasei* F19 [112], *L. rhamnosus* GG [114] a combination with *L. rhamnosus* HN001 or *B. lactis* HN019 [38], *L. rhamnosus* LGG and *B. longum* BL999 [40], synbiotic mix of *L. rhamnosus* GG and LC705, *B. breve* Bb99 and *Proprionibacterium freudenreichii ssp. Shermani* JS plus prebiotic galactooligosaccharides [36] had no effects on these measures. Haemoglobin values decreased during administration of the synbiotic mix but at age 2 the hematologic values in both groups were equal [115]. In summary, probiotic supplementation during pregnancy and infancy may be considered safe.

The effect of probiotics on gut microbiota composition

Probiotic supplementation has been hypothesised to have a beneficial effect on the gut microbiota. However, when comparing the results from different studies it is important to acknowledge how varying methodologies may affect the findings. Traditional culture based methods are hard to compare with the next generation sequencing tools that are available today. There is some evidence for a bifidogenic effect of probiotic supplementation [116, 117], although this has not been consistently observed [29, 32]. Also, the probiotic strain has been detected in faeces during but not after the administration period in several studies (Supplementary Table 1).

The effect of prenatal *L. rhamnosus* GG supplementation on infant gut microbiota development was evaluated by quantitative Polymerase Chain Reaction (qPCR) for *Bifidobacterium* quantity [116] and Terminal Restriction Fragment Length Polymorphism (T-RFLP) for *Bifidobacterium* [116] or overall species composition [118]. At one week, diversity was not promoted by *L. rhamnosus* GG supplementation [118], and at 90 days of age infants of supplemented mothers were more often colonised with *B. longum* [116]. Furthermore, pre- and postnatal supplementation with *L. rhamnosus* GG enhanced the early bifidobacterial diversity in infants in another cohort [117]. Higher counts of bifidobacteria were found at 2 years of age after supplementation with *L. reuteri* compared with placebo to the mother from gestational week 36 to delivery and to the child during the first 12 months [75], while no effects on gut microbiota diversity was detected by next generation sequencing [8]. *L. reuteri* was found in the majority of supplemented infants stool, with the highest recording at 5-6 days of age [75]. Increased faecal counts of all supplemented bacteria were observed when feeding infants a mix of *L. rhamnosus* GG and LC705, *B. breve* Bb99 and *Proprionibacterium freudenreichii ssp. Shermani JS* plus prebiotic galactosaccharides for 6 months, while no differences between groups were observed at 2 years of age [25]. In another study investigating the effect of maternal supplementation with *L. rhamnosus* GG, *B. animalis* subsp. *lactis* Bb-12 and *L. acidophilus* La-5 from 36 weeks gestation and during breastfeeding for three months, *L. rhamnosus* GG was detected more frequently by qPCR in infant stool samples in the supplemented group than the placebo group at 10 days and 3 months but not at 1 and 2 years, while the other

strains were not detected more frequently in the probiotic than the supplemented group at any time point [88]. Gut microbiota diversity was not affected by the intervention, as analysed by next generation sequencing [88]. It may be possible that certain strains of probiotics are more efficient colonisers than other supplemented strains also after direct administration to the infant. One study comparing *L. rhamnosus* HN001 and *B. lactis* HN019 supplementation found that *L. rhamnosus* was more likely than *B. lactis* to be present in stool samples at 3 months, although detection rates were similar at 24 months, at the end of the supplementation period [27]. In addition, in another study *L. lactis* and *B. bifidum* but not *B. lactis* were detectable more often in the probiotic group (*L. lactis, B. lactis* and *B. bifidum*) compared with placebo at 3 months of age [29]. Infants supplemented with *L. acidophilus* were more often colonised with lactobacilli at 6 months but no other significant differences were observed [32].

Long term follow up of gut microbiota development has been performed so far in one study [119] up to the age of six years, where only minor and short term differences were observed between the probiotic and placebo groups using 16S–23S rDNA interspace region based profiling. Children were reported to have a gut microbiota development determined by age rather than intervention and atopic status.

In conclusion, while the probiotic strain may be transiently detected during the supplementation period in most studies, clear gut microbial diversity promoting effects early in life have not been observed. Long-term effects remain to be investigated, as few such studies have been performed. The effects on gut microbiota composition seem to depend on choice of strain and treatment duration, which is consistent with the reported strain-specific differences also for immunomodulatory and clinical outcomes.

