Clinical reviews in allergy and immunology

Series editors: Donald Y. M. Leung, MD, PhD, and Dennis K. Ledford, MD

Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates

Petra Ina Pfefferle, PhD, DrPH,[a,b] Susan L. Prescott, MD, PhD,[b,c] and Matthias Kopp, MD[d] *Marburg and Lübeck, Germany, and Perth, Australia*

INFORMATION FOR CATEGORY 1 CME CREDIT

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the following instructions.

Method of Physician Participation in Learning Process: The core material for these activities can be read in this issue of the Journal or online at the JACI Web site: www.jacionline.org. The accompanying tests may only be submitted online at www.jacionline.org. Fax or other copies will not be accepted.

Date of Original Release: June 2013. Credit may be obtained for these courses until May 31, 2014.

Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation: The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The AAAAI designates this journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

List of Design Committee Members: Petra Ina Pfefferle, PhD, DrPH, Susan L. Prescott, MD, PhD, and Matthias Kopp, MD

Activity Objectives

1. To discuss why interventions aimed at achieving a more favorable microbiome are likely to have multisystem benefits.
2. To discuss the challenges in defining optimal colonization patterns.
3. To discuss the evidence that the early environment and early nutritional patterns are major determinants in initiating a long-lasting microbial colonization pattern.

Recognition of Commercial Support: This CME activity has not received external commercial support.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations: S. L. Prescott has received research support from the NHMRC and is on the board for Nestlé, Danone, and ALK-Abelló. M. Kopp has received consulting fees from Nestlé Health Care Nutrition and Infectopharm GmbH; is on the Xolair Advisory Board for Novartis Pharma GmbH; has received lecture fees from Novartis, GlaxoSmithKline, Chiesi, Bencard, and Nutricia; and has received travel support from Chiesi. P. I. Pfefferle declares that she has no relevant conflicts.

Epidemiologic studies indicate that microbes and microbial components are associated with protection against chronic inflammatory disease. Consequently, a plethora of clinical approaches have been used to investigate the benefits of a range of microbial products on inflammatory conditions in human trials. Centered particularly on the use of prebiotics, probiotic bacteria, and bacterial lysates in early life, this review provides an overview on clinical approaches aimed at reducing the global burden of allergic disease through primary prevention. Microbial interventions beginning before birth and in early infancy are discussed in the context of underlying mechanisms of oral tolerance and the establishment of gut colonization as a critical early homeostatic influence. We explore both the findings and challenges faced in existing studies with a view toward improving future clinical studies of the application of microbial compounds for the prevention of allergic disease and other inflammatory diseases. (J Allergy Clin Immunol 2013;131:1453-63.)

Key words: *Microbiome, probiotics, prebiotics, allergy, prevention, immune tolerance, bacterial lysates, allergic disease, Developmental Origins of Health and Disease*

Discuss this article on the JACI Journal Club blog: www.jaci-online.blogspot.com.

On the basis of Robert Koch's perception of human health being influenced by the host, potential pathogenic agents, and the environment, the concept of the "health or epidemiologic triad"[1] evolved: human subjects were seen within a homeostatic ecosystem affected by environmental and host-determined factors. With the (re)-discovery of the human microbiome, the contrast between

From [a]the Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University Marburg, Marburg; [b]the University of Gießen and Marburg Lung Center (UGMLC), Marburg; [c]the School of Paediatrics and Child Health, University of Western Australia and Princess Margaret Hospital for Children, Perth; and [d]the Department of Pediatric Allergy and Pulmonology, Children's Hospital, University Lübeck.

Received for publication December 14, 2012; revised February 19, 2013; accepted for publication March 22, 2013. Available online May 2, 2013.

Available online May 2, 2013.

Corresponding author: Petra Ina Pfefferle, PhD, DrPH, Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics Biomedical Research Centre, Philipps University Marburg, Hans-Meerweinstr. 2, 35043 Marburg, Germany. E-mail: pfefferl@med.uni-marburg.de.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.03.020

Abbreviations used
ARTI: Acute respiratory tract infection
BL: Bacterial lysate
FOS: Fructo-oligosaccharide
GOS: Galacto-oligosaccharide
NCD: Noncommunicable disease
PNG: Papua New Guinea
RR: Relative risk

host and environment resolved into an integral view of human subjects themselves being an entire ecosystem.[2,3] The microbiome (ie, the entirety of microorganisms colonizing epithelial surfaces) seems to be highly diverse between subjects but also between habitats at different body sites. Although ultimately acquired from the environment, the composition of this internal ecosystem is distinct from that of the biosphere.[4] We are only starting to understand these interactions between human subjects and microbes; nevertheless, the importance of a well-balanced microbial environment for human health is evident.

In parallel, our view of the immune system has moved from the mere defense against pathogenic or hazardous agents to an interface between host and environment, actively organizing the interactions with all sorts of microorganism, from pathogens over harmless commensal agents to helpful symbionts. As a key function of the immune system, tolerance developed through coevolutionary phylogenetic adaptation of human populations to their microbial environment.[5] From the dawn of mankind, the biosphere incessantly stimulated the human immune system. The development of affluent lifestyles and postmodern habitats has led to skewed microbial diversity and disturbance of the human-microbial balance only within the last century, with health consequences subsumed under the hygiene hypothesis.[6] Altered microbial exposure in early life predisposes populations in industrialized countries and increasingly in developing regions of the globe to disease development.[7] In particular, chronic inflammatory diseases driven by immune dysregulation, such as allergies, autoimmune diseases, and other noncommunicable diseases (NCDs), have become a major health problem. As a preventive strategy, early supplementation of infants with microbial compounds was proposed for priming the developing immune system toward more tolerance.[8]

In many cultures nutrients fermented by microorganisms are a traditional part of the everyday diet. Originally used to preserve food, fermented nutrients were also recognized to provide health benefits.[9] Metchnikoff[10] initially postulated that dairy products protect the gut from toxic bacteria and thereby prolong life. Medical application of viable bacteria was first reported for *Escherichia coli* Nissle 1917, a gut bacterium that was isolated from a soldier who safely survived a strong epidemic of diarrhea during World War I.[11] Despite these early approaches, the related term probiotic was introduced only in the 1960s. Favoring pharmaceutical approaches, the therapeutic and preventive potential of live microorganisms was vastly neglected. This paradigm was changed by the increasing success of the hygiene hypothesis and recent insights into functional aspects of the microbiome.[12] In the example of allergy and asthma, preventive strategies are now changing from avoidance of allergens to the establishment of tolerance.[13]

PRENATAL STRATEGIES TO MODIFY MATERNAL COLONIZATION PATTERNS

There is now little doubt that the foundations for immune tolerance are established during fetal life and that factors in the maternal environment during this period can have a significant influence. Importantly, the modern environmental lifestyle changes associated with failing tolerance and immune disease are also implicated in the increase of many other chronic inflammatory NCDs. Allergy is arguably the earliest and most common manifestation of the increasing propensity for inflammation and highlights the specific vulnerability of the immune system to environmental changes. Although the focus here is primarily on changing microbial exposure as a modifiable risk factor, this cannot realistically be examined in isolation. This is integrally determined by changes in behavior, lifestyle, and nutritional patterns,[14] and therefore prevention strategies will ultimately need to be considered in this wider context. Similarly, although the focus of these discussions might center around allergic disease, it should be recognized that similar environmental risk factors for many other modern NCDs could require common solutions.

