

REVIEW

Practical prebiotics, probiotics and synbiotics for allergists: how useful are they?

H. Johannsen and S. L. Prescott

School of Paediatrics and Child Health Research, Princess Margaret Hospital, University of Western Australia, Perth, WA, Australia

Clinical & Experimental Allergy

Summary

With the advent of the hygiene hypothesis, probiotics have provided an avenue of hope in curbing the allergic epidemic. The initial enthusiasm has been tempered by recognition of the inherent complexities of this approach. This review examines the current clinical evidence and practical issues in using probiotics and related products, for the prevention and treatment of allergic disease. So far, probiotics have shown more promise, albeit limited, in the primary prevention of allergic disease rather than in the treatment of established disease. These effects have largely been limited to the prevention of early childhood conditions such as eczema, with no consistent effects on other allergic outcomes. There is emerging evidence that clinical effects may be strain specific, but again these findings have been inconsistent. While there have been several meta-analyses to examine probiotics in both the prevention and the treatment of allergic disease, these have been hampered by significant heterogeneity between studies, including wide variations in the strains used, the methods and timing of administration and the age and assessment of allergic outcomes. In any case, these have also become outdated by a series of new studies published in the last year. Although it is not yet clear exactly how the growing number of new studies will modify the results of meta-analyses, it is likely that these will add yet further heterogeneity that will continue to make interpretation of pooled data difficult. At this stage, the effects of prebiotics, synbiotics and postbiotics are even less clear. Thus, while there is little doubt that microbiota modulate immune development and can prevent the allergic phenotype, the optimal way of achieving this is far from clear. Given the current level of evidence, it is not appropriate to recommend prebiotics/probiotics/synbiotics or postbiotics as a part of standard therapy or for the prevention of any allergic conditions. Further studies are needed to address the growing speculation that supplementation with a single probiotic strain may be oversimplistic and that approaches that have a more global effect on colonization may be warranted.

Correspondence:

Susan L. Prescott, School of Paediatrics and Child Health Research, Princess Margaret Hospital, University of Western Australia, Perth, WA, PO Box D184, Perth, WA 6001, Australia.
E-mail: sprescott@meddent.uwa.edu.au

Cite this as: H. Johannsen and S. L. Prescott, *Clinical & Experimental Allergy*, 2009 (39) 1801–1814.

Introduction

Gut microbiota are an integral part of the gastrointestinal system, playing an essential role in the development of both local immune maturation and systemic immune programming. Complex symbiotic relationships have evolved between the human host and gut microflora to the evolutionary advantage of each. Disruption of this balance has been implicated in many disease processes, including local gastrointestinal disorders (such as necrotizing enterocolitis, irritable bowel syndrome and inflammatory bowel diseases) as well as the epidemic rise in systemic immune diseases (autoimmunity and allergy) [1]. This review focuses primarily on strategies aimed at promoting colonization and immune

tolerance in the context of preventing and treating allergic diseases.

The vast and complex intestinal ecosystem contains $> 10^{14}$ microorganisms, at least 10 times the entire number of cells in the human host, with a huge potential for local and systemic influence. These effects appear to be most critical during early infancy when colonization is first established and patterns of immune response are evolving. Over the first months of life, there is an enormous transition from the 'sterile' *in utero* environment to reach full microbial colonization. During the same period, the functionally immature neonatal immune system must also develop to achieve a complex and finely regulated balance between host defence and immune tolerance. It is now clear that this normal immune

homeostasis is dependent on gut microflora and may be influenced by differences in colonization patterns. Animal studies under germ-free conditions demonstrate that oral tolerance cannot be induced in the absence of gut microbiota [2, 3]. Although restoration of full-immune function and oral tolerance can be achieved by providing normal intestinal microbiota, this is age-dependent and cannot be achieved in mature animals [3]. These observations *firstly* highlight the pivotal role of gut microbiota in the development of the immune system, and *secondly* illustrate the importance of the timing of microbial exposure.

In humans, a range of environmental factors can lead to wide individual variations in early colonization patterns, which then tend to persist throughout life. It has been proposed that the environmental changes responsible for the epidemic rates of both autoimmunity and allergic disease may be mediated, at least in part, through changes in early colonization [1, 4]. It was therefore highly logical to investigate the potential of pre- and/or post-natal dietary supplements to favourably modify the gut microbiota for disease prevention. With >400–500 different bacterial species, each with a multitude of strains, and the enormous heterogeneity of this microbiome between individuals, there is growing speculation that supplementation with a single probiotics strain may be oversimplistic and that approaches that have a more global effect on colonization may be warranted.

Rationale and history of pre/probiotics in allergic disease

Allergic disorders such as asthma, eczema, rhino-conjunctivitis and food allergies have dramatically increased in the last 40 years, becoming the most common chronic childhood diseases in industrialized countries. Although the environmental changes that are responsible for this epidemic have not been clearly defined, one of the most appealing explanations, as proposed by Gerrard et al. [5], has been the reduction in general microbial exposure during early childhood, with parallel changes in commensal flora [6]. Epidemiological associations between declining microbial exposure and allergic disease have been supported by animal models demonstrating that early exposure to both pathogenic [7, 8] and non-pathogenic microbial products [9] either prevents or modifies allergic responses. The specific immuno-protective effects of gut microflora are likely to be mediated through multiple pathways within (and potentially beyond) the gut-associated lymphoid tissue (GALT), the largest immune organ of the human host. This includes effects on local IgA production, induction of tolerogenic dendritic cells and regulatory T cell populations with the production of immunomodulatory cytokines such as IL-10 and TGF- β (as reviewed by Prescott and Björkstén [10]). Although these mechanisms are still not well understood, they appear to collectively inhibit local inflammation, improve

gut integrity, and thereby reduce the risk of inappropriate systemic immune responses.

Apparent differences in perinatal colonization patterns with both the level of industrialization and the subsequent allergic outcomes [11, 12] provided a strong rationale for intervention studies designed to modify immediate post-natal colonization and prevent allergic disease. The logical approach for preventing allergic disease, first used by the Isolauri group in Finland, was to give probiotics in pregnancy and the early post-natal period [13]. A reported 50% reduction in infantile eczema was cause for great initial enthusiasm, and this has been followed by a series of studies using different strains under different conditions and in different populations (as summarized below). The same group was also the first to report a benefit on the treatment of early allergic disease [14]. Although the findings of many subsequent studies have been inconsistent and disappointing, the novel concepts behind this approach remain important, and the limitations of the existing strategies may likely lie in our limited understanding of the complex enteric ecosystem. It may be premature to dismiss these strategies as inherently oversimplistic while our knowledge of the vast, yet intricate, interactions in the gut is still so limited. Indeed, while supplementing with a few selected probiotic strains may be insufficient, further research in this field remains important.