How may the WAO recommendations be received and handled by clinicians and parents?

When giving advice in medical care it is important to have a discussion about the ethics in giving recommendations. When is it ethical to give advice and recommendations? The enormous amount of information that parents are required to

handle and process when attending the medical care during pregnancy is also an important consideration. As previously mentioned, WAO has given conditional guidelines for probiotic use [50], concluding 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. There was a lack of evidence that probiotics prevented any other allergy, however. According to the document conditional recommendations mean that the majority of patients may want the suggested course of action, but others may not. Clinicians are required to guide families in making decisions consistent with their values and preferences. Good scientific support is required in when translating general recommendations to 'specific' practical guidance, and this is still lacking (regarding exactly which strains to use, when exactly to start these and when to cease them). The balance is that variations in these parameters are unlikely to cause harm, if families choose to use these products. Families should also be made aware that the protective effects are limited and so far only apply to eczema.

What is needed to address these uncertainties - for more specific recommendations to consumers

The fact that the WAO recommendations are supported by low quality evidence by the GRADE guidelines [50] does not mean that the studies are necessarily of low quality, but rather that they are very heterogeneous in design. This contributes to the difficulty in translating WAO recommendations to specifics regarding choice of strains, dose, timing, mode of administration and duration. Further research is warranted to determine the differential effects of these factors on immune modulation and gut microbiota composition. One way to address this is a well-coordinated multicentre collaborative effort, which could include harmonised studies focused on different aspects of this issue but collectively with sufficient power to look at both long term outcomes and assess the differential effects in different risk groups (*i.e.* such as caesarean delivery), in different genetic backgrounds and in environmental contexts where the risk of disease may also be different. Similar designs of these harmonised studies regarding strains, dose, timing, mode of administration and duration are important. We contend that most previous studies have focused on only

late pregnancy – largely with the focus of achieving vertical transmission of the microbiota, rather than on the direct immunomodulatory effects of optimising the maternal microbiome *in utero*. Together with prebiotics, probiotics (studied separately and/or together) is an important avenue of investigation. Importantly, probiotics are regarded as safe during pregnancy [111], and even in premature neonates where they have become standard practice in many centres to reduce the risk of necrotizing enterocolitis [120]. Thus, supplementing women earlier in pregnancy is both feasible and reasonable and should be an important element of multicentre efforts. While this is an ideal scenario, cross-continental/jurisdictional studies face many challenges – including substantive funding and regulatory challenges. If researchers work together in consortia these challenges will become more surmountable.

Conclusion

Meta-analyses show a benefit of probiotics for prevention of eczema but not other allergic symptoms, and the WAO guidelines suggest using probiotics in pregnant and lactating women and in infants when there is high risk of allergy in the children. Further research is required to be able to translate the WAO recommendations into practice guidelines, however, as specific advice on choice of strains, dose, timing, mode of administration and duration is not possible to give due to the great heterogeneity between studies performed so far [1, 17, 121, 122]. Replication of the promising results in collaborative well-coordinated multicentre harmonised studies with multidisciplinary expertise in paediatrics, immunology and microbiology would thus be of great importance to enable future evidence-based implementation.

Table 1				
Study population and probiotic intervention	***Effect on eczema***	***Effect on sensitization***	***Effect on respiratory symptoms***	***Effect on lung function measures***
MATERNAL ADMINISTRATION ONLY				
Huurre *et al*, 2008 [28] Maternal allergic disease *L. rhamnosus* GG and *B. lactis* Bb-12 $1x10^{10}$ CFU daily from first trimester and then to breastfeeding mother until cessation of exclusive breastfeeding	No Long term outcomes not reported	Not reported	Not reported	Not reported
Dotterud *et al*, 2010 [20] and **Simpson *et al*, 2015** [43] Unselected - about 2/3 with family history of allergic disease *L. rhamnosus* GG, *L. acidophilus* LA5, and *B. lactis* Bb-12 (5 x 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*, 2011 [19] Any first degree relative with allergic disease *L. rhamnosus GG* 1.8 x 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*, 2012 [21] Maternal allergic disease *L. rhamnosus* LPR and *B. longum* BL999 or *L. paracasei* and *B. longum* BL9 – each probiotic at a daily dose of 1x 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	Not reported	Not reported
PERINATAL ADMINISTRATION TO MOTHER AND/OR CHILD				
Kalliomäki *et al*, 2001 [23] and **Kalliomäki *et al*, 2007** [35] Any first degree relative with allergic disease *L. rhamnosus GG* 1x10^{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	No
Abrahamsson *et al*, 2007 [26] and **Abrahamsson *et al,* 2013** [37] Any first degree relative with allergic disease *L. reuteri* 1 x 10^8 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*, 2007 [25] and **Kuitunen *et al*, 2009** [36] Any first degree relative with allergic disease	Eczema reduction in the probiotic group at 2 years	No	No	No differences in FeNO levels between the groups at 5 years in a randomized