Until recently, the dominant focus of early microbial exposure was in the immediate postnatal period. Even intervention strategies, such as probiotics, commenced in the final stages of pregnancy were generally aimed at modifying initial postnatal colonization patterns. Although the fetus resides in the relatively "sterile" uterine environment, it is becoming clearer that maternal microbial exposures and colonization patterns throughout pregnancy can also have an influence on the developing fetal immune system and many other developing systems. Animal models have clearly demonstrated how maternal exposure to both pathogenic[15] and nonpathogenic[16] microbial products in pregnancy can prevent allergic outcomes in the progeny. In human subjects the now well-known allergy-protective effects of animal exposure in Alpine farmhouses has provided a natural model to examine the effects of early microbial exposure.[17] Within this setting, high prenatal microbial exposure has an independent protective effect on subsequent allergic outcomes.[18] This has been associated with differences in innate immunity,[4] as well as increased numbers and function of cord blood regulatory T cells.[19] Investigating this further in an animal model, prenatal administration of a cowshed-derived bacterium, *Acinetobacter lwoffii* F78, can prevent the development of an asthmatic phenotype in progeny. Furthermore, this effect is IFN-γ dependent and appears to be mediated through epigenetic mechanisms.[20] The asthma-preventive effect has also been associated with modification of Toll-like receptor expression in the placenta[21] and establishes a direct relationship between maternal bacterial exposures, functional maternal Toll-like receptor signaling, and asthma protection in the progeny. Human studies also provide preliminary evidence that prenatal microbial exposure is associated with epigenetic variations (decreased methylation) of the forkhead box protein 3 regulatory gene *(FOXP3)*, again in the farming environment.[19] This is consistent with observations in other populations that subsequently nonallergic children are likely to have higher placental forkhead box protein 3 expression.[22] Extensive differences in both innate[23] and adaptive[23,24] immune function at birth in children who go on to have allergy also point to the importance of *in utero* exposures.

Studies of neonates born in areas of the developing world with very high microbial burden, such as Papua New Guinea (PNG), also show extensive differences in neonatal immune function

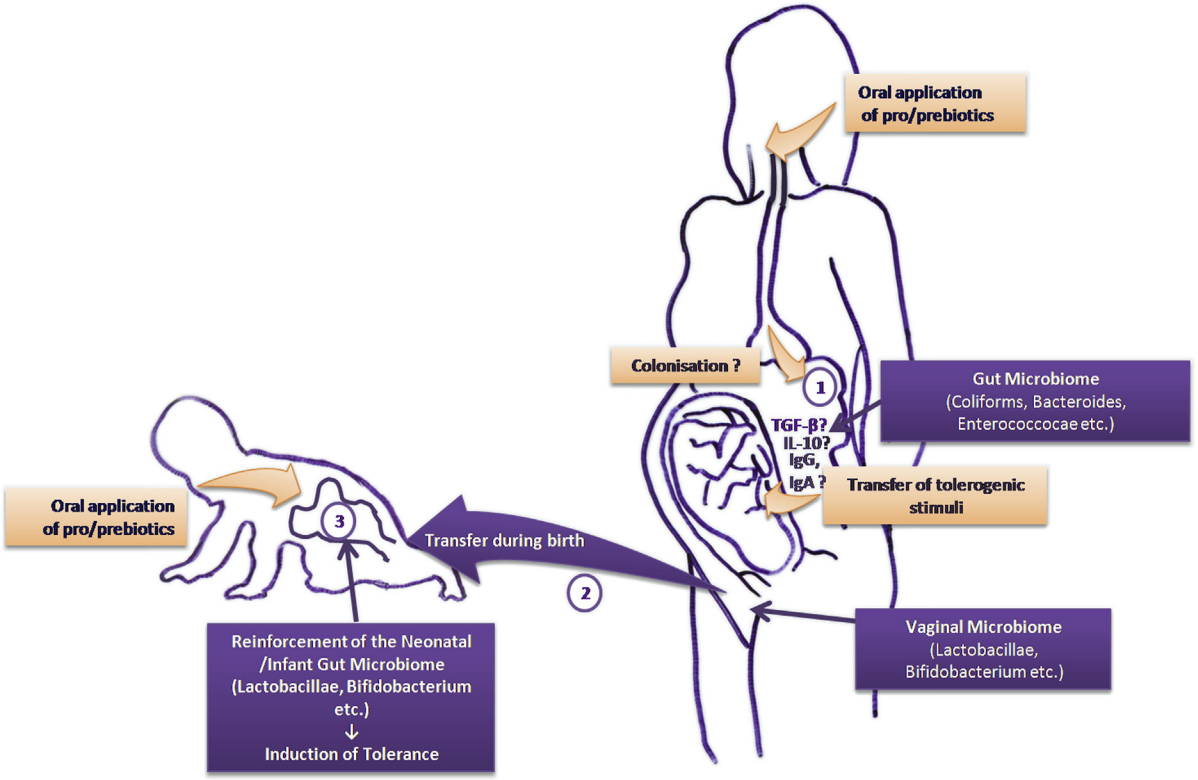

FIG 1. Possible mechanisms of prenatal and postnatal induction of tolerance by microbial components. *1*, Prebiotics and probiotics can influence the mother's gut microbiome, potentially inducing regulatory cytokines that might pass the placenta and prime the fetal immune system toward tolerance. *2*, Through vaginal birth, the newborn's gut acquires the maternal vaginal microbiome. *3*, Oral application of prebiotics/probiotics might support a homeostatic colonization of the infant's gut.

compared with newborns in highly developed hygienic regions, such as Australia.[25,26] At the end of gestation, antigen-presenting cells from PNG neonates already show much higher baseline expression of markers of activation (HLA-DR and CD86) and inhibition (immunoglobulin-like transcripts 3 and 4) compared with Australian newborns.[25] However, on activation, antigen-presenting cells from PNG cord blood are relatively quiescent with reduced activation and antigen processing and evoke an attenuated T-cell response compared with Australian neonates.[25] Although there are many environmental differences between these settings, microbial burden is one of the most striking, and the more tolerogenic responses of PNG newborns might protect against the development of harmful inflammatory responses in early life. These and other experiments of nature all strongly support the role of the prenatal maternal environment in early immune programming and raise the challenging question of how to harness this to improve immune health in the setting of globally increasing propensity for allergy and other chronic inflammatory diseases.

Thus the effects of maternal microbial exposures on offspring are multifaceted, including direct effects on the maternal immune system, how these effects can modulate events at the maternofetal interface to influence patterns of fetal immune programming, and the more obvious effects on postnatal colonization of the infant (Fig 1).

To date, most of the intervention studies using microbial products in pregnancy for allergy prevention have been limited to probiotics, and the clinical findings are discussed further below, with other clinical trials. Because prenatal use of prebiotic and probiotic products has been largely in combination with postnatal supplementation, the specific antenatal effects are difficult to determine in many studies. In some of these studies, examination of cord blood immune function has been one strategy to examine the immunologic effects of antenatal probiotics. Although animal studies with probiotics show antenatal effects,[16] the effects of maternal probiotics on cord blood immune responses in human randomized controlled trials are conflicting.[25,26] In one study the administration of probiotic bacteria during the final weeks of pregnancy was associated with an increase in cytokine (IFN-γ) detection in cord blood,[27] whereas another study performed a much more comprehensive investigation of immune function and found no effects on any aspect of neonatal cellular profile of function.[28] Importantly, these studies examined different strains, and the inconsistencies between strains might reflect the strain-specific clinical effects that have also been seen (as discussed below).[29]

OUTCOMES OF CLINICAL TRIALS WITH PROBIOTICS AND PREBIOTICS FOR PRIMARY PREVENTION

When it comes to clinical intervention studies using microbial products in human subjects, these have been mainly focused on the use of probiotic bacteria, although the prebiotics fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS) are also used to modify colonization patterns (Table I).[29-42] Most of these studies looked primarily at early outcomes of allergic disease, such as eczema and IgE-mediated food allergy, with far fewer looking at longer-term outcomes on respiratory allergic disease.