In addition to the recent enthusiasm for probiotics, there has also been a growing interest in prebiotics, based on the concepts that changing the substrate for bacterial growth may have a more generalized effect on colonization than merely adding single probiotic strains. Again, while initial studies have been promising [15], this approach is still under investigation.

Finally, it must be remembered that despite the tantalizing notions behind the hygiene hypothesis, we have still not ascertained definitively the extent to which disruptions in colonization are responsible for the allergic epidemic. However, even if other more powerful environmental forces are ultimately found to be responsible for the allergy epidemic, ways of promoting optimal enteric conditions for immune development may still be a useful strategy in the face of other environmental pressures. Further research is needed to understand events during early immune development as, if anything, these strategies are likely to be more successful in early life before colonization and immune phenotype are established.

Definitions

Probiotics have been defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host [16].

Prebiotics are non-digestible, fermentable food ingredients that can affect the host by selectively stimulating

the growth and activity of bacteria in the colon and improve host health.

Synbiotics are a combination of probiotics and prebiotics. In this combination the prebiotic substrate could promote survival of both the probiotic organism and other 'favourable' endogenous species.

Postbiotics are the biologically active by-products of probiotic cultures (i.e. short-chain fatty acids produced by bacterial fermentation such as butyrate), which can be added to health supplements. At this stage, there have been too few studies in allergic disease to warrant a review of postbiotic products specifically.

Summary of evidence of pre/probiotic supplements in the prevention of allergic disease

The most ambitious but most cost-effective strategies to tackle the growing burden of allergic disease centre on primary prevention. As the allergic phenotype is often expressed within months of life, prevention strategies must be targeted in pregnancy and/or the immediate post-natal period. In the absence of accurate allergy predictors, these strategies have been generally directed at children perceived to be at a 'high risk'. High-risk infants are usually characterized by a positive family history of allergic disease. Research for better preventive measures needs to go hand in hand with research on better methods for predicting allergic disease.

Current evidence for probiotics in allergy prevention

To our knowledge, there are at least 17 randomized-controlled trials (RCT) and two meta-analyses examining the role of maternal and/or infant probiotic supplementation for primary allergy prevention. These are summarized individually in Tables 1a and b, and include five studies cited in previous meta-analyses [13, 17–20], eight new studies published in the last 2 years [21–28] (Table 1a) and a further four studies that are, to our knowledge, still in progress (Table 1b). There have also been several follow-up publications to previous studies [29, 30]. As noted, there is considerable heterogeneity in virtually all aspects of these studies, including the strains used, the timing of administration, the duration of administration, the method of probiotic delivery and the outcome measures.

Most of the current studies looked primarily at early outcomes of allergic disease such as eczema and IgE-mediated food allergy. Half of these have shown some reduction in the development of eczema, but there was little evidence of this in the remaining studies (Table 1a). The majority of studies have shown no reduction in sensitization, food allergy or allergic disease in general (Table 1a). At present, long-term outcomes such as allergic rhinitis (AR) or asthma have not been satisfactorily assessed.

Lactobacillus rhamnosus strains have featured heavily in existing studies, most notably in the studies documenting a beneficial effect on eczema reduction, particularly the LGG strain [13, 19, 28], but also other *L. rhamnosus* strains [24]. However, other studies using *L. rhamnosus* strains have not shown any reduction in infant allergic disease or sensitization [17, 22, 23, 25, 29]. Although some have shown no overall benefits there was some reduction in allergic outcomes in subgroups born by caesarean section [29] or those with maternal sensitization [25]. Similarly, one earlier study also found a benefit (reduced sensitization) with *Lactobacillus reuteri* in a subgroup with maternal sensitization, although there was no overall benefit [20]. One new study has reported a beneficial effect of a combination of *Lactobacillus lactis*, *Bifidobacterium infantis* and *Bifidobacterium bifidum*, with a significant reduction of eczema [26], while another study has shown clear differences in efficacy between two species [24].

The duration of treatment has also varied quite substantially. The majority of studies included a pre-natal treatment period, typically for 4–6 weeks, but the period of post-natal supplementation has ranged from 1 month [21] to 2 years [24]. It has been proposed that, in addition to strain differences, antenatal supplementation may also explain differences between studies, as this is a feature of studies showing a benefit [13, 19, 24, 25, 26, 28] compared with those that did not [18, 23]. Animal studies with probiotics show antenatal effects [9], but the effects of maternal probiotics on cord blood immune responses are conflicting [31, 32], and a specific study to address this is still in progress (Tang et al., Table 1b). Only one newly reported Swedish study has specifically examined the effects of probiotic supplementation (*Lactobacillus F19*) during the weaning period (4–13 months of age) and found an impressive 50% reduction in eczema [27].

Although there have been two meta-analyses on probiotics for allergy prevention, these are difficult to interpret [33, 34]. These generally only included data from the first five studies published [13, 17–20] and are therefore already outdated, with seven new studies [22–28] and at least two follow-up studies [29, 30] reported since. One earlier study was also not included [21]. Both systematic reviews found that probiotics reduce the risk of eczema, but found no effect of probiotics in preventing any other allergic outcome including allergic sensitization and food allergy. The analysis by Lee et al. [33] has been criticized [35] for including the data from the Isolauri study three times over [13, 36, 37], and amplifying the effect. A number of other methodological issues have been raised, including the outcome measures used [35]. The systematic review by Osborn and Sinn [34] in 2007 was generally more robust, and also appears to have included the Isolauri data more than once. Nevertheless, their conclusions were more conservative, indicating that although

Table 1. Summary of primary allergy prevention studies using probiotics (completed and in progress): (a) completed published studies[†] and (b) studies still in progress[§]