Mix of *L. rhamnosus* GG and LC705 (both 5 x 10^9) and *B. breve* Bb99 and *Proprionibacterium freudenreichii ssp. shermani JS* (both 2 x 10^9) plus prebiotic galactooligosaccharides; given twice daily to mother 2-4 weeks before delivery and then to infant for 6 months	No eczema reduction at five years			subpopulation
Kopp *et al*, 2008 [24] Any first degree relative with allergic disease *L. rhamnosus* GG 1x10^{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	No at 2 years Long term outcomes not reported	No	No	Not reported
Wickens *et al*, 2008 [27] and **Wickens *et al*, 2013** [38] Any first degree relative with allergic disease *L. rhamnosus* HN001 or *B. lactis* HN019 1x10^{10} CFU daily from 2-5 weeks before delivery and then to infant directly for 2 years	Eczema reduction in the *L. rhamnosus* group at 2 years which remained until 6 years No benefit of *B. lactis*	Lower cumulative sensitisation in the group receiving *L. rhamnosus* at 6 years No benefit of *B. lactis*	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 6 years
Niers *et al*, 2009 [29] and **Gorissen *et al*, 2014** [42] Allergic disease of either parent and in at least one sibling *Lactococcus lactis* W58, *B. lactis* W52 and *B. bifidum* W23 1 x 10^9 CFU each daily six weeks before delivery and then directly to infant for 12 months	Reduced cumulative incidence of eczema in the first three months of life No difference at 6 years	No	No	Not reported
Kim *et al*, 2010 [30] Any first degree relative with allergic disease	Reduced cumulative incidence and prevalence	Not reported	Not reported	Not reported

B. bifidum BGN4, *B. lactis* AD011, and *L. acidophilus* AD031(1.6 x 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	of eczema at 12 months Long term outcomes not reported			
Ou *et al*, 2012 [22] Maternal allergic disease *L. rhamnosus* GG 1 x 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
Allen *et al*, 2014 [31] Any first degree relative with allergic disease *L. salivaris* CUL61*, L. paracasei* CUL08, *B. animalis ssp lactis* CUL34 and *B. bifidum* CUL20, 10^{10} CFU daily in total starting 2-4 weeks before delivery and then to the infant for six months	No reduction of eczema, but a reduction of IgE-associated eczema at 2 years of age in the probiotic group	Not reported	No	Not reported
POSTNATAL ADMINISTRATION				
Taylor *et al*, 2007 [32] and **Jensen *et al*, 2012** [39] Maternal allergic disease *L. acidophilus* (LAVRI-A1) 3 x 10^8 CFU given within 48 hours, and then for six months, directly to infant	No reduction at 1 year nor at the or 5 year follow-up	No Sensitisation more common in the probiotic group at 1 year, but not at the later follow-ups	No	Not reported

Soh *et al*, 2009 [33] and **Loo *et al*, 2014** [40] Any first degree relative with allergic disease, *L. rhamnous LPR* 1 x 10^9 CFU and *B. longum* (BL999) 6 x 10^8 CFU daily to infant (in infant formula) for 6 months	No reduction at 2 or 5 years	No	No	Not reported
West *et al*, 2009 [34] **West *et al*, 2013** [41] Mixed (2/3 with at least one first grade relative with allergic disease) *L. paracasei ssp paracas*ei F19 1 x 10^9 CFU daily to infant (in infant cereal) during weaning from 4-13 months	Reduced cumulative incidence of eczema at 13 months No difference at 8 years	No	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 8 years