TABLE I. Data from clinical trials that included either prenatal or the prenatal and postnatal or postnatal intervention phase of probiotics, prebiotics, or both

Author	Intervention	Country	Inclusion criteria	Participants and dropout rate
Only prenatal intervention				
Boyle et al,[41] 2011	*Lactobacillus* GG	Australia	Mother, father, or a previous child affected by a doctor-diagnosed allergic disease	NR = 250 mothers/infants NE = 212 infants Dropout: 15%
Prenatal and postnatal intervention				
Kalliomaki et al,[30] 2001	*Lactobacillus* GG	Finland	One family member (mother, father, or older sibling) with an atopic disease	NR = 159 NE = 132 Dropout: 17%
Abrahamsson et al,[35] 2007	*Lactobacillus reuteri*	Sweden	One or more family members with eczema, asthma, gastrointestinal allergy, allergic urticaria, or allergic rhinoconjunctivitis	NR = 232 NE = 188 Dropout: 19%
Kukkonen et al,[33] 2007	*Lactobacillus rhamnosus* GG, *L rhamnosus Bifidobacterium breve Propionibacterium freudenreichii* plus galacto-oligosaccharides	Finland	At least 1 parent of the unborn child had a doctor-diagnosed allergic disease, as evaluated in telephone interviews by trained personnel	NR = 1223 NE = 925 Dropout: 24%
Kopp et al,[31] 2008	*Lactobacillus* GG	Germany	One family member (mother, father, or older sibling) with an atopic disease	NR = 105 NE = 94 Dropout: 10%
Niers et al,[38] 2009	*Bifidobacterium bifidum*, *Bifidobacterium lactis*, and *Lactococcus lactis*	The Netherlands	Allergic diseases of mother or father plus an older sibling	NR = 156 NE = 102 (3 mo) Dropout: 34% NE = 89 (12 mo) Dropout: 43%
Huurre et al,[32] 2008	*Lactobacillus rhamnosus* strain GG and *Bifidobacterium lactis* Bb12	Finland	Healthy mothers and mothers with an allergic disease (probiotic group, 80%; placebo group, 77%)	NR = 171 NE = 140 Dropout: 18%
Wickens et al,[29] 2008	*Lactobacillus rhamnosus* or *Bifidobacterium animalis* HN019	New Zealand	Mother or father with a history of treated asthma, eczema, or hay fever	NR = 512 NE = 446 Dropout: 13%
Kim et al,[37] 2010	*Bifidobacterium bifidum* and *B lactis* and *Lactobacillus acidophilus*	Korea	Family history of allergic diseases	NR = 112 mothers NE = 68 infants Dropout: 39%
Dotterud et al,[36] 2010	*Lactobacillus rhamnosus* GG and *L acidophilus* La-5 and *Bifidobacterium lactis* Bb-12	Norway	Nonselected population	NR = 415 mothers NE = 278 infants Dropout: 33%
Rautava et al,[42] 2012	*Lactobacillus rhamnosus* and *Bifidobacterium longum* (I) or *L paracasei* ST11 and *B longum* (II)	Finland	Mothers with allergic diseases and sensitization	NR = 241 NE = 205 Dropout: 15%
Only postnatal intervention				
Taylor et al,[39] 2007	*Lactobacillus acidophilus*	Australia	Mothers with allergy	NR = 231 NE = 178 Dropout: 23%
Soh et al,[34] 2009	*Lactobacillus rhamnosus* and *Bifidobacterium longum*	Singapore	Family history of allergic disease; first-degree relative with a doctor's diagnosis of asthma, allergic rhinitis, or eczema and a positive skin prick test response	NR = 253 NE = 245 Dropout: 3%
Grüber et al,[40] 2010	Prebiotic mixture of neutral oligosaccharides and pectin-derived acidic oligosaccharides	Europe	Families with low atopy risk; formula-fed infants were randomized.	NR = 830 NE = 735 Dropout: 11.5% A total of 300 infants were followed in the breast-feeding group.

NE, Number of participants followed to the primary end point; *NR*, number of randomized participants; *OR*, odds ratio.

TABLE I. *(Continued)*

Primary end point	Allergic sensitization	Follow-up	Comments
Cumulative incidence of eczema during the first year: probiotic group, 34%; placebo group, 39% (RR, 0.88; 95% CI, 0.63-1.22)	No effect on allergic sensitization	No	
Chronic recurrent atopic eczema at age 2 y: probiotic group, 23%; placebo group, 46% (R, 0.51; 95% CI, 0.32-0.84)	No effect on allergic sensitization	4- and 7-y Follow-up: persistent effects	
Allergic diseases at ages 12 and 24 mo: no differences between groups Cumulative incidence of eczema: probiotic group, 36% Placebo group, 34%	No effect on allergic sensitization	No	Less IgE-associated eczema during second year: 8% vs 20% ($P = .02$)
Cumulative incidence of allergic diseases (food allergy, eczema, asthma, and allergic rhinitis): no effect (OR, 0.85; 95% CI, 0.64-1.12)	No effect on allergic sensitization	5-y Follow-up: no difference between groups	Probiotic treatment reduced eczema (OR, 0.74; 95% CI, 0.55-0.98; $P = .035$) and atopic eczema (OR, 0.66; 95% CI, 0.46-0.95; $P = .025$).
Atopic eczema at age 2 y: probiotic group, 28%; placebo group, 27.3% (OR, 0.96; 95% CI, 0.38-2.33)	No effect on allergic sensitization	No	Same design and intervention as Kalliomäki et al
Parent-reported eczema at age 3 mo: Probiotic groups, 12%; placebo group, 29% (OR, 0.322; 95% CI, 0.108-0.960); no difference between the groups at ages 12 and 24 mo	No effect on allergic sensitization	No	High dropout rate; parent-reported eczema only in a small group was confirmed by doctor's diagnosis
Infant sensitization at age 12 mo; no difference between infant sensitization between probiotic (29%) and placebo (31%) groups (OR, 0.92; 95% CI, 0.45-1.90)	Subgroup of of infants with a maternal sensitization: probiotic group had a smaller number of sensitizations (26%) compared with placebo group (50%; OR, 0.34; 95% CI, 0.13-0.88)	No	Part of an ongoing study with nutrition modulation by dietary counseling and probiotic supplementation
Infant's cumulative prevalence of eczema at age 2 y Infants receiving *L rhamnosus* HN001 had a significantly reduced risk of eczema by 2 y (14.8%) compared with infants in the placebo group (26.8%; hazard ratio, 0.51; 95% CI, 0.30-0.85) No effect was seen in the *Bifidobacterium* species group	No effect on allergic sensitization	4-y Follow-up: persistent effect	
Cumulative incidence of eczema during first 12 mo: significantly reduced in probiotic group (36.4%) over placebo group (62.9%; $P = .029$)	No effect on allergic sensitization	No	High dropout rate
Cumulative incidence of atopic dermatitis at age 2 y age; reduced in the probiotic group (OR, 0.51; 95% CI, 0.30-0.87)	No effect on allergic sensitization	No	High dropout rate
Cumulative incidence of eczema during first 24 mo: significantly reduced in infants of mothers receiving probiotics (I: OR, 0.17; 95% CI, 0.08-0.35; II: OR, 0.16; 95% CI, 0.08-0.35)	No effect on allergic sensitization	No	Only supplementation of the mother 2 mo before delivery and during first 2 mo of breast-feeding
Atopic dermatitis at ages 6 and 12 mo; no difference between probiotic group (25.8%) and placebo group (22.7%)	No effect on allergic sensitization	Yes	
Eczema at age 1 y; atopic eczema: probiotic (7.3%) vs placebo (5.8%) group ($P = 0.86$)	No effect on allergic sensitization	No	
Cumulative incidence of atopic dermatitis at age 12 mo; significantly reduced incidence in prebiotic group (5.7%) compared with placebo group (9.7%; $P = .04$)	No effect on allergic sensitization	No	Incidence of atopic dermatitis in prebiotic group was in the low range of breast-feeding group (7.3%)