Investigator(s) (and location of study)	Study protocol	Findings						
	Population characteristics	Organism(s) and dosage	Pre-natal (duration)	Post-natal (duration)	Reduction in eczema sensitization	Reduction in other AD	Effect on colonization	Comments
(a) Kalliomäki, Isolauri and colleagues (2001, 2002 and 2003) Turku, Finland (references [13, 36, 37])	Any first degree relative with allergic disease N = 132 completed (of initial 159)	<i>Lactobacillus rhamnosus</i> GG, (1×10 ¹⁰ CFU daily) Only to mother if breast feeding post-natally	Yes 2–4 weeks before delivery	6 months *Only directly to baby if not breastfeeding	Yes At 2 and 4 years	No	Yes	
	Rautava, Isolauri and colleagues Turku, Finland (reference [17])	Any need for artificial feeding before 2 months of age N = 72 completed (of initial 81)	<i>Lactobacillus rhamnosus</i> GG, 1×10 ¹⁰ CFU daily <i>Bifidobacterium lactis</i> Bb-12 (1×10 ¹⁰ CFU daily) Added to infant formula	No	Before 2 months (depending on age started formula) until 12 months	No	No	–
Kocourková and colleagues 2006 Prague, Czech republic (reference [21])	3 groups: Allergic mother Colonized Allergic mother Not colonized Non-allergic mother N = 144 completed	<i>Escherichia coli</i> serotype O83:K24:H31 (8×10 ⁸ CFU given 3 times/week) from day 2 to 1 month of age	No	1 month Direct to infant	Yes ^Δ	No	Yes ^Δ	^Δ Decreased number of pathogenic organisms at 3 months ^Δ Possible allergy preventing effect (mostly eczema) noted
Taylor, Prescott and colleagues 2007 Perth, Australia (references [18, 30])	Mother with SPT+ allergic disease N = 189 completed (of initial 230)	<i>Lactobacillus acidophilus</i> (3×10 ⁸ CFU daily)	No	6 months Direct to infant	No	No ^Φ	Yes	^Φ Sensitization more common in probiotic group at 1 year, but no difference at 2.5 years.
Kukkonen, Kuitunen and colleagues 2007 Helsinki/Tampere, Finland (reference [19])	One or both parents with allergic disease N = 925 completed (of initial 1223)	<i>Lactobacillus rhamnosus</i> GG & LC705 (both 5×10 ⁹ CFU twice daily); and <i>Bifidobacterium breve</i> and <i>Propionibacterium freudenreichii</i> (both 2×10 ⁹ CFU twice daily)	Yes 2–4 weeks before delivery	6 months Direct to infant*	Yes At 2 years	No [≠]	Yes	*Probiotic group also received prebiotic supplement (galacto- oligosaccharides) [≠] Trend for less IgE-associated allergic disease (P=0.052)
Abrahamsson, Oldaeus and colleagues 2007 Linköping, Sweden (reference [20])	Any first degree relative with allergic disease N = 188 completed (of initial 232)	<i>Lactobacillus reuteri</i> (1×10 ⁸ CFU daily)	Yes 2–4 weeks before delivery	12 months Direct to infant	No ^{**} At 2 years	No	Not known	**Probiotics: less IgE-associated eczema in the second year, and less sensitization in subgroup with atopic mothers

Kopp, Urbanek and colleagues 2007 Freiburg, Germany (reference [22])	Any first degree relative with allergic disease N = 94 completed (of initial 105)	<i>Lactobacillus rhamnosus</i> GG, 1×10^{10} CFU daily To mother if breast feeding post-natally for 3 months and than to the neonates for additional 3 months	Yes 4–6 weeks before delivery	6 months Direct to infant	No [†] At 2 years	No	No [†]	Not known	†Probiotics: increased rate of recurrent episodes of wheezing bronchitis
Wickens, Crane and colleagues 2008 Wellington and Auckland, New Zealand (reference [24])	One or both parents with allergic disease N = 474 (of initial 512)	<i>Lactobacillus rhamnosus</i> HN001 (n = 157) (1×10^{10} CFU daily) OR <i>Bifidobacterium lactis</i> (1 $\times 10^{10}$ CFU daily) HN019 (n = 158)	Yes 2–5 weeks before delivery	2 years To infant regardless of feeding method	Yes At 2 years	No	–	Yes	Reduction in eczema prevalence and severity with <i>L. rhamnosus</i> but not <i>B. lactis</i>
Soh, Skek, Aw and colleagues 2008 Singapore (reference [23])	Any first degree relative with SPT+ allergic disease N = 245 (of 253 randomized)	<i>Lactobacillus rhamnosus</i> (1 $\times 10^9$ CFU daily) and <i>Bifidobacterium longum</i> (6×10^8 CFU daily)	No	6 months In infant formula	No At 1 year	No	–	Not known	
Huurre, Laitinen, and colleagues 2008 Turku, Finland (reference [25])	Mother with current atopic disease N = 140 completed (of initial 171)	<i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i> (1×10^{10} CFU daily)	Yes From first trimester	Yes End of exclusive breastfeeding	No	No [†]	–	Not known	†Probiotics had no effect on infant sensitization at 12 months (except in subgroup with maternal sensitization)
Kuitunen, Kukkonen and colleagues 2009 Helsinki, Finland (reference [29])	Same study as [19] N = 891 (of initial 1223)	<i>Lactobacillus rhamnosus</i> (5×10^9 CFU) and <i>Bifidobacterium breve</i> (2×10^8 CFU) and <i>Propionibacterium freudenreichii</i> (2×10^9 CFU) twice daily plus Prebiotics	Yes 4 weeks before delivery	Yes For 6 months	No 5 years follow-up	No 5 years follow-up	No 5 years follow-up	Not known	No overall differences in allergy-prevention at 5 years. In subgroup of Caesarean-delivered children: probiotics associated with reduction in eczema and sensitization.
Niers, Rijkers, Hoekstra and colleagues 2009 Utrecht, Netherlands (reference [26])	Atopic disease in either mother or father plus at least one sibling N = 98 completed (of initial 156)	<i>Lactococcus lactis</i> W58 <i>Bifidobacterium lactis</i> W52 <i>Bifidobacterium bifidum</i> W23 (1×10^9 CFU each daily)	Yes 6 weeks before delivery	Yes 12 months (direct to infant)	Yes	No (trend for more sensitization with probiotics)	No	Yes	
West, Hernel, and colleagues 2009 Umeå, Sweden (reference [27])	Atopic disease in either mother, or sibling N = 171 completed (of initial 179)	<i>Lactobacillus paracasei</i> strain F19 (1×10^8 CFU daily in weaning cereal)	No	Yes 4–13 months (during weaning)	Yes	No	No	Not known	A significant difference in Th1/Th2 responses at 13 months (higher Th1/Th2 in probiotic group)
Dotterud, Johnsen, and colleagues 2009 Trondheim, Norway (abstract only [28])	Unselected population N = 278 (of 415 randomized)	<i>Lactobacillus rhamnosus</i> GG <i>Lactobacillus acidophilus</i> LA5 <i>Bifidobacterium lactis</i> Bb-12 (5×10^{10} CFU of each daily)	Yes From 36 weeks	No Given to the breastfeeding mother for 3 months	Yes	No	No	Not known	Reduced eczema in first 2 years compared with placebo group