The table was modified from West CE, Probiotics for allergy prevention, Beneficial Microbes 2016; 7: 171-9

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Supplementary Table 1						
Study population and probiotic intervention	***Effect on eczema***	***Effect on sensitization***	***Effect on respiratory symptoms***	***Effect on l ung function measures***	***Immunomodulatory effects***	***Effects on gut microbiota***
MATERNAL ADMINISTRATION ONLY						
Huurre *et al*, 2008 [28] Maternal allergic disease *L. rhamnosus* GG and *B. lactis* Bb-12 $1x10^{10}$ CFU daily from first trimester and then to breastfeeding mother until cessation of exclusive breastfeeding	No Long term outcomes not reported	Not reported	Not reported	Not reported	**Huurre 2008**; The breast milk TGF-β2, sCD14, IFN-γ, TNF, IL-10, IL-6, IL-4 and IL-2 levels were measured from samples taken immediately after birth and 1 month after delivery. The colostrum TGF-β2 levels tended to be higher in the probiotic than the placebo group, while the other mediators were not affected [28].	Not reported
Dotterud *et al*, 2010 [20] and **Simpson *et al*, 2015** [43] Unselected - about 2/3 with family history of allergic disease *L. rhamnosus* GG, *L. acidophilus* LA5, and *B. lactis* 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	Not reported	**Dotterud 2015**; Infant stool samples from 10 days, 3 months, 1 year and 2 years, were analysed by qPCR and 16S rRNA gene deep sequencing on the Illumina MiSeq platform. Only the LGG bacteria colonized the children at 10 days and at 3 months of age. There were no significant differences in the abundance of the probiotic bacteria between the groups at 1 and 2 years of age, nor for the bacterial classes and genera, alpha and beta diversity [88].
Boyle *et al*, 2011 [19] Any first degree relative with allergic disease *L. rhamnosus 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	**Boyle 2008**; CBMCs from 73 neonates were cultured with heat-killed LGG, ovalbumin (OVA) or without stimulus. LGG treatment of pregnant women did not influence CD4+ T cell proliferation, FoxP3 expression, DC phenotype or cytokine secretion in CBMCs cultured with heat-killed LGG or OVA [94]. **Boyle 2011**; CBMCs from 73 neonates were examined for DC and Treg numbers and LTA and LPS induced production of TGF-β, IL-10, IL-12p40, IL-13, IFN-γ and TNF. Prenatal probiotic treatment was not associated with any change in cord blood immune markers or cytokine secretion. Breast milk samples from the probiotic group had lower levels of total IgA at day 28 and lower sCD14 at day 7, while breast milk TGF-β1 levels were not affected [19].	**Lahtinen 2009**; Investigated infant faecal samples from 7 and 90 days using qPCR and T-RFLP. At 90 days of age, infants whose mothers received LGG were more often colonized with species belonging to the *B longum* group. Bifidobacterial species colonization at 7 days or *Bifidobacterium* levels did not differ between the 2 groups [116]. **Ismail 2012;** using T-RFLP analysis showed that prenatal LGG failed to modulate diversity of early infant gut microbiota despite promoting a beneficial bifidobacteria profile [118].
Rautava *et al*, 2012 [21] Maternal allergic disease	Reduction of eczema at 2	No	Not reported	Not reported	Not reported	Not reported