When considering probiotic studies collectively,[29-39,43-49] around half showed a significant reduction in the development of eczema (from 25% to 50% reduction), whereas there were no benefits in the remaining studies, even when the same probiotic strains and similar protocols were used. There have been no consistent effects on other allergic outcomes, and fewer studies have assessed the longer-term effects on respiratory allergic disease. Although 2 follow-up studies observed a persistent protective effect of probiotics on eczema prevention after 4[44,50] and 7[43] years, another study saw no sustained effect of a mixture of prebiotics and probiotics after 5 years.[45] Several meta-analyses have now been performed, generally concurring that probiotics reduce the risk of eczema but not other allergic outcomes,[51-55] although more specific conclusions have varied (see below). The most recent meta-analysis[55] included 13 prevention studies[30,31,33-35,37-39,46] and found that probiotic treatment reduced the incidence of eczema by 21% (relative risk [RR], 0.79; 95% CI, 0.71-0.88). This effect was still evident when the analysis was restricted to patients with IgE-associated eczema (RR, 0.80; 95% CI, 0.66-0.96).

For GOS/FOS prebiotics, the focus of the allergy-preventive effects has largely been in the postnatal period. However, animal studies have shown that maternal consumption of FOS prebiotics during pregnancy and lactation diminished eczema-like skin lesions in offspring.[56] In this model the maternal intervention was more effective than direct feeding to offspring after weaning. Human studies with GOS/FOS prebiotics have also shown early promise in reducing some allergic manifestations. Again, it is also important to consider the other systemic benefits of these interventions in pregnancy. For example, maternal consumption of high-prebiotic fiber during pregnancy can also have favorable effects on glucose and lipid metabolism in offspring,[57] and this might have lasting benefits for the risk of obesity and type 2 diabetes and other chronic inflammatory diseases. In one of the first major studies to investigate the effect of prebiotics on the incidence of eczema, a mixture of GOS/FOS prebiotics was administered during the first 6 months of life to formula-fed infants at high risk of atopy.[58] After 6 months, 10 (9.8%; 95% CI, 5.4% to 17.1%) infants in the prebiotic group and 24 (23.1%; 95% CI, 16.0% to 32.1%) infants in the placebo group had eczema. The follow-up assessment at 2 years of age, although limited to approximately half of the original population, showed a significant reduction in the cumulative incidence of eczema, recurrent wheeze, and allergic urticaria in the treatment group compared with the control group.[59] Further studies are also needed to confirm these findings, although there is another large study in a population at low risk of allergic disease with randomized formula-fed infants given a formula with a mixture of neutral oligosaccharides and pectin-derived acidic oligosaccharides or regular formula. Breast-fed infants served as a nonrandomized reference group. The cumulative incidence of eczema at 12 months of age was significantly reduced in the prebiotic group compared with the placebo group (5.7% vs 9.7%), and the cumulative incidence of eczema in the breast-fed group was 7.3%.[40] More studies of GOSs/FOSs are needed before any recommendations can be made in either high- or low-risk infants.

Many of the inconsistencies in the findings between these studies, particularly of probiotics, can be attributed to the wide range of methodological heterogeneity, which hampers both direct comparison and meta-analysis (ie, different probiotic strains or combinations, method of delivery, dose, inclusion criteria, timing and duration of the intervention, and measurement of clinical outcomes). The results are conflicting, even for *Lactobacillus rhamnosus* GG, the most extensively studied strain.[30,31,41,60]

Differences in the timing of the intervention have been proposed as one major reason for differences in study outcomes, with some earlier speculation that favorable effects might be more likely if a prenatal component were included.[35] The protective effect of probiotics on infantile eczema is evident both when all eligible studies are examined collectively (regardless of strain and regardless of prenatal supplementation, postnatal supplementation, or both)[51,55] and when analyses are limited to studies that included a prenatal component.[55] However, there has also only been 1 randomized trial to specifically examine the effects of probiotics administered only in the prenatal period on subsequent allergic disease. In that study mothers were given *Lactobacillus rhamnosus* GG or placebo for the last 2 to 4 weeks of pregnancy (ie, without any subsequent postnatal supplement); however, there was no difference in allergic outcomes between the groups.[41] With only 1 study giving a single strain for a limited time, it is difficult to base clear conclusions on this. The majority of studies that included a prenatal treatment typically only used this for 4 to 6 weeks before delivery. There is only 1 study that has used longer-term supplementation with probiotics in pregnancy (for the last 2 months of gestation and in lactation), and this study found a reduction in eczema but no effect on other outcomes.[42] On the other hand, there are also several studies that used probiotics exclusively after birth. Two commenced probiotics in the neonatal period with no effect on any allergic outcomes,[34,39] and one delayed the probiotic until weaning and still achieved a reduction in eczema.[47] In contrast, 6 of 9 studies with combined prenatal and postnatal prebiotics (with or without prebiotics) reduced the incidence of eczema.[29-33,35,37,38,42-44]

Collectively, these findings suggest that a combined antenatal and postnatal approach supplementation seems to be the most promising approach in families at risk of atopic diseases.

Another key point of heterogeneity is in the strains used and whether these are used alone or in combination. Recently, Doege et al[54] compared monotherapy with various combinations of different probiotic strains and concluded that only monotherapy with lactobacilli resulted in a significant risk reduction for atopic eczema (RR, 0.82; 95% CI, 0.71-0.95),[29,31,35,43] whereas a mixture of different probiotic strains did not (RR, 0.92; 95% CI, 0.83-1.02).[32,33,45] However, despite their quality control criteria of excluding follow-up publications from the same populations, the data from one large Finnish study were included twice.[33,45] Moreover, a more recent study (data not published at the time of their analysis) shows the efficacy of several combinations of probiotics in reducing eczema, namely (1) *L rhamnosus* and *Bifidobacterium longum* and (2) *Lactobacillus paracasei* and *B longum*, respectively.[42] Finally, *in vitro* studies have demonstrated synergistic effects for a combination of lactobacilli and bifidobacteria, which challenge the proposal that monotherapy with lactobacilli is the best strategy.[61]

Variation in population characteristics is another important point of difference between studies. This is illustrated in the 2 clinical trials (one in Finland and the other in Germany) that used the same probiotics and the same study protocol but found differing results.[30,31] The most striking difference between these 2 trials is the background prevalence of eczema in the placebo group. Although 46% (n = 31/68) of the Finnish children had eczema during the first 2 years of life, only 27.3% (n = 12/44) of the German children had doctor-diagnosed eczema. More recently,

TABLE II. List of the concepts and therapeutic implications

Key concept	Therapeutic implications
Oral application of probiotic bacteria and prebiotics during pregnancy	There appears to be a moderate risk reduction for eczema (but not other allergic outcomes) in studies that have included an antenatal component. Although studies are limited, favorable effects might be more likely when combined with postnatal supplementation.
Oral application of probiotic bacteria and prebiotics in early infancy	Collectively, studies suggest a moderate risk reduction for eczema but not for any other allergic outcomes that is likely to be more successful when combined with antenatal supplementation.
Oral application of bacterial lysates in childhood	Moderate risk reduction for recurrent ARTIs and wheezing is seen, but there is only minor evidence for prevention of allergic outcomes.

another prevention study from Finland also observed a high prevalence of eczema (as high as 71% [n = 44/62]) in a high-risk population based on maternal atopic disease.[42] Differences in both genetic background and lifestyle (which might contribute to different epigenetic effects) could explain these discrepancies. In particular, background differences in colonization patterns could be important environmental differences in these populations. In support of this, stool analysis of a small cohort (n = 30) of fully breast-fed neonates in Finland and Germany[62] showed that German infants had a more heterogeneous stool flora, with significantly more *Akkermansia muciniphila* and a tendency for less bifidobacteria compared with the Finnish population. This is a key environmental factor in considering regional differences in effectiveness of any intervention to modify colonization. Other background behaviors can also contribute to difficulties in adequate placebo control in the study population. Probiotics are widely used as supplements in infant formulas, yogurts, and other milk products confounding adequate placebo control. Recently, published trials[52] gave the participants advice to avoid these products, which might be a way to control for inadvertent consumption of probiotics in the control group.