Table 1. continued

Study protocol					
Investigator(s) (and location of study)	Population characteristics	Organism(s) and dosage	Pre-natal (duration)	Post-natal (duration)	Comments
(b) Thornton, Morgan and colleagues Swansea, UK	Any first degree relative with allergic disease N = 600 intended	<i>Lactobacillus salivarius</i> (6.25×10 ⁸ CFU daily) and <i>Lactobacillus paracasei</i> and <i>Bifidobacterium infantis</i> and <i>Bifidobacterium bifidum</i> (each at 1.25×10 ⁸ CFU daily)	Yes 2–4 weeks before delivery	6 months (direct to infant regardless of feeding method)	Study not complete Outcomes will be assessed at 6 months, 2 and 5 years
Tang and colleagues (Unpublished data) Melbourne, Australia	Any first degree relative with allergic disease N = 200 intended	<i>Lactobacillus rhamnosus</i> (2×10 ¹⁰ CFU daily)	Yes 2–4 weeks before delivery	No (only to mother)	Study not complete Outcomes will be assessed at 3, 6 12 and 24 months
Lau and colleagues Berlin, Germany	Infants at age 4 weeks with at least one atopic parent N = 650	<i>Streptococcus faecalis</i> DSM 16440 and <i>Escherichia coli</i> DSM 17252 (combined at 1.5–4.5×10 ⁷ daily)	No	6 months (week 5–7 months (direct to child))	Study not complete Primary outcome: effect on eczema at age 7 and 12 months of age. Follow-up period after 6 month treatment period up to age 3 Note: this study is using heat killed (rather than live) bacteria
Cabana and colleagues San Francisco, USA (reference [60])	Either parent with asthma N = 276 intended	<i>Lactobacillus rhamnosus</i> GG (1×10 ¹⁰ CFU daily)	No	0–6 months (direct to infant)	Study not complete Primary outcome: atopic dermatitis in first 3 years

[†]This table shows a comprehensive list of the published randomized-controlled trials, which have used probiotics for the primary prevention of allergic disease.

Search strategy for published studies (a): Our strategy included the following keywords: [probiotic OR Lactobacillus OR Bifidobacterium OR prebiotic OR synbiotic] AND [allergy OR allergic disease OR atopic dermatitis OR eczema]. We then selected all randomized controlled trials using the product of interest in humans and assessed allergic outcomes. This list was also cross-referenced against studies cited in previous publications including the Cochrane meta-analyses, reviews articles, previous studies and conference abstracts.

[§]For completeness we have also included studies still in progress (to our knowledge). This is based on web searches, personal communications and conference discussions.

N, number; CFU, colony forming unit; s, soluble; CD, cluster of differentiation; SPT, skin prick testing; SCORAD, scoring atopic dermatitis; Th 1/2, T helper cells 1/2; AD, allergic disease.

there was a reduction in clinical eczema in infants, this effect is not consistent between studies, and they advised caution in view of methodological concerns of a number of included studies. This systematic review is currently in the process of being revised. At this stage, few studies have assessed the long-term effects on asthma and respiratory allergic disease, but those that have, have found no effect [29, 37].

Both these meta-analyses identified the marked heterogeneity between studies and the need for more definitive studies. However, we have previously questioned the role of the meta-analyses method in this setting where the interventions are diverse and not necessarily comparable [10]. With at least four more studies in progress (Table 1b), this will remain an ongoing issue as these systemic reviews are revised.

Current evidence for prebiotics in allergy prevention

Non-digestible, fermentable oligosaccharides are of interest because of their capacity to selectively favour colonization with favourable microflora, particularly bifidobacteria [15, 38]. This could theoretically have more global effects on colonization than adding single strains. Commercial preparations include polydextrose, fructo-oligosaccharides, galacto-oligosaccharides and mixtures of these. Although these prebiotics can be added to infant formula, these unfortunately do not exactly replicate the unique prebiotics in human breast milk.

So far, the studies in allergy prevention are limited. There has really only been one study specifically designed to assess the effects of prebiotics on allergy prevention [15, 39]. This Italian study by Moro, Arslanoglu and colleagues enrolled 259 infants at a high risk of allergic disease and assigned them to a formula containing either a placebo or a prebiotic mixture of 90% short-chain galacto-oligosaccharides (GOS) and 10% long-chain fructo-oligosaccharides (FOS), designed to resemble human milk. Mothers and infants were only recruited into the study if they decided not to breastfeed and the average age of the first feed was around 11 days in each group. At both 3 and 6 months of age, there was a significant increase in colonization with bifidobacteria (in the 206 children completing follow-up) and a significant decrease in the cumulative incidence of eczema at 6 months of age in the GOS/FOS supplementation group compared with the placebo group [15]. A further follow-up assessment has been reported for this population at 2 years of age, although this was limited to approximately half of the original population ($n = 134$), showing a significant reduction in the cumulative incidence of eczema, recurrent wheezing and allergic urticaria compared with the control group [39]. Furthermore, the prebiotics intervention group also had a smaller number of upper respiratory tract infections, fever episodes and courses of antibiotic therapy [39].

Other allergic disease or food hypersensitivity outcomes were not reported.

The only other study to address this issue was designed to examine the effects of prebiotics on infant growth and stool consistency, but this was not in a population at risk of allergic disease and eczema was only assessed as a secondary adverse event with few details provided [40]. They examined the effect of two different combinations of prebiotic ingredients, polydextrose, GOS and lactulose, at two different intake levels, and noted that eczema was more common in one prebiotic group than the control group, but tended to be lower in the other. In the Cochrane systematic review that combined these studies, no effect was found but this may have been due to differences in infant risk, prebiotic formulation and measurement of eczema [41]. Essentially, more studies are needed.

Role of synbiotics in allergy prevention

So far, only one, albeit large, study ($n = 1223$) has assessed a synbiotic mix in the context of allergy prevention. Kukkonen et al. [19] used a prebiotic (GOS) in combination with three different probiotics. This was administered to mothers for 2–4 weeks before delivery and then to the infant for the first 6 months post-natally. At 2 years of age ($n = 925$), there was a reduction in eczema, particularly atopic eczema, in the synbiotic group. There were no effects on other allergic outcomes or sensitization, although IgE-associated (atopic) diseases tended to occur less frequently in the probiotic group [19]. However, a recent follow-up of this population at 5 years of age did not show any difference in the cumulative incidence of eczema, atopic eczema, sensitization, AR and asthma between the treatment and the placebo group [29].

Summary of evidence of pre/probiotics in the treatment of established allergic disease

It may be argued that once both colonization and the allergic phenotype are established, the therapeutic potential of these interventions may diminish or at least may be less effective than at younger ages when there is more inherent plasticity. For these reasons, most treatment studies have been in infancy and early childhood.