L. rhamnosus LPR and *B. longum* BL999 or *L. paracasei* and *B. longum* BL9 – each probiotic at a daily dose of 1x 10^9 CFU from two months before delivery and during two months to breastfeeding mother	years in both probiotic groups Long term outcomes not reported					
PERINATAL ADMINISTRATION TO MOTHER AND/OR CHILD						
Kalliomäki *et al*, 2001 [23] and **Kalliomäki *et al*, 2007** [35] Any first degree relative with allergic disease *L. rhamnosus GG* 1x10^{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	No	**Rautava 2002**; Probiotic administration increased the amount of anti-inflammatory TGF-β2 in the milk at three months in mothers receiving probiotics as analysed by ELISA. Infants with elevated IgE in cord blood benefited most of the probiotic supplementation [89].	**Guiemonde 2006**; At 5 days of age, infants whose mothers received *L. rhamnosus* GG showed a significantly higher occurrence of *B. breve* and lower of *B. adolescentis* than those from the placebo group, as determined by PCR. In addition, *L. rhamnosus* GG consumption increased the bifidobacterial diversity at 3 weeks in infants [117].
Abrahamsson *et al*, 2007 [26] and **Abrahamsson *et al,* 2013** [37] Any first degree relative with allergic disease *L. reuteri* 1 x 10^8 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	**Abrahamsson 2011**; There were no significant difference in chemokine levels between the *L. reuteri* and the placebo-treated group, at CB, 6-, 12- and 24 months. The presence of *L. reuteri* in stool the first week of life was associated with lower CCL22 and CCL17 and higher CXCL11 levels at 6 months of age [103]. **Böttcher 2008**; Probiotic supplementation was associated with low levels of TGF-β2 and slightly increased levels of IL-10 in colostrum. Infants receiving breast milk with low TGF-β2 levels were less likely to become sensitized during their first 2 yr of life. The levels of total IgA, SIgA, TGF-β1, TNF, sCD14, and Na/K ratios in breast milk were not affected by *L. reuteri* intake [90]. **Forsberg 2013;** Probiotic treatment was associated with low cat-induced IL-5 and IL-13 responses at 6 months, with a similar trend for IL-5 at 12 months. Cat-induced IFN-γ responses were also lower after probiotic than after placebo treatment at 24 months, with similar findings for IL-10 at birth and at 12 months. At 24 months, birch induced CCL22 levels were lower in the probiotic than in the placebo group [96]. **Forsberg 2014;** Probiotic supplementation was	**Abrahamsson 2009**; The prevalence of *L reuteri* was higher during the first year of life in the stool samples from infants in the probiotic group. The highest prevalence was recorded at 5 to 6 days of age (82% in the treated vs 20% in the placebo group). Supplementation affected neither the prevalence nor the counts of bifidobacteria or *C difficile*, except for higher counts of bifidobacteria in the treated group at 2 years. At 12 months the prevalence of *L reuteri* was lower in breast-fed than formula-fed infant. *L. reuteri* was isolated from 12% and 2% of the colostrum samples in the probiotic and placebo group, respectively [75]. **Abrahamsson 2012**; Probiotic supplementation did not affect gut microbiota diversity at 1 and 12 months, as determined by 16S rDNA 454-pyrosequencing [8].

					associated with decreased LTA induced CCL4, CXCL8, IL-1β and IL-6 responses at 12 months and decreased CCL4 and IL-1β secretion at 24 months. TLR2 and TLR4 mRNA expression and responses to LPS were not affected by probiotic treatment [97].	
Kukkonen *et al*, 2007 [25] and **Kuitunen *et al*, 2009** [36] Any first degree relative with allergic disease Mix of *L. rhamnosus* GG and LC705 (both 5 x 10^9) and *B. breve* Bb99 and *Proprionibacterium freudenreichii ssp. shermani JS* (both 2 x 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 No eczema reduction at five years	No	No	No differences in FeNO levels between the groups at 5 years in a randomized subpopulation	**Kukkonen 2006;** In the probiotic compared with the placebo group, protective antibody concentrations to Haemophilus influenzae type b (Hib) occurred more frequently at 6 months, and geometric mean IgG titres to Hib tended to be higher. IgG titres to diphtheria and tetanus were similar in the 2 groups [106]. **Marschan 2008;** Infants receiving probiotic bacteria had higher plasma levels of CRP, total IgA, total IgE, and IL-10 than infants in the placebo group [102]. **Kuitunen 2009;** Probiotic supplementation caused a gut mucosal inflammation with decreased Hb values at 6 months, but Hb and the other hematologic values were similar at 2 years in the 2 groups [115]. **Kuitunen 2012**; Probiotic supplementation was associated with less IgA to casein and more IL-10 in mature BM (3 month samples) but not in colostrum, and with reduced colostral but not mature BM TGF-β2 levels. Probiotic supplementation did not affect colostrum or mature BM total IgA levels, nor levels of IgA antibodies to CM, BLG and OVA [91]. **Savilathi 2015**; Colostrum and 3 month BM sCD14, human neutrophil peptide (HNP) 1–3 and β-defensin 2 (HBD2) levels were not affected by probiotic supplementation [123].	**Kukkonen 2007**; Faecal counts of all the supplemented microbes were significantly higher at 3 and 6 months. At 2 years, no differences were observed between study groups in faecal bacterial colonization using agar culturing and PCR [25]. **Kukkonen 2009**; Faecal IgA, α1-AT, TNF and calprotectin was measured at the age of 3 and 6 months. Probiotics tended to augment faecal IgA and significantly increased faecal a1-AT. High intestinal IgA associated with reduced allergy risk [124].
Kopp *et al*, 2008 [24] Any first degree relative with allergic disease *L. rhamnosus* GG 1x10^{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	No at 2 years Long term outcomes not reported	No	No	Not reported	**Kopp 2008**; CBMC and PBMC of the corresponding mother were isolated from cord blood and peripheral blood (n=68). Cells were stimulated with IL-2, β-lactoglobulin or LGG and IFN-γ, IL-10 and IL-13 in the supernatants were measured with ELISA. LGG induced IL-10 and IFN-γ secretion *in vitro*, but independently of probiotic supplementation [95].	Not reported
Wickens *et al*, 2008 [27] and **Wickens *et al*, 2013** [38]	Eczema reduction in	Lower cumulative	No	No differences between the	**Prescott 2008;** Neonates of mothers who received *L. rhamnosus* but not *B. lactis* had	**Wickens 2008**; *L. rhamnosus* (71.5%) was more likely than *B. lactis* (22.6%) to be