Achieving adequate longer-term follow-up is another major challenge in all of these studies. However, where possible, it is important that clinical trials with 39%[38] and 40%[37] of the randomized children lost to follow-up also provide information about an "intention-to-treat" analysis rather that reporting only results by "per protocol" for the retained group. Moreover, measures of control for the loss of follow-up should be calculated in the statistical analysis. In the subsequent considerations about the efficacy of clinical trials, studies with a dropout rate of greater than 35% and no information about the intention-to-treat population are tagged with a specific comment.

Both clinical definitions of risk (inclusion criteria) and definitions of childhood disease (primary outcome measures) are important differences between studies. The effectiveness of intervention can also depend on the genetic risk of allergic disease. Most, but not all, trials targeted populations at risk of allergy with a first-degree family member with an atopic disease but included a variable proportion of uniparental and biparental allergic history. Primary outcome measures also varied, and included doctor-diagnosed eczema, parent-reported eczema, or any atopic disease.

In conclusion, there are some encouraging results from clinical trials that prebiotics, probiotics, or both might have a potential for preventing atopic eczema but not asthma, allergic rhinitis, or allergic sensitization (Table II). Most probably, intervention should best include both antenatal and postnatal components. However, more than 10 years after the first reported clinical trials, there is still uncertainty about the ideal strain or strains, the target population, the timing of introduction, dosing and the role of background factors (eg, feeding habit), and a range of complex genetic factors.

POSTNATAL ADMINISTRATION OF BACTERIAL LYSATES TO PREVENT PRECONDITIONS OF ASTHMA

Although treatment with probiotics is based on application of viable bacteria, therapeutic approaches with bacterial lysates (BLs) focus on application of lyophilized extracts from bacterial cultures. Lyophilisates are obtained from single strains or from a cocktail of bacterial species either through chemical or mechanical disruption. Mechanical lysis is thought to provide more immunogenic lysate components because no denaturation of proteins occurs during mechanical disruption of the cells.[63] On the basis of the concept that microbial immunogenic components are capable of improving the host defense against pathogens, BL application has been recommended for the prevention of respiratory and gut infections and subsequent inflammatory conditions.[64]

In contrast to application of viable bacteria, BLs seem not to act through direct modification of colonization patterns. Clinical studies, as well as animal experiments, have indicated that increases in (secretory) IgA levels seem to be the most important immunomodulating activity of BLs.[65-67] According to the age-dependent development of the IgA repertoire,[68] improved IgA release observed after BL application is attributed to a more unspecific T cell–driven enhancement of IgA maturation in early life and an antigen-defined induction of IgA in patients of preschool age and older (Fig 2).[69] However, this mode of mucosal immunization against lung pathogens might at least affect the microbial colonization pattern in the lung and thereby contribute to long-lasting protective effects against airway inflammation.

OM-85 BV, the best clinically evaluated BL, is composed of an endotoxin-low alkaline-extracted fraction of pathogens causing respiratory tract infections of the upper airways.* OM-85 BV has been predominantly developed to prevent recurrent acute respiratory tract infections (ARTIs), namely acute viral infections, including the common cold, which are recognized as closely linked to asthma exacerbations in patients if preschool age.[70] Moreover, recurrent ARTIs are preconditions for persistent wheezing in childhood and thereby linked to the development of asthma later in life.[71]

Evidence that orally administered OM-85 BV is able to stimulate a selective T_H1-driven response came from studies in neonate rats[72] indicating that OM-85 BV can induce maturation of

*_Haemophilus influenzae_, _Diplococcus pneumonia_, _Klebsiella pneumoniae_, _Klebsiella ozaenae_, _Staphylococcus aureus_, _Streptococcus pyogenes_, _Streptococcus viridans_, and _Neisseria catarrhalis_.

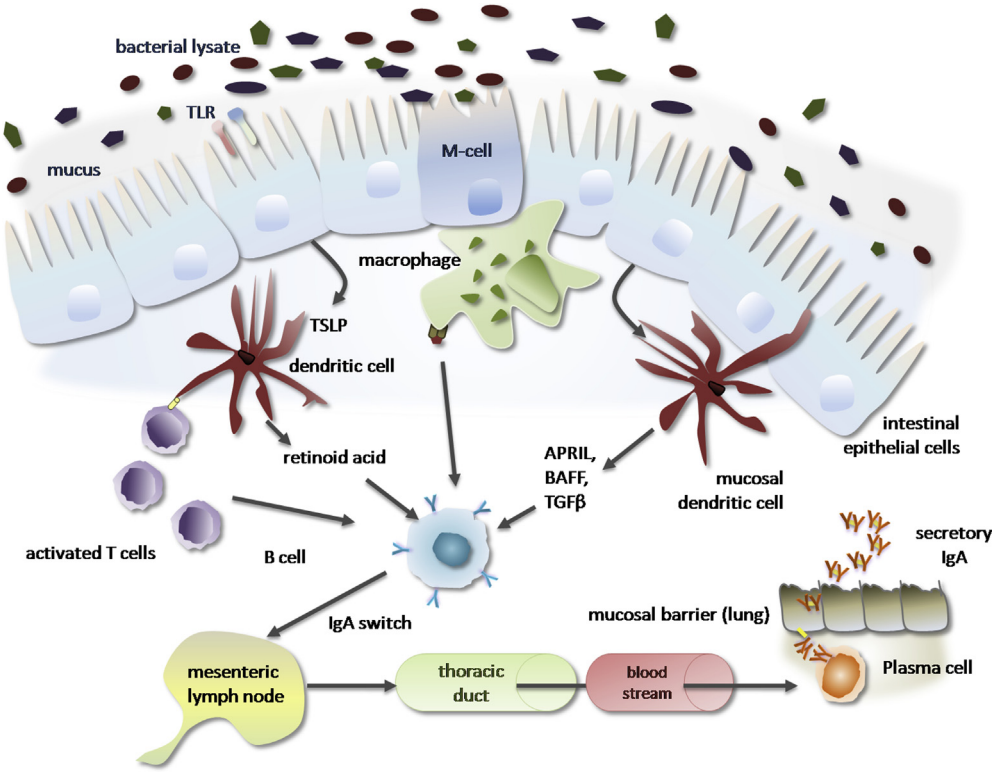

FIG 2. Gut-associated lymphoid tissue at the intestinal epithelium initiates microbial recognition. First, M cells transfer antigens from the intestinal lumen to dendritic cells and macrophages. Second, epithelial cells produce thymic stromal lymphopoietin *(TSLP)* on Toll-like receptor *(TLR)* signaling. Third, TSLP release induces production of a proliferation-inducing ligand *(APRIL)*, *B-cell* activating factor of the TNF family *(BAFF)*, TGF-β, and retinoic acid by dendritic cells promoting IgA class-switching in B cells with help from activated T cells. Fourth, B cells migrate from the intestinal submucosa through the mesenteric lymph nodes and lymphatic and blood vessels to the target tissues and mature to plasmocytes. Finally, release of IgA at mucosal surfaces supports preserving local homeostasis and immune exclusion.

dendritic cells and skew the perinatal T_H2 milieu toward T_H1 maturation. Exposure of OM-85 BV in rodent models of experimental asthma pointed to an attenuation of the functional hallmarks of asthma. Strickland et al[73] demonstrated that OM-85 BV–pretreated and ovalbumin-sensitized rats displayed significantly diminished airway inflammation accompanied by an improvement in lung function. Similarly, Navarro et al[74] showed that the abolishment of the asthmatic phenotype in mice was associated with a selective recruitment of $CD4^+CD25^+Foxp3^+$ regulatory T cells in the tracheal compartment.[74] Both studies suggest that orally applied OM-85 BV has the capacity to affect the immune repertoire of the lung along the mucosal lung-gut axis toward tolerance.