The role of probiotics and prebiotics in eczema

The therapeutic effects of probiotics have been best studied in infants with atopic dermatitis. At least 13 studies have been published in more than a decade (Table 2). To our knowledge, there are three more RCT currently ongoing to further examine probiotics for the treatment of eczema as well (Murray and colleagues, Goossens and colleagues, and Land and colleagues. as summarized by Lee et al. [33] and Boyle et al. [42]). Again, there is marked

Table 2. Summary of studies using probiotics for treatment of eczema in children

Study protocol				Findings			
Investigator(s) (and location of study)	Population characteristics (all with eczema)	Organism(s) and dosage	Duration	Outcome measures	Reduction in SCORAD ^a	Improved quality of life [*]	Comments
Majamaa, Isolauri and colleagues 1997 Turku, Finland (reference [14])	N = 27 Age: 2–16 months Median SCORAD (baseline): placebo = 21; probiotic = 26	Lactobacillus GG (5×10 ⁸ CFU/g extensively hydrolysed formula (eHF) OR Placebo (eHF)	4 weeks	SCORAD at 4 & 8 weeks	No [*]	-	[*] Improved SCORAD only in the treatment group, but no difference reported in relation to placebo group. Reduced fecal α1–antitrypsin, and TNF-α levels in the treatment group
Isolauri and colleagues 2000 Turku, Finland (reference [48])	N = 27 Mean age: 4.6 months Median SCORAD = 16	Lactobacillus GG (5×10 ⁸ CFU/g eHF) OR <i>Bifidobacterium lactis</i> Bb–12 (1×10 ⁹ CFU/g eHF) OR Placebo (eHF)	Not clear	SCORAD at 8 weeks Follow-up at 6 months	Yes	-	SCORAD decreased in the <i>B. lactis</i> Bb–12 and in the LGG (lower than placebo group at 2 months). No differences at 6 months (most children had shown complete resolution). Reduction in serum sCD4 and urine EPX in probiotics groups.
Kirjavainen, Isolauri and 2003 Turki, Finland (reference [61])	N = 35 Mean age = 5.5 months Median SCORAD (baseline): placebo = 13; active probiotic = 19; heat inactivated = 15	Lactobacillus GG (1×10 ⁹ CFU/g eHF) OR Heat inactivated LGG (3×10 ¹⁰ CFU/g eHF) OR placebo (eHF)	Not clear (trial terminated early)	SCORAD	No [Ⓛ]	-	[Ⓛ] Decrease in all three study groups, greater in active group. Trial prematurely terminated after complaints from children's parents concerning adverse gastrointestinal symptoms.
Rosenfeldt and colleagues, 2003 Copenhagen, Denmark (reference [49])	N = 43 Age: 1–13 years SCORAD (15–66) (Crossover design)	<i>Lactobacillus rhamnosus</i> 19070-2 and <i>Lactobacillus reuteri</i> DSM 122460 (1×10 ¹⁰ CFU of each strain, twice daily) OR dextrose placebo	6 weeks	SCORAD, subjective evaluation	No	More perceived improvement in the active group	Extent of the eczema decreased during active treatment. Response was more pronounced in allergic patients. Serum ECP levels decreased.
Hattori, Taniuchi, et al. (2005) Japan (reference [44])	N = 15 Age: 3–18 months SCORAD: not done	Synbiotic with <i>Bifidobacterium breve</i> M-16V (5–15×10 ⁹ CFU/day) + prebiotic containing eHF OR placebo (same prebiotic containing eHF)	12 weeks	Investigator-rated severity scoring scale	Unclear [‡]	-	[‡] Significant reduction in symptom scores from baseline in both probiotic and placebo groups, but no statistical comparison between the groups.

Weston, Prescott and colleagues (2005) Perth, Australia (reference [47])	N = 56 Mean age: 11.5 months Mean SCORAD = 41	<i>Lactobacillus fermentum</i> VR1-003PCC 2×10^9 CFU/day as a sachet reconstituted by parents with 5–10 mL water twice daily OR Maltodextran placebo	8 weeks	SCORAD, family impact questionnaire, global assessment by parent.	Yes	No	Reduction SCORAD was significant in the probiotic group but not the placebo group. Significantly more children receiving probiotics had improvement compared with the placebo group.
Viljanen, Savilahti and colleagues (2005) Helsinki, Finland (reference [45])	N = 230 Mean age: 6.5 months Mean SCORAD = 32.5	Lactobacillus GG capsules (10^{10} CFU/day) OR Mixture of probiotics: (<i>LGG</i> 10^{10} CFU/day; <i>B. breve</i> 4×10^8 CFU/day, <i>L. rhamnosus</i> 10^{10} CFU/day and <i>Propionibacterium JS</i> 4×10^9 CFU/day) OR Microcrystalline cellulose placebo	4 weeks	SCORAD at 4 and 8 weeks	No	–	All groups improved. No difference between probiotic groups and placebo at end of intervention or 4 weeks later. Beneficial effects in subgroup of IgE + dermatitis.
Passeron, Ortonne and colleagues (2006) Nice, France (reference [43])	N = 48 Age: 2–12 years Mean SCORAD = 39.7	Synbiotic with <i>Lactobacillus rhamnosus</i> Lcr35 (3.6×10^9 CFU/day) + prebiotic and fermentation broth OR Prebiotic and fermentation broth alone	12 weeks	SCORAD, parent (and investigator) severity assessment at 4, 8 and 12 weeks	No	No	Both groups improved. No difference between groups (no placebo group without supplement)
Sistek, Crane and colleagues (2006) New Zealand (reference [50])	N = 59 Age: 1–10 years Mean SCORAD: Placebo = 35.1 Probiotics = 26	<i>Lactobacillus rhamnosus</i> and <i>Bifidobacterium lactis</i> capsules at total dose of 2×10^{10} CFU/day. OR Microcrystalline cellulose placebo	12 weeks	SCORAD, at 2, 12 and 16 weeks	No	Yes[#]	[#]Parent (subjective) scores more improved in probiotic group. No difference in total SCORAD overall, but subgroup of food sensitized children had significant improvement with probiotics. No effect on antibiotic or topical steroid use and parents could not identify if patient on probiotic or placebo