Any first degree relative with allergic disease *L. rhamnosus* HN001 or *B. lactis* HN019 1x10^{10} CFU daily from 2-5 weeks before delivery and then to infant directly for 2 years	the *L. rhamnosus* group at 2 years which remained until 6 years No benefit of *B. lactis*	sensitisation in the group receiving *L. rhamnosus* at 6 years No benefit of *B. lactis*		groups when evaluated by spirometry reversibility test and FeNO levels at 6 years	higher CB IFN-γ levels, compared with the placebo group. Colostrum TGF-β1 levels were increased after *B. lactis* supplementation, with a similar tendency for *L. rhamnosus*. Increased colostrum IgA levels were observed after both *B. lactis* and *L. rhamnosus* administration. Neonatal plasma sCD14 levels were lower in the *B. lactis* group compared with the placebo group [93].	present in the faeces at 3 months, although detection rates were similar by 24 months [27].
Niers *et al*, 2009 [29] and **Gorissen *et al*, 2014** [42] Allergic disease of either parent and in at least one sibling *Lactococcus lactis* W58, *B. lactis* W52 and *B. bifidum* W23 1 x 10^{9} CFU each daily six weeks before delivery and then directly to infant for 12 months	Reduced cumulative incidence of eczema in the first three months of life No difference at 6 years	No	No	Not reported	**Niers 2009**; Reduced anti-CD2/CD28 induced IL-5 and IL-13 levels were found in whole blood cultures at 3 months of age in the probiotic compared with the placebo group. The *in vitro* lymphocyte proliferative response to either anti CD2/CD28 or PHA did not differ between the groups [29].	**Niers 2009;** Using T-RFLP, qPCR and DGGE, *L. lactis* and *B. bifidum* but not *B. lactis* were more easily detectable in the probiotic compared with the placebo group at 3 months of age [29] **Rutten 2015**; Only minor and short term differences in composition of microbiota between the probiotic and placebo group were found using 16S–23S rDNA interspace region based profiling. Gut microbiota development continued between two and six years, then approaching a more adult-like composition [119].
Kim *et al*, 2010 [30] Any first degree relative with allergic disease *B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031(1.6 x 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	Reduced cumulative incidence and prevalence of eczema at 12 months Long term outcomes not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Ou *et al*, 2012 [22] Maternal allergic disease *L. rhamnosus* GG 1 x 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 2012**; There were no significant differences in plasma IgE levels in the children at 6, 18, and 36 months of age, while mean plasma IgE was higher in the cord blood of the LGG group. Maternal plasma IL-13, IL-10, IFN-γ, CXCL10, and TGF-β levels were not affected [22].	Not reported
Allen *et al*, 2014 [31]	No reduction	Not	No	Not reported	Not reported	Not reported