Human studies on the efficacy of OM-85 BV as an immunostimulating agent have mainly focused on attenuation of ARTIs in early childhood. Within the last 3 decades, a number of clinical studies investigated the efficacy of OM-85 BV in diminishing and preventing ARTIs in children. Recently, 2 systematic reviews provided a quantified overview of the clinical evidence. In 2007, Steurer-Stey et al[75] meta-analyzed 13 randomized and controlled trials including 2721 patients. Because efficacy end points varied widely between studies, pooling of data was limited to data from up to 2 trials. A beneficial trend was observed as recurrence of infections was reduced. Use of antibiotics and lengths of episodes were significantly reduced in OM-85 BV–treated children in at least 2 studies. The authors concluded that OM-85 BV application in young children has only weak effects on the prevention of ARTIs. A more recent meta-analysis provided by Schaad[76] included 8 randomized controlled trials (n = 851) on secondary prevention of pediatric ARTIs. This revealed a significant and consistent reduction of ARTI recurrence in OM-85 BV–treated children (32% receiving active treatment vs 58.2% receiving placebo with ≥3 ARTIs/6 months). Data from an exploratory analysis indicated that benefit from OM-85 BV treatment was greater in high-risk children. These results were underlined recently by a well-conducted study on the prevention of wheezing episodes in children with recurrent ARTIs. Razi et al[71] observed a 37.9% reduction in wheezing attacks ($P < .001$) accompanied by a significant reduction of ARTIs in the intervention arm.

Comparable results have been described for other BLs proved in clinical application.[77] For example, LW 50020 and ribosomal extracts are mixtures of slightly different BLs† and were shown to activate T_H1-polarizing innate immune responses and increase locally and systemically specific immunoglobulin levels in human subjects.[78-80] Placebo-controlled clinical trials on the efficacy of LW 50020 and ribosomal extract reported similar results for the

†*LW 50020 = Staphylococcus aureus, Streptococcus mitis, Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Moraxella (Branhamella) catarrhalis, and Haemophilus influenzae*. Ribosomal extract= *Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes*, and *Haemophilus influenza* in combination with cell wall proteoglycan KpOmpA from *Klebsiella pneumonia*.

recurrence and duration of ARTIs in children as found in studies with OM-85 BV.[81-83] A pilot study applying ribosomal extract to children with allergic (IgE-associated) and nonallergic eczema revealed an improvement in clinical manifestations of eczema after 20 weeks of treatment.[84] The safety of all of these treatments was estimated as good, with no severe adverse events observed in the trials.[85]

There is increasing evidence that BLs from aeropathogenic bacteria might be protective against ARTIs and thereby might support the primary prevention of asthma (Table II). However, none of the studies has shown whether there is a reduction of risk for allergic outcomes. Randomized controlled trials specifically in atopic children are needed to evaluate the allergoprotective potential of BLs under standardized conditions.

A novel clinical approach to prevent children from having asthma is based on the epidemiologic farm studies demonstrating that protection against allergies and asthma is strongly associated with increased concentrations of environmental endotoxin.[86] Accordingly, Lau et al[87] examined the preventive efficacy of a BL derived from 2 bacterial gut isolates on infant eczema. This BL (Pro-Symbioflor; SymbioPharm, Herborn, Germany) containing heat-killed, nonpathogenic, gram-negative *E coli* Symbio Deutsche Sammlung für Mikroorganismen 17252 and nonpathogenic, gram-positive *Enterococcus faecalis* Symbio Deutsche Sammlung für Mikroorganismen 16440 is already used in patients with infections and inflammatory conditions of the gut and in the prevention of recurrent ARTIs in infancy. The randomized, placebo-controlled trial included 606 newborns with atopic heredity. Infants were treated orally with BLs or placebo from week 5 until the end of month 7 and then followed until 3 years of age. Although there were no effects on the primary outcome (atopic dermatitis rates between the groups), children with 1 affected parent displayed a significantly lower prevalence of AD at 32 weeks of age when treated with the BLs. This finding was more pronounced in infants with paternal heredity for atopy.

The renaissance of BLs in the primary prevention of asthma is only just emerging. In contrast to older trials, newer study designs generally should fulfill the Jadad criteria,[88] including randomization, placebo control, double blinding, and an adequate description of reasons for withdrawals.[76] New approaches, such as the study conducted by Lau et al,[87] highlight that BLs might be successful not only in the prevention of early risk factors for bronchial asthma but also in the protection against subsequent allergic outcomes. Future study designs should therefore include clinical end points of allergic diseases in high- and low-risk children to clearly demonstrate a benefit for allergic conditions.

CONCLUSIONS, FUTURE PERSPECTIVES, AND CHALLENGES

It is intriguing that many of the same environmental risk factors (including changes in microbial exposure, diet, and environmental pollutants) are implicated in such a broad range of inflammatory NCDs, including allergic disease, suggesting early fundamental effects on common immunoregulatory pathways. Of these, the changing diversity of microbial exposure remains one of the leading explanations for the increase in many of these diseases. In this context it is notable that manipulation of the microbiome can prevent not only allergic[89] and autoimmune phenomena but also the longer-term risk of obesity and cardiovascular and metabolic disease[90] and even have effects on mood and behavior.[91,92] This means that interventions aimed at achieving a more favorable microbiome are likely to have multisystem benefits, and it is therefore logical that we take a more interdisciplinary approach in overcoming the increasing global burden of these and other NCDs. In recent years, there have been major advances in our knowledge of the vast and complex human microbiome, with some insights into patterns that are less favorable[93-95] and more likely to induce inflammation and metabolic dysregulation through changes in host gene expression.[96] We have also seen how these physiologic and metabolic adaptations can be reversed through restoring a more favorable gut microbiome.[97] However, there is still considerable uncertainty in defining optimal colonization patterns,[98] how these might vary with the broader environmental context, and how variations might differentially determine the risk of various diseases. These interrelationships need to be further investigated, particularly in early life, when patterns of physiologic, immunologic, and even behavioral response are being established. Even once a potential favorable range is understood, the greatest challenge will be how to achieve this. Clearly, the early environment and early nutritional patterns are major determinants in initiating a long-lasting microbial colonization pattern. Ultimately, it would be both ideal and logical if optimal colonization for multiple health outcomes could be achieved by optimal nutrition and other optimal environmental conditions, rather than by taking a pill or other more invasive strategies to manipulate the microbiome. Working toward this goal requires a more holistic interdisciplinary approach, with a clear recognition of the importance of the developing immune system as one of the key pillars in this process.

What do we know?