Table 2. continued

Investigator(s) (and location of study)	Study protocol		Findings		
	Population characteristics (all with eczema)	Organism(s) and dosage	Duration	Outcome measures	Reduction in SCORAD* Improved quality of life* Comments
Brouwer, Duijverman and colleagues (2006) Groningen, Netherlands (reference [62])	N = 50 Mean Age: 3.8 months Mean SCORAD: 20.3	<i>Lactobacillus</i> <i>rhamnosus</i> NP-Lrh (5×10^9 CFU/100 mL eHF) OR <i>Lactobacillus</i> GG NP- LGG (5×10^9 CFU/ 100 mL eHF) OR Placebo (eHF alone)	12 weeks	SCORAD, at 4, 8 and 12 weeks	No - All groups improved. No effect of probiotics on eosinophil protein X in urine, fecal a-1-antitrypsin or production of IL-4, IL-5 and IFN- γ by peripheral blood mononuclear cells
Folster-Holst, Weichenhath and colleagues (2006) Germany (reference [63])	N = 54 Age: 1–55 months Mean SCORAD: Placebo = 41.4 Probiotics = 43.3	<i>Lactobacillus</i> <i>rhamnosus</i> LGG (1×10^{10} CFU/day) OR Microcrystalline cellulose placebo	8 weeks	SCORAD, QOL score, parent rating	No No At the end of treatment there were no significant differences in SCORAD, pruritus, medication requirements, immune parameters, or quality of life.
Gruber, Niggemann and colleagues (2007) Berlin, Germany (reference [64])	N = 106 Age: 3–12 months SCORAD: 15–40	<i>Lactobacillus</i> <i>rhamnosus</i> LGG (1×10^{10} CFU/day) OR Placebo	12 weeks	SCORAD, topical steroid requirement	No - Improvements in both groups without significant differences between groups (SCORAD or medication requirements)
Hol, Nieuwenhuis, and colleagues (2008) Netherlands (reference [65])	N = 116 Age: 4.2 months All with cows milk protein allergy	<i>Lactobacillus</i> <i>paracasei</i> CRL431 and <i>B. lactis</i> Bb-12 (1×10^7 CFU/g eHF) OR Placebo (eHF alone)	6 months	Clinical tolerance to cows milk, SCORAD (at 6 and 12 months)	No No No effect of probiotics on development of cow's milk tolerance. Improvements in SCORAD both groups without significant differences between groups.
Gueniche Biedermann and colleagues (2008) Germany (reference [46])	N = 75 Age: 6–70 years SCORAD: 30	Cream containing a 5% lysate of the nonpathogenic bacteria <i>Vitreoscilla</i> <i>filiformis</i> . OR Placebo (vehicle alone)	Topical Once daily for 30 days	SCORAD, sleep loss, permeability, <i>Staphylococcus aureus</i> colonization	Yes Yes (less sleep loss) Improvements in SCORAD in treatment group compared with placebo group (also reduced <i>Staphylococcus aureus</i> colonization)

This table shows a comprehensive list of the published randomised controlled trials, which have used probiotics for the treatment of established allergic disease.

**Outcomes measured*: Improved SCORAD or quality of life (QOL, including parent perception) in probiotic supplementation relative to the placebo group

Search strategy: Our strategy included the following keywords: [probiotic OR Lactobacillus OR Bifidobacterium OR prebiotic OR synbiotic] AND [allergy OR allergic disease OR atopic dermatitis OR eczema OR asthma OR allergic rhinitis]. This list was also cross-referenced against studies cited in previous publications including the Cochrane meta-analyses, reviews articles and previous studies. N, number; CFU, colony forming unit; s, soluble; CD, cluster of differentiation; SPT, skin prick testing; SCORAD, scoring atopic dermatitis; QOL, quality of life; eHF, eosinophil cationic protein X; ECP, eosinophil cationic protein; eHF, extensively hydrolysed formula.

heterogeneity between studies. Most have used probiotic species alone, but two studies have used synbiotics [43, 44]. None has examined prebiotics alone. The study populations have varied significantly in the baseline severity of eczema (Table 2), and study sizes have differed widely from 15 [44] to 230 participants [45]. Almost all studies have given probiotics orally, but it is noteworthy that one study used a topical lysate of a non-pathogenic gram-negative bacterium (*Vitreoscilla filiformis*) and observed a significant reduction in Scoring Of Atopic Dermatitis Severity Index (SCORAD) and in cutaneous *Staphylococcus aureus* colonization, compared with the placebo group [46].

L. rhamnosus strains have been most widely used, but the duration of intervention in all studies varied from 4 weeks to 6 months. In most studies, SCORAD has been used as the primary measure for determining eczema severity. To our knowledge, only two studies have reported a reduction in SCORAD with probiotic supplementation [47, 48]. One of these used the *L. rhamnosus* strain (LGG) [48] and the other used an *Lactobacillus fermentum* strain [47]. Several other studies have reported effects on quality-of-life measures although these were not standardized [49, 50].

Several authors have performed meta-analyses on these studies [33, 42, 51]. The recent Cochrane systematic review by Boyle et al. [42] found no evidence that probiotics are effective for the treatment of established eczema, but emphasized the problems with heterogeneity and the quality of some studies. However, a further review by Michail et al. [51] described that probiotic treatment showed a small but significant difference in improvement in SCORAD score. These contrasting conclusions between meta-analyses [42, 51] are the result of differences in the inclusion criteria, definitions and outcome measures examined. However, while a three-point difference in the SCORAD score reported by Michail et al. [51] might be statistically significant, the clinical relevance is doubtful. Thus, at present, there are insufficient data to support pre- or probiotics as effective treatments for eczema.

The role of probiotics in the treatment of allergic respiratory disease

Asthma and AR tend to affect older individuals with more established bowel flora and more established patterns of immune response, and there has been some anticipation that the benefits of probiotics in these conditions may be more limited. This may be reflected by the relative paucity of good studies in this area. Vliagoftis et al. [52] recently undertook a systematic review of all RCT that studied probiotics for the treatment of AR and asthma. Yet again, marked heterogeneity was a confounding feature. For the studies that were collectively examined for asthma treatment, there were no apparent benefits of probiotics

[53–56] (as summarized by Vliagoftis et al. [52]). For AR, the use of probiotics was associated with lower symptoms scores and medication use in many of the studies. However, the wide variation and lack of standardization in the assessment methods, study protocols and population characteristics made the collective data difficult to interpret. The main conclusion was that while the data suggest a beneficial effect of probiotics in AR, this is not conclusive and more studies are needed [52]. At present, there is insufficient evidence to support probiotics for treatment of either AR or asthma.