Any first degree relative with allergic disease *L. salivaris* CUL61*, L. paracasei* CUL08, *B. animalis ssp lactis* CUL34 and *B. bifidum* CUL20, 10^{10} CFU daily in total starting 2-4 weeks before delivery and then to the infant for six months	of eczema, but a reduction of IgE-associated eczema at 2 years of age in the probiotic group	reported				
POSTNATAL ADMINISTRATION						
Taylor *et al*, 2007 [32] and **Jensen *et al*, 2012** [39] Maternal allergic disease *L. acidophilus* (LAVRI-A1) 3 x 10^8 CFU given within 48 hours, and then for six months, directly to infant	No reduction at 1 year nor at the or 5 year follow-up	No Sensitisation more common in the probiotic group at 1 year, but not at the later follow-ups	No	Not reported	**Taylor 2006;** Infant cytokine (IL-5, IL-6, IL-10, IL-13, TNF or TGF-β) responses to TT, HDM, OVA, BLG, SEB and PHA were measured at 6 months of age. Probiotic supplementation was associated with reduced production of IL-5 and TGF-β in response to SEB stimulation, lower IL-10 responses to TT vaccine antigen and reduced TNF and IL-10 responses to HDM allergens [98]. **Taylor 2006**; Mononuclear cell samples were available from 118 infants and stimulated using ligands for TLR2 and TLR4/CD14, finding no effects of the probiotic supplementation on cytokine responses. Circulating DC subset frequencies and antigen presenting capacity were similar between the groups [99]. **Taylor 2007;** Infant regulatory T-cell function was examined at 6 months. Probiotic supplementation did not affect the proportion of circulating CD4+CD25+CTLA4+ cells or FoxP3 mRNA expression [100].	**Taylor 2007**; At 1 month of age, infants in the probiotic group were almost twice as likely to show culturable levels of Lactobacillus species. By 6 months of age, the rate of Lactobacillus colonization was significantly higher in the probiotic than the placebo group. The rates of colonization with Bifidobacterium not affected by the treatment [32].
Soh *et al*, 2009 [33] and **Loo *et al*, 2014** [40] Any first degree relative with allergic disease, *L. rhamnous LPR* 1 x 10^9 CFU and *B. longum* (BL999) 6 x 10^8 CFU daily to infant (in infant formula) for 6 months	No reduction at 2 or 5 years	No	No	Not reported	**Soh 2010**; Compared with placebo, probiotic supplementation improved hepatitis B (HepB) surface antibody responses at 12 months in subjects receiving monovalent doses of HepB vaccine at 0, 1 month and a DTPa–HepB combination vaccine at 6 months, but not those who received 3 monovalent doses [104].	Not reported

West *et al*, 2009 [34] **West *et al*, 2013** [41] Mixed (2/3 with at least one first grade relative with allergic disease) *L. paracasei ssp paracase*i F19 1 x 10^9 CFU daily to infant (in infant cereal) during weaning from 4-13 months	Reduced cumulative incidence of eczema at 13 months No difference at 8 years	No	No	No differences between the groups when evaluated by spirometry reversibility test and FeNO levels at 8 years	**West 2008**; Antibody concentrations to Hib capsular polysaccharide (HibPS), diphtheria toxin (D) and tetanus toxoid (T) before and after the second and third doses was measured. LF19 enhanced antibody concentrations to D and T, especially in infants breastfed less than 6 months. Conversely, breastfeeding duration influenced the anti-HibPS concentrations, with no effect by LF19 [105] **West 2009;** At 13 months of age, the anti-CD3/CD28 induced IFN-γ/IL4 mRNA ratio was higher in the probiotic compared with the placebo group [34]. **West 2012;** At 13 months of age, the anti-CD3/CD28 induced IFN-γ/IL-2 and IL-17A/IL-2 mRNA ratios were higher in the probiotic than the placeco group, as was the TT induced IL17A expression. No differences were observed between the two groups at 5.5 months [101].	**West 2008**: Using RAPD-PCR, *L. paracasei* ssp *paracasei* F19 was detected in stool in 90% of the infants in the probiotic group at 6 months and the frequency remained high throughout the intervention [105].