- **The gut is a central player in the ontogenic development of immune tolerance.**
- **A well-balanced microbial intestinal colonization in early postnatal life is necessary to establish immune homeostasis later in life.**
- **Prenatal and postnatal environmental stimuli are capable of balancing the immune system toward a tolerogenic response.**

What is still unknown?

- **Whether orally administered probiotic bacteria are able to colonize the gut in adults and thereby affect an already established colonization pattern**
- **How tolerogenic effects are transferred from mother to fetus during prenatal treatment with orally administered probiotic bacteria**
- **Which are optimal conditions (eg, prenatal and/or postnatal application, duration, composition, and dose) for clinical microbial application**
- **Who should be treated (low- or high-risk patients)**
- **Whether clinically applied microbes are able to prevent moderate inflammatory conditions with an onset later in life (eg, rhinitis and asthma)**

REFERENCES

1. Thrusfield MV. Veterinary epidemiology. 2nd ed. Oxford (United Kingdom): Blackwell Science; 1985.

2. Gonzalez A, Clemente JC, Shade A, Metcalf JL, Song S, Prithiviraj B, et al. Our microbial selves: what ecology can teach us. EMBO Rep 2011;12:775-84.
3. Stephens C. Microbiology: intimate strangers. Curr Biol 2000;10:R272-5.
4. Ursell LK, Clemente JC, Rideout JR, Gevers D, Caporaso JG, Knight R. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. J Allergy Clin Immunol 2012;129:1204-8.
5. Willing BP, Antunes LC, Keeney KM, Ferreira RB, Finlay BB. Harvesting the biological potential of the human gut microbiome. Bioessays 2011;33:414-8.
6. Jarchum I, Pamer EG. Regulation of innate and adaptive immunity by the commensal microbiota. Curr Opin Immunol 2011;23:353-60.
7. von Mutius E. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: farm lifestyles and the hygiene hypothesis. Clin Exp Immunol 2010;160:130-5.
8. von Hertzen L, Hanski I. Haahtela T Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Rep 2011;12:1089-93.
9. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. Am J Clin Nutr 2001;73(suppl):361S-4S.
10. Metchnikoff E. The prolongation of life. Optimistic studies. London: Butterworth-Heinemann; 1907.
11. Nissle A. Die antagonistische Behandlung chronischer Darmstörungen mit Colibakterien. Med Klin 1918;2:29-33.
12. McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol 2011;127:1097-109.
13. Hatzler L, Hofmaier S, Papadopoulos NG. Allergic airway diseases in childhood—marching from epidemiology to novel concepts of prevention. Pediatr Allergy Immunol 2012;23:616-22.
14. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol 2012; 129:1187-97.
15. 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.
16. Blumer N, Sel S, Virna S, Patrascan CC, Zimmermann S, Herz U, et al. Perinatal maternal application of *Lactobacillus rhamnosus* GG suppresses allergic airway inflammation in mouse offspring. Clin Exp Allergy 2007;37:348-57.
17. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.
18. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol 2006;117:817-23.
19. Schaub B, Liu J, Hoppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol 2009;123:774-82.e5.
20. Brand S, Teich R, Dicke T, Harb H, Yildirim AO, Tost J, et al. Epigenetic regulation of in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol 2011;128:618-25.
21. Conrad ML, Ferstl R, Teich R, Brand S, Blumer N, Yildirim AO, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. J Exp Med 2009;206:2869-77.
22. Prescott SL, Tulic M, Osei-Kumah A, Richman T, Crook M, Martino D, et al. Reduced placental FOXP3 associated with subsequent infant allergic disease. J Allergy Clin Immunol 2011;128:886-7.e5.
23. Tulic M, Forsberg A, Hodder M, McCarthy S, Martino D, de Vaz N, et al. Differences in the developmental trajectory of innate microbial responses in atopic and normal children: new insights into immune ontogeny. J Allergy Clin Immunol 2011;127:470-8.
24. Prescott S, Macaubas C, Holt B, Smallacombe T, Loh R, Sly P, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T-cell responses towards Th-2 cytokine profile. J Immunol 1998;160:4730-7.
25. Lisciandro JG, Prescott SL, Nadal-Sims MG, Devitt CJ, Richmond PC, Pomat W, et al. Neonatal antigen-presenting cells are functionally more quiescent in children born under traditional compared with modern environmental conditions. J Allergy Clin Immunol 2012;130:1167-74.e10.
26. Lisciandro JG, Prescott SL, Nadal-Sims MG, Devitt CJ, Pomat W, Siba PM, et al. Comparison of neonatal T regulatory cell function in Papua New Guinean and Australian newborns. Pediatr Allergy Immunol 2012;23:173-80.
27. Prescott SL, Wickens K, Westcott L, Jung W, Currie H, Black PN, et al. Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon-gamma and breast milk transforming growth factor-beta and immunoglobin A detection. Clin Exp Allergy 2008;38: 1606-14.
28. 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.
29. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW, 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.
30. Kalliomaki 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.
31. Kopp MV, Hennemuth I, Dietschek A, Goldstein M, Ihorst G, Heinzmann A, et al. A randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical or immunological effects of *Lactobacillus* GG supplementation. Pediatrics 2008;121:e850-6.
32. 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.
33. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa 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.
34. Soh SE, Aw M, Gerez I, Chong YS, Rauff M, Ng YP, 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 2009;39:571-8.
35. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B, et al. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 2007;119:1174-80.
36. Dotterud CK, Storrø O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. Br J Dermatol 2010;163: 616-23.
37. Kim JY, Kwon JH, Ahn SH, Lee SI, Han YS, Choi YO, et al. Effect of probiotic mix (*Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial. Pediatr Allergy Immunol 2010;21:e386-93.
38. Niers L, Martín R, Rijkers G, Sengers F, Timmerman H, van Uden N, et al. The effects of selected probiotic strains on the development of eczema (the PandA study). Allergy 2009;64:1349-58.
39. 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.
40. Grüber C, van Stuijvenberg M, Mosca F, Moro G, Chirico G, Braegger CP, et al. Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. J Allergy Clin Immunol 2010;126:791-7.
41. Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah LJ, et al. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. Allergy 2011;66:509-16.
42. Rautava S, Kainonen E, Salminen S, Isolauri E. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. J Allergy Clin Immunol 2012;130:1355-60.
43. Kalliomaki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. J Allergy Clin Immunol 2007;119:1019-21.
44. Kalliomaki 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.
45. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, 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.
46. Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res 2006;60:221-4.
47. West CE, Hammarström ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. Pediatr Allergy Immunol 2009;20:430-7.
48. Lodinová-Zádníková R, Prokesová L, Kocourková I, Hrdý J, Zizka J. Prevention of allergy in infants of allergic mothers by probiotic *Escherichia coli*. Int Arch Allergy Immunol 2010;153:201-6.
49. Rose MA, Stieglitz F, Köksal A, Schubert R, Schulze J, Zielen S. Efficacy of probiotic Lactobacillus GG on allergic sensitization and asthma in infants at risk. Clin Exp Allergy 2010;40:1398-405.
50. Wickens K, Black P, Stanley TV, Mitchell E, Barthow C, Fitzharris P, et al. A protective effect of *Lactobacillus rhamnosus* HN001 against eczema in the first 2 years of life persists to age 4 years. Clin Exp Allergy 2012;42:1071.
51. Tang ML, Lahtinen SJ, Boyle RJ. Probiotics and prebiotics: clinical effects in allergic disease. Curr Opin Pediatr 2010;22:626-34.