General issues, limitations and challenges

This remains an important ongoing area of research, in terms of both the safety and the effectiveness of probiotics. Probiotics are generally considered safe in the community where they are widely available and often considered as supplements to existing therapies. Long-term safety data from primary prevention studies using post-natal probiotics and prebiotics showed no differences in side-effects in the treated children [57]. However, in the context of treating children with established allergic disease, there are some reports of serious adverse effects (anaphylaxis) in milk-allergic children as a result of contamination with cow's milk proteins [58]. It is not uncommon for commercially available probiotics to be contaminated with cow's milk protein [58, 59] and for milk-allergic children to show sensitization to these probiotics [59]. Clinicians should promote awareness of these risks. More research is needed to provide a clearer message on the benefits (if any) and limitations of probiotics, to avoid potentially unnecessary use.

As we have indicated previously [10], a variety of factors contribute to the conflicting findings between studies. We propose that, as with any environmental exposure, the effects of probiotics are likely to vary according to a range of host and environmental factors. The same factors may explain benefits in sub-populations (i.e. such as different effects according to maternal phenotype, delivery method and infant phenotype). Some of these observations may reflect clinically relevant benefits that are obscured when data are pooled. *Host factors* include differences in genetic predisposition to allergic disease (across multiple loci), specific polymorphisms in microbial recognition pathways that may influence effects and susceptibility to colonization. A range of *environmental influences* may have secondary effects on colonization including maternal flora, delivery method, feeding practices and weaning diet, antibiotics, general microbial burden and other immunomodulatory influences. Finally, differences in the probiotic supplement such as the strain, dose, viability, timing/duration and method of administration may

result in differences in clinical effects. All these variations underpin the difficulties in performing and interpreting meta-analyses.

Future directions

Cohort studies are slow and expensive and generally only able to examine one or two variables. Ideally, these could be more cost effective if coordinated through an international consortium. Such studies need to be undertaken in tandem with detailed mechanistic studies that advance our still limited concepts of oral tolerance. It is important for future studies to also examine the effects of microflora in the context of other complex environmental changes, which are also likely to modify allergic propensity.

Conclusions

At present, based on the currently available evidence, probiotics, prebiotics or other microbial products are not indicated in the standard treatment or the primary prevention of allergic diseases. For primary prevention, initial meta-analyses suggest a benefit of probiotics in reducing the development of eczema, but not other allergic outcomes. However, at least some of these effects may have been exaggerated by methodological approaches [35], and do not include the effects of an equal number of more recent studies. Prebiotics (and synbiotics) may play a potential role in allergy prevention but at this stage more studies are needed. For treatment of established disease, meta-analyses have generally concluded that probiotics do not reduce symptoms of eczema or asthma and the effects on AR are inconclusive. These meta-analyses (of treatment or prevention studies) do not exclude the effects of selected strains in selected subgroups of patients, as suggested by a number of studies. While heterogeneity may be problematic when combining data, it may provide clues for understanding the optimal context for these strategies and suggest directions for further research.

The present strategies of modulating colonization are restricted by a still limited understanding of the complex gut microbiome, including the 'optimal' patterns of colonization for promoting immune tolerance. Finally, while there is no doubt that colonization is critical for normal immune development, the contribution of 'cleaner environments' relative to other complex environmental changes is not yet clear and a better understanding of these complex early interactions is also essential. These will be major challenges and underscore the need for novel approaches, which are likely to entail interdisciplinary collaborations.

Acknowledgements

Prof. Prescott receives funding from the National Health and Medical Research Council (NHMRC) of Australia.

Disclosures: Prof. Prescott has been a speaker at meetings sponsored by SHS/Nutricia and Nestlé. She has been a member of the independent Scientific Advisory Board of Nestlé Nutrition Institute Oceania, an expert panel on Cows Milk Allergy for Nutricia Australia, and an expert panel on omega-3 fatty acids for Mead and Johnson. She has received travel assistance and speaker fees from these companies to present at or attend scientific meetings.

References

- 1 Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001; 1:69–75.
- 2 Crabbe PA, Nash DR, Bazin H, Eyssen H, Heremans JF. Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. *Lab Invest* 1970; 22:448–57.
- 3 Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–45.
- 4 Björkstén B. Perinatal events in relation to sensitization in the human. *Am J Respir Crit Care Med* 2000; 162:S105–7.
- 5 Gerrard JW, Geddes CA, Reggin PL, Gerrard CD, Horne S. Serum IgE levels in white and metis communities in Saskatchewan. *Ann Allergy* 1976; 37:91–100.
- 6 Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998; 53:20–5.
- 7 Blumer N, Herz U, Wegmann M, Renz H. Prenatal lipopolysaccharide-exposure prevents allergic sensitisation and airway inflammation, but not airway responsiveness in a murine model of experimental asthma. *Clin Exp Allergy* 2005; 35:397–402.
- 8 Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000; 22:604–12.
- 9 Blumer N, Sel S, Virna S *et al.* Perinatal maternal application of lactobacillus rhamnosus GG suppresses allergic airway inflammation in mouse offspring. *Clin Exp Allergy* 2007; 37:348–57.
- 10 Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. *J Allergy Clin Immunol* 2007; 120:255–62.
- 11 Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108:516–20.
- 12 Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107:129–34.
- 13 Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076–9.

- 14 Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997; **99**:179–85.
- 15 Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006; **91**:814–9.
- 16 Food and Agriculture Organization/World Health Organization (FAO/WHO). *Joint FAO/WHO working group guidelines for the evaluation of probiotics in food*. London, ON, Canada: FAO/WHO, 2002.
- 17 Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. *Pediatr Res* 2006; **60**:221–4.
- 18 Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007; **119**:184–91.
- 19 Kukkonen K, Savilahti E, Haahtela T *et al*. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007; **119**:192–8.
- 20 Abrahamsson TR, Jakobsson T, Bottcher MF *et al*. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007; **119**:1174–80.
- 21 Kocourková I, Zádňíková R, Žižka J, Rosová V. Effect of oral application of a probiotic *E. coli* strain on the intestinal microflora of children of allergic mothers during the first year of life. *Folia Microbiol* 2007; **52**:189–93.
- 22 Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics* 2008; **121**:e850–6.
- 23 Soh SE, Aw M, Gerez I *et al*. Probiotic supplementation in the first 6 months of life in at risk Asian infants – effects on eczema and atopic sensitization at the age of 1 year. *Clin Exp Allergy* 2008; **39**:571–8.
- 24 Wickens K, Black PN, Stanley TV *et al*. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2008; **122**:788–94.
- 25 Huurre A, Laitinen K, Rautava S, Korkeamäki M, Isolauri E. Impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization: a double-blind placebo-controlled study. *Clin Exp Allergy* 2008; **38**:1342–8.
- 26 Niers L, Martin R, Rijkers G *et al*. The effects of selected probiotic strains on the development of eczema (The PandA study). *Allergy* 2009, in press.
- 27 West CE, Hammarstrom ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol* 2009; **20**:430–7.
- 28 Dotterud C, Oien T, Storro O, Johnsen R. Probiotic supplementation given to mothers in primary prevention of allergic diseases in early childhood – a randomised controlled trial in an unselected population. *Allergy* 2009; **64** (Suppl. 90):64.
- 29 Kuitunen M, Kukkonen K, Juntunen-Backman K *et al*. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009; **123**:335–41.
- 30 Prescott SL, Wiltschut J, Taylor A *et al*. Early markers of allergic disease in a primary prevention study using probiotics. *Allergy* 2008; **63**:1481–90.
- 31 Boyle RJ, Mah LJ, Chen A, Kivivuori S, Robins-Browne RM, Tang ML. Effects of lactobacillus GG treatment during pregnancy on the development of fetal antigen-specific immune responses. *Clin Exp Allergy* 2008; **38**:1882–90.
- 32 Prescott SL, Wickens K, Westcott L *et al*. Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon- γ and breast milk transforming growth factor- β and immunoglobulin A detection. *Clin Exp Allergy* 2008; **38**:1606–14.
- 33 Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. *J Allergy Clin Immunol* 2008; **121**:116–21 e111.
- 34 Osborn D, Sinn J. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev* 2007; **4**:CD006475.
- 35 Boyle RJ, Leonardi-Bee J, Bath-Hextall FJ, Tang ML. Probiotics for the treatment or prevention of eczema. *J Allergy Clin Immunol* 2009; **123**:266–7.
- 36 Rautava S, Kalliomäki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; **109**:119–21.
- 37 Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003; **361**:1869–71.
- 38 Boehm G, Lidestri M, Casetta P *et al*. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2002; **86**:F178–81.
- 39 Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J Nutr* 2008; **138**:1091–95.
- 40 Ziegler E, Vanderhoof JA, Petschow B *et al*. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J Pediatr Gastroenterol Nutr* 2007; **44**:359–64.
- 41 Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev* 2007:CD006474.
- 42 Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J, Murrell DF, Tang ML. Probiotics for treating eczema. *Cochrane Database Syst Rev* 2008:CD006135.
- 43 Passeron T, Lacour JP, Fontas E, Ortonne JP. Prebiotics and synbiotics: two promising approaches for the treatment of atopic dermatitis in children above 2 years. *Allergy* 2006; **61**:431–7.
- 44 Hattori K, Yamamoto A, Sasai M *et al*. Effects of administration of bifidobacteria on fecal microflora and clinical symptoms in infants with atopic dermatitis. *Arerugi* 2003; **52**:20–30.
- 45 Viljanen M, Savilahti E, Haahtela T *et al*. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy* 2005; **60**:494–500.

- 46 Gueniche A, Knaudt B, Schuck E *et al.* Effects of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. *Br J Dermatol* 2008; **159**:1357–63.
- 47 Weston S, Halbert A, Richmond P, Prescott SL. Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child* 2005; **90**:892–7.
- 48 Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000; **30**:1604–10.
- 49 Rosenfeldt V, Benfeldt E, Nielsen S *et al.* Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 2003; **111**:389–95.
- 50 Sistek D, Kelly R, Wickens K, Stanley T, Fitzharris P, Crane J. Is the effect of probiotics on atopic dermatitis confined to food sensitized children? *Clin Exp Allergy* 2006; **36**:629–33.
- 51 Michail SK, Stolfi A, Johnson T, Onady GM. Efficacy of probiotics in the treatment of pediatric atopic dermatitis: a meta-analysis of randomized controlled trials. *Ann Allergy Asthma Immunol* 2008; **101**:508–16.
- 52 Vliagoftis H, Kouranos VD, Betsi GI, Falagas ME. Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. *Ann Allergy Asthma Immunol* 2008; **101**:570–9.
- 53 Wheeler JG, Shema SJ, Bogle ML *et al.* Immune and clinical impact of *Lactobacillus acidophilus* on asthma. *Ann Allergy Asthma Immunol* 1997; **79**:229–33.
- 54 Giovannini M, Agostoni C, Riva E *et al.* A randomized prospective double blind controlled trial on effects of long-term consumption of fermented milk containing *Lactobacillus casei* in pre-school children with allergic asthma and/or rhinitis. *Pediatr Res* 2007; **62**:215–20.
- 55 Stockert K, Schneider B, Porenta G, Rath R, Nissel H, Eichler I. Laser acupuncture and probiotics in school age children with asthma: a randomized, placebo-controlled pilot study of therapy guided by principles of traditional Chinese Medicine. *Pediatr Allergy Immunol* 2007; **18**:160–6.
- 56 Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *Allergy* 2002; **57**:243–6.
- 57 Kukkonen K, Savilahti E, Haahtela T *et al.* Long-term safety and impact on infection rates of postnatal probiotic and prebiotic (synbiotic) treatment: randomized, double-blind, placebo-controlled trial. *Pediatrics* 2008; **122**:8–12.
- 58 Lee TT, Morisset M, Astier C *et al.* Contamination of probiotic preparations with milk allergens can cause anaphylaxis in children with cow's milk allergy. *J Allergy Clin Immunol* 2007; **119**:746–7.
- 59 Bruni FM, Piacentini GL, Peroni DG, Bodini A, Fasoli E, Boner AL. Cow's milk allergic children can present sensitisation to probiotics. *Acta Paediatr* 2009; **98**:321–3.
- 60 Cabana MD, McKean M, Wong AR, Chao C, Caughey AB. Examining the hygiene hypothesis: the trial of infant probiotic supplementation. *Paediatr Perinat Epidemiol* 2007; **21** (Suppl. 3): 23–8.
- 61 Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. *J Pediatr Gastroenterol Nutr* 2003; **36**:223–7.
- 62 Brouwer ML, Wolt-Plompen SA, Dubois AE *et al.* No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. *Clin Exp Allergy* 2006; **36**: 899–906.
- 63 Folster-Holst R, Muller F, Schnopp N *et al.* Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. *Br J Dermatol* 2006; **155**: 1256–61.
- 64 Gruber C, Wendt M, Sulser C *et al.* Randomized, placebo-controlled trial of *Lactobacillus rhamnosus* GG as treatment of atopic dermatitis in infancy. *Allergy* 2007; **62**:1270–6.
- 65 Hol J, van Leer EH, Elink Schuurman BE *et al.* The acquisition of tolerance toward cow's milk through probiotic supplementation: a randomized, controlled trial. *J Allergy Clin Immunol* 2008; **121**:1448–54.