52. Osborn DA, Sinn JK. Prebiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev 2007;(4):CD006474.
53. 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.e11.
54. Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ. Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood—a meta-analysis. Br J Nutr 2012;107:1-6.
55. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. Epidemiology 2012;23:402-14.
56. Fujiwara R, Takemura N, Watanabe J, Sonoyama K. Maternal consumption of fructo-oligosaccharide diminishes the severity of skin inflammation in offspring of NC/Nga mice. Br J Nutr 2010;103:530-8.
57. Maurer AD, Reimer RA. Maternal consumption of high-prebiotic fibre or -protein diets during pregnancy and lactation differentially influences satiety hormones and expression of genes involved in glucose and lipid metabolism in offspring in rats. Br J Nutr 2011;105:329-38.
58. 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.
59. 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;138:1091-5.
60. Ou CY, Kuo HC, Wang L, Hsu TY, Chuang H, Liu CA, et al. Prenatal and postnatal probiotics reduces maternal but not childhood allergic diseases: a randomized, double-blind, placebo-controlled trial. Clin Exp Allergy 2012;42:1386-96.
61. Ouwehand AC, Isolauri E, Kirjavainen PV, Tölkko S, Salminen SJ. The mucus binding of Bifidobacterium lactis Bb12 is enhanced in the presence of *Lactobacillus* GG and *Lact. delbrueckii* subsp. bulgaricus. Lett Appl Microbiol 2000;30:10-3.
62. Grześkowiak Ł, Grönlund MM, Beckmann C, Salminen S, von Berg A, Isolauri E. The impact of perinatal probiotic intervention on gut microbiota: double-blind placebo-controlled trials in Finland and Germany. Anaerobe 2012;18:7-13.
63. Cazzola M, Rogliani P, Curradi G. Bacterial extracts for the prevention of acute exacerbations in chronic obstructive pulmonary disease: a point of view. Respir Med 2008;102:321.e7.
64. Roży A, Chorostowska-Wynimko J. Bacterial immunostimulants—mechanism of action and clinical application in respiratory diseases. Pneumonol Alergol Pol 2008;76:353-9.
65. Puigdollers JM, Serna GR, Hernandez del Rey I, Barruffet MT, Torroella JJ. Immunoglobulin production in man stimulated by an orally administered bacterial lysate. Respiration 1980;40:142-9.
66. Rossi GA, Peri C, Raynal ME, Defilippi AC, Risso FM, Schenone G, et al. Naturally occurring immune response against bacteria commonly involved in upper respiratory tract infections: analysis of the antigen-specific salivary IgA levels. Immunol Lett 2003;86:85-91.
67. Huber M, Mossmann H, Bessler WG. Th1-orientated immunological properties of the bacterial extract OM-85-BV. Eur J Med Res 2005;10:209-17.
68. Rogosch T, Kerzel S, Hoß K, Hoersch G, Zemlin C, Heckmann M, et al. IgA response in preterm neonates shows little evidence of antigen-driven selection. J Immunol 2012;189:5449-56.
69. Cazzola M, Capuano A, Rogliani P, Matera MG. Bacterial lysates as a potentially effective approach in preventing acute exacerbation of COPD. Curr Opin Pharmacol 2012;12:300-8.
70. Weinberger M. Can we prevent exacerbations of asthma caused by common cold viruses? J Allergy Clin Immunol 2010;126:770-1.
71. Razi CH, Harmancı K, Abacı A, Özdemir O, Hızlı S, Renda R, et al. The immunostimulant OM-85 BV prevents wheezing attacks in preschool children. J Allergy Clin Immunol 2010;126:763-9.
72. Bowman LM, Holt PG. Selective enhancement of systemic Th1 immunity in immunologically immature rats with an orally administered bacterial extract. Infect Immun 2001;69:3719-27.
73. Strickland DH, Judd S, Thomas JA, Larcombe AN, Sly PD, Holt PG. Boosting airway T-regulatory cells by gastrointestinal stimulation as a strategy for asthma control. Mucosal Immunol 2011;4:43-52.
74. Navarro S, Cossalter G, Chiavaroli C, Kanda A, Fleury S, Lazzari A, et al. The oral administration of bacterial extracts prevents asthma via the recruitment of regulatory T cells to the airway. Mucosal Immunol 2011;4:53-65.
75. Steurer-Stey C, Lagler L, Straub DA, Steurer J, Bachmann LM. Oral purified bacterial extracts in acute respiratory tract infections in childhood: a systematic quantitative review. Eur J Pediatr 2007;166:365-76.
76. Schaad UB. OM-85 BV, an immunostimulant in pediatric recurrent respiratory tract infections: a systematic review. World J Pediatr 2010;6: 5-12.
77. Cazzola M, Anapurapu S, Page CP. Polyvalent mechanical bacterial lysate for the prevention of recurrent respiratory infections: a meta-analysis. Pulm Pharmacol Ther 2012;25:62-8.
78. Ruedl C, Frühwirth M, Wick G, Wolf H. Immune response in the lungs following oral immunization with bacterial lysates of respiratory pathogens. Clin Diagn Lab Immunol 1994;1:150-4.
79. Faure GC, Bene MC, Simon C, Quantain A. Increase in specific antibody-forming cells in human tonsils after oral stimulation with D-53, a ribosomal vaccine. Int J Immunopharmacol 1990;12:315-20.
80. Jongmans W, Tiemessen DM, van Vlodrop IJ, Mulders PF, Oosterwijk E. Th1-polarizing capacity of clinical-grade dendritic cells is triggered by Ribomunyl but is compromised by PGE2: the importance of maturation cocktails. J Immunother 2005;28:480-7.
81. Rutishauser M, Pitzke P, Grevers G, van Aubel A, Elsasser U, Kämmereit A. Use of a polyvalent bacterial lysate in patients with recurrent respiratory tract infections: results of a prospective, placebo-controlled, randomized, double-blind study. Adv Ther 1998;15:330-41.
82. Ruah SB, Ruah C, van Aubel A, Abel S, Elsasser U. Efficacy of a polyvalent bacterial lysate in children with recurrent respiratory tract infections. Adv Ther 2001; 18:151-62.
83. Boyle P, Bellanti JA, Robertson C. Meta-analysis of published clinical trials of a ribosomal vaccine (ribomunyl) in prevention of respiratory infections. BioDrugs 2000;14:389-408.
84. Brunetti L, Francavilla R, Tesse R, Fiermonte P, Dambra P, Massagli M, et al. Effects of oral bacterial immunotherapy in children with atopic eczema/dermatitis syndrome: a pilot study. BioDrugs 2005;19:393-9.
85. Podleski WK. Immunomodulation of allergic autocytotoxicity in bronchial asthma by a bacterial lysate—Broncho-Vaxom. Int J Immunopharmacol 1985; 7:713-8.
86. Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129-33.
87. Lau S, Gerhold K, Zimmermann K, Ockeloen CW, Rossberg S, Wagner P, et al. Oral application of bacterial lysate in infancy decreases the risk of atopic dermatitis in children with 1 atopic parent in a randomized, placebo-controlled trial. J Allergy Clin Immunol 2012;129:1040-7.
88. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996;17:1-12.
89. 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.
90. Boerner BP, Sarvetnick NE. Type 1 diabetes: role of intestinal microbiome in humans and mice. Ann N Y Acad Sci 2011;1243:103-18.
91. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31.
92. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 2011;141:599-609, e1-3.
93. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol 2012;129:434-40, e1-2.
94. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol 2011;128:948-55, e1-3.
95. Bisgaard H, Li N, Bonnelykke K, Chawes BL, Skov T, Paludan-Muller G, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. J Allergy Clin Immunol 2011; 128:646-52, e1-5.
96. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. Neurogastroenterol Motil 2012;24:405-13.
97. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008;3:213-23.
98. Harris JK, Wagner BD. Bacterial identification and analytic challenges in clinical microbiome studies. J Allergy Clin Immunol 2012;129:441-